YUKON RIVER DRAINAGE FISHERIES ASSOCIATION

725 Christensen Drive, Suite 3-B, Anchorage, Alaska 99501
Tel: 907-272-3141 Toll free: 877-999-8566
Fax: 907-272-3142 E-mail: joe-yrdfa@alaska.com

Yukon River/Bering Sea *Ichthyophonus* Scoping and Planning Meeting
October 13 -14, 2004

MEETING NOTES and RESULTS

SUMMARY: Representatives from the Alaska Department of Fish and Game, the US Fish and Wildlife Service, the US Geological Survey, the Department of Fisheries and Oceans Canada, the University of Washington, Oregon State University, the University of Alaska, the Bering Sea Fisherman's Association, the Association of Village Council Presidents, the Yukon River Drainage Fisheries Association, Yukon River subsistence and commercial fishers from coastal, downriver, middle river and upriver locations, and salmon processors from downriver and upriver locations met in Anchorage on October 13 and 14, 2004. They evaluated the impacts of the fish parasite *Ichthyophonus* to salmon and fisheries, described what is known about the pathogen and the disease it causes, identified important knowledge gaps, and set action priorities for addressing the most important needs. Those are to investigate pre-spawning mortality, to monitor the prevalence of the parasite and disease, to determine the undocumented harvest that results from discarded diseased fish, to educate the Yukon fishers about this disease, to develop quantitative diagnostics to measure the severity of the disease, to determine spawning success of diseased fish, to determine the time, means and place of infection, and to investigate possible freshwater horizontal transmission. Collaborative and lead agencies were identified to investigate these areas and a working group was established with YRDFA as the lead. Several project proposals related to *Ichthyophonus* were submitted to funders just before and after the meeting.

Introduction and Purpose of the Meeting:

The fish parasite *Ichthyophonus* was first observed in the flesh of Yukon River Chinook salmon in the mid-1980's while I (Joe Sullivan, YRDFA Program Director) was working as a fish pathologist for the Alaska Dept. of Fish and Game (ADFG). At the time, the person submitting the fish for examination was concerned about the human health aspects of eating this fish. The person was advised that there were none and the investigator dismissed the case as interesting but of little larger consequence. Approximately fifteen years later, however, many more infected fish were observed, the fish smelled strange to subsistence users and they observed that they did

not dry properly as uninfected fish did. Dr. Richard Kocan, University of Washington, was asked to investigate the prevalence and impact of this pathogen, which he did (Kocan and Hershberger, 2003). Dr. Kocan's research suggests a significant pre-spawning mortality among adult salmon in at least some stocks, perhaps as high as 30%, and what appears to be an increasing proportion of diseased fish relative to overall prevalence of infection. Further, some subsistence fishers have reported throwing away up to a third of their catch because of the fruity smell and poor drying ability of infected fish. Because of this, the ADFG received funding to investigate *Ichthyophonus* in Yukon River fish and their research focuses on more accurate assessment of pre-spawning mortality related to this disease and whether escapement numbers need to be increased to account for this. However, even with the answers to these questions, many others remain for stakeholders, the principal one being how will this pathogen affect the quality and quantity of the fish they catch in the future? How much more effort will it take for them to meet their needs and will they be able to meet their needs if the prevalence and severity of the disease increases over time? To answer those questions, a number of aspects of the life cycle, epizootiology and pathogenesis of *Ichthyophonus* need to be investigated. Those aspects could include among many others: investigating the source and timing of the infections (freshwater, marine, what forage fish, are adult carcasses involved, etc.), the impacts of water temperature (is *Ichthyophonus* prevalence and disease increasing due to global warming?), the course of the disease in salmon with intact and compromised immune systems, and so forth.

For these reasons, the Yukon River Drainage Fisheries Association (YRDFA), on October 13 and 14, 2004, brought together the various investigators along with stake holders to assess what we do know, identify the important information gaps, and create a research plan to investigate the priority elements identified. Funding to support those projects may come from several sources, but a unified planned effort of researchers and stakeholders will be more likely to succeed than individual independently planned projects.

Impacts to Subsistence Fishers, Commercial Fishers and Processors:

Lester Wilde, one of the YRDFA co-chairs and a representative of coastal fishers said that this parasite was only seen in burbot, not salmon, and that only a few burbot were caught. He also noted that burbot were caught in the rivers while the salmon were caught in saltwater. When asked how he knew that the spots in burbot were, in fact, *Ichthyophonus*, he said did not know for sure, but that people there saw it mainly in the spring.

Ragnar Alstrom, a processor from 6 miles inland from the coast (Alakanuk) said that he bought 32,000 Chinook salmon in 2003 and 27,000 in 2004 for top-end Japanese and domestic fresh, flaked and smoked markets. Someone in Denver complained about one fish this year having an off smell, thus that was one fish in almost 60,000 fish in two years. Thus, it is not a problem for fish where he catches them. He is also a subsistence fisher and only saw fish of concern in 1998 or 1999 in two fish. One had white cysts and the other had black cysts. His family makes some fillets and freezes some whole fish. Bill Fliris, an up-river subsistence fisher said that he had heard of one fish with white spots noted in the Japanese market of Yukon Chinook. Alstrom then noted that the Japanese have a stricter grading system now than they used to. They won't take red-skinned fish or belly meat anymore.

Billy Charles, a subsistence fisher from Emmonak, also near the coast, said that his family puts up 100 Chinook a year and has not had a problem, but that if anyone from that area would know, it would be Emmonak's natural resource person, Ted Hamilton. Dr. Richard Kocan, who has

been investigating *Ichthyophonus* on the Yukon River for several years, noted that there were very few infections in the flesh in the lower river. Jennifer Hooper, Association of Village Council Presidents (AVCP), noted that many of the lower river villages do not have a natural resources person.

Benedict Jones, a subsistence fisher from Koyukuk, said that he first saw it in 2000 and 2001, and that there was not much around this year, but there were two fish with *Ichthyophonus* near the villages of Hughes and Allakaket. These fish wouldn't dry properly and were soft. *Ichthyophonus* is showing up in whitefish, a non-salmon. Because of low water, whitefish haven't shown up this year like they used to. The water started dropping before the leaves fell. Leaves falling are a sign to the fish to get out of lakes, but because they fell late, the fish got stuck in the lakes. The catch was down from 175 fish to 30 fish per day and had fewer eggs than the previous year. *Ichthyophonus* was also found in moose meat, he said, in the hindquarters under the skin. *[Editor's note: Ichthyophonus does not infect moose, but there are other parasites which do cause white spots in moose]*. When asked what he saw in whitefish, he said that one of 100 fish were infected and had dead skin on the outside. When asked if the heart was infected, Jones said he did not look. When asked what species, he said broad whitefish.

Bill Fliris, a fishwheel operator from Tanana, said that he first saw *Ichthyophonus* in 1986 in one Chinook salmon. The strips he made from it did not dry right so he gave it to the dogs. He saw several fish with it the next year towards the end of the run. The following year he found spots in fruity smelling fish. He sent samples to the state lab but they got inconclusive results. Monty Millard (US Fish and Wildlife Service, deceased) gave him some preservative; he sent the samples to Oregon and they identified the parasite as *Ichthyophonus hoferi*. He went to a YRDFA meeting and told them he was throwing away 25% of his catch. He talked with Russ Holder (US Fish and Wildlife Service) who told him to talk to Dr. Dick Kocan (University of Washington). Dan Senecal-Albrecht (then YRDFA Executive Director) paid Kocan to come up and look which is how he got involved. Now there are problems with canned fish and frozen fish when they have commercial fishing. Virgil Umphenour (buyer/processor from Fairbanks who uses up-river fish) has had to reject fish due to *Ichthyophonus.* So things have changed over the years and now there is a lot of impact. Don Rivard (US Fish and Wildlife Service, Office of Subsistence Management) wondered about the 25% while Kocan had mentioned 5%. This figure was a matter of disease, not infection rates. Dr. Kocan explained that the 25% was a function of Tanana being much further upriver, the 5% rate being what was observed downriver shortly after fish had entered the Yukon. Infection rates were most likely did not change from downriver to upriver locations.

Stan Zuray, who runs a fishwheel upriver from Bill Fliris, said that people now see a lot and there is heightened awareness of the disease in the area. It is different than down at the mouth because of the progression of the disease by the time fish reach his area. **He especially noted that *Ichthyophonus* shuts down the subsistence fishery every year.** It affects people differently. When a third to a half of the fish are infected, he doesn't want to put them on the drying racks. Everyone has the line that they draw relative to *Ichthyophonus*. One person he knows chlorinates the cutting table and throws the fish on the far shore. Some people who say they don't have a problem with it, but he can smell it even before he walks into their smokehouse. But for those who do have some discriminating values, fishers stop fishing when the proportion of seriously diseased fish and discarded fish exceeds what they feel their time is worth. If commercial fishing happens at a late time and people sell their fish to Umphenour, he may have to throw half away. This has the effect of destroying markets. Kids at Zuray's wife's

science camp found 25% of males, females and jacks were visibly infected. They are also seeing white spots in chum salmon, whitefish and other species. The white spots in chum are in a specific part of the run. He thought it was *Ichthyophonus*. He sees other white spots in almost 100% of the humpback whitefish and 75% of the sheefish, but he doesn't know what they are. It could be something like *Ichthyophonus*, but he is concerned that if they don't do anything about this now, they could have the same sort of problems in 10 years with these parasites that they currently have with *Ichthyophonus* now. Dr. Kocan noted that he cultured these white spots and got no *Ichthyophonus* out of the samples. At the meeting, Zuray projected some photographs of white spots in fish other than Chinook salmon. Chum salmon with white spots did look macroscopically identical to infected Chinook. When asked how much he discarded, Zuray said 5% to 24% and that subsistence fishing shuts down as a result at about the 2/3 to ¾ point of the run. Ragnar Alstrom said they get sheefish twice a year but never see them in estuarine waters. Lester Wilde speculated that perhaps infected fish were dying before coming downriver. Dick Kocan remarked that it is possible, but what looks like *Ichthyophonus* in sheefish could be something else. Another participant wondered if it could be related to *Henneguya*, another protozoan parasite that Kocan then described as tailed things that don't culture out *(Editor's note: Henneguya is very easy to identify microscopically, but not with the naked eye)*. Wally Evans, a microbiologist for the ADFG Fish Pathology Laboratory, noted that the smell was what was distinctive for subsistence users of salmon and asked the group whether that same smell had been noticed in other fishes. Stan Zuray said that occasionally when he is drying fish (chums) for dog food, he notices that distinctive smell in one or two fish and thinks there are true examples in sheefish. Most are surface spots and probably not *Ichthyophonus*, but some are imbedded all through the gut and both look and smell like *Ichthyophonus*. Wally Evans offered the ADFG Fish Pathology Lab's diagnostic services to determine what was causing the spots, but others suggested there had been some problems getting samples to them.

Asked to clarify what "shutting down the run" meant relative to meeting people's needs, Zuray said that in general, they did meet their needs but stop fishing at least a week before they otherwise would have because of this. John Hilsinger noted that then affected the timing of the harvest. Zuray said that all the fish he froze were from the first part of the run as a result, but people are still getting fish later in the run. Terry Reeve, University of Alaska, Marine Advisory Program said that in 1988 and 1989 he was buying fish, but stopped buying up-river fish after that because there was too much of this parasite in them. Pat Milligan, Department of Fisheries and Oceans, Canada, took punch biopsies early and late in the Chinook salmon run and had the same observations as Zuray. The early period was just before high water temperatures and the later period just after them. Hilsinger wondered whether this did not contradict the finding that the prevalence rate remained the same throughout the run. Milligan thought perhaps the stress of a fish's weakened condition caused it to be expressed. Kocan suggested that heart samples rather than muscle biopsy samples would probably have been closer to the same prevalence.

Virgil Umphenour, an up-river buyer and processor, next spoke about how *Ichthyophonus* had affected his business. He first noticed it in 1998 when a Steven's Village fisher sold him fish from the late part of the run. He eventually had to discard half the fish even though he tried many different ways of preparing them, none of which resulted in acceptable product. In 1999, the commercial season opened late in areaY-5. The Tanana Chiefs Conference wanted Chinook salmon for a function, so his company got 29 fish, immediately put them into cold water, but still had to discard 15 of them. When he has to throw away half the fish, he makes no profit from selling the rest. In 2002, someone from Kaltag sent in some fish for relatives. About half were infected, but only in the hearts and the flesh was good. In 2003, there were several commercial

openings, then one more towards the end of the run. Although the fish were flown in, over half of these late fish were thrown into the garbage. In 2004, a friend of his got his subsistence fish late and over half were no good. In Eagle, near the Canadian border, Andy Bassich also has the same observation, so people try to get their fish early. Umphenour was particularly grieved when he finds the few really big fish are infected and has to throw them away. Another participant observed that this could also be *Henneguya*, but Kocan noted that it didn't matter if the fish still had to be thrown away. **Umphenour noted that he no longer sells Chinook salmon steaks nor whole big salmon anymore and is forced to fillet every fish.** When David Daum, US Fish and Wildlife Service, asked Umphenour about Tanana River fish, he replied that they don't seem to have the pus spots. Though there are lots of infected fish, not so many of them are thrown away.

Benedict Jones said that the fish always seem to be good when ice is still in the river, so does temperature matter? Kocan said that there is more dissemination of the parasite into the fish in warmer waters because it grows faster and consequently kills more fish. Relating this to global warming, Jones then noted that glaciers that were there 10 to 30 years ago are gone now. Kocan said it doesn't take much. David Daum observed data recorder spikes to 21^{o}C but didn't know how long salmon could take those temperatures. Kocan noted that different places have different temperatures and thus different results. Evans added that perhaps sometimes the problem is not *Ichthyophonus* so much as *Henneguya*, and therefore there are regional differences. ADFG biologist Paul Salomone said that archive tags showed the fish were largely swimming in 16^{o} – 18^{o}C water (choosing where they are most comfortable) and that tributaries generally tend to be cooler than the larger rivers. Nevertheless, Kocan noted, above 15^{o}C, *Ichthyophonus* is almost always lethal so around that temperature, you could also expect problems.

Fliris asked Umphenour what the color of the meat was from Tanana-caught fish. He replied that it ranged from nice to tablecloth white, with more nice earlier than later and jacks seem to have pale meat more often than normal kings (Chinook). Fliris wondered whether people in Nenana and Fairbanks might not see as much, but Umphenour speculated that the fish might just be dying before they get there. Zuray emphasized that the quality of the meat meant something different to different people. At least three people in his area have no problems with any fish even though he himself can smell *Ichthyophonus* just driving by their places. Umphenour does observe infected hearts, but if the meat is fine visually, he can get a good product from that fish.

Pat Milligan (Department of Fisheries and Oceans, Canada) spoke on behalf of the First Nations and commercial fishers from Canada's Yukon Territory. The Yukon River at the border with Canada is 1,200 miles from the mouth and has another 450 miles to go to the spawning areas. Between 3,000 and 10,000 salmon are taken and he has not heard of many reports of fish that can't be eaten but he does receive samples of diseased fish taken from the spawning areas. Prevalences there are low and so there is also the question of pre-spawning mortalities from Canada as well. People are not discarding a lot of fish, but if they die en route, you would never see them anyway. They haven't tracked egg development, but it seems to be okay and the hatchery is near the spawning area. If it is related to water temperatures and stress, there could be more fish discarded if higher temperatures made diseased fish more common. When asked if DFO had looked at offspring to see whether they were infected, Milligan replied that they had tested 100 mortalities and found none. If vertical transmission is occurring, he doesn't see it. And they have been doing this for four years. Simon Jones, DFO fish pathologist, asked Kocan how much inter-annual variability there had been in infection prevalence to which Kocan replied that it was fairly constant. The disease rate, however, changes until it almost approaches the

infection rate. Regarding the question of pre-spawning mortality, however, Gene Sandone, ADFG Commercial Fisheries Regional Supervisor, wanted to know why so many tagged fish were accounted for and did not seem to suffer so much pre-spawning mortality. Perhaps diseased fish were more susceptible to harvest. He needs to know their fate. On the Canadian side, Milligan said that they did not do a telemetry program this year, but that fish can enter the tributaries after the counting towers and he doesn't know whether they make it to where they need to be.

Research Results:

Dick Kocan observed that according to the Center For Disease Control (CDC) *Ichthyophonus* is an emerging disease. It is in a new geographic area (the Yukon River) in a new host (Pacific salmon). It was previously unrecognized in an area undergoing ecological transition (global warming). It could be either a new infection due to changes or evolution or an old infection that is re-emerging as a result of antimicrobial resistance. Though the percentage of female infection rates have remained the same over five years, the males' rate has increased to match the females'. By 2003, most infected fish were also clinical near the mouth, but the rate had remained stable at The Rapids near Tanana. It appears that all fish are being exposed at the same time at the same stage of the infection. Jones asked whether age was assessed differently than size to which Kocan no, that larger fish were assumed to be older. **Lester Wilde noted that coastal water temperatures had been going up which might account for the higher recent prevalence of disease near the mouth.** Dave Daum wanted to know whether jacks got into the Gulf of Alaska or not (i.e.: if not, they couldn't be infected where it is known to exist). **Kocan identified a critical information gap as where, when and how fish get infected.** John Hilsinger, ADFG research supervisor, wanted to know whether there was enough information to compare Emmonak and Tanana sites to which Kocan directed him to Cliff Schleusner (USFWS, OSM) who could make that information available to him. Using only muscle culture from the mouth, his laboratory had gotten only 6.8% positives even though the hearts from the same fish were 30% positive. In the early part of the Canadian samples, they see twice as many positive muscle samples and four times as many later in the run. Again the question was asked as to why more radio tagged fish made it to the spawning grounds to which Kocan replied that he did not have access to that data. He infected rainbow trout experimentally, could find no positive fish at the end of the first week but 100% positive by the end of the fifth week.

Kocan listed what we know about *Ichthyophonus*:

- *Ichthyophonus* can kill wild and cultured fish.
- Plasma cortisol levels are elevated in Chinook salmon during their freshwater migration.
- Plasma cortisol levels suppress the immune response.
- *Ichthyophonus* damages cardiac muscle in Chinook.
- *Ichthyophonus* causes anemia in salmonids (reduced hemoglobin and red blood cells).
- As the temperature goes up the dissolved oxygen decreases (31% decrease from 5^{o}C to 20^{o}C.
- Low blood O_2 increases the demand on the heart to meet tissue needs.

He also noted that when looking at the Upper Yukon versus the Tanana infection prevalence, there was no difference between these two and the rate at Emmonak, but, of fish caught on the North and South shores of the Yukon near Tanana, the North Shore fish had a significantly higher prevalence than the South Shore fish.

Paul Hershberger (US Geological Survey) began by saying that *Ichthyophonus* is a protozoan in the Class Protozoa and the Order Ichthyophonida. It is not known what triggers the organism to break out of its cyst and disseminate. Pacific herring and Yukon Chinook seem to have the same isolate. Rockfish isolates from Washington, Oregon and British Columbia are a different haplotype, but Puget Sound rockfish had the same isolate as herring and Yukon Chinook. From 1898 to 1957, there were six major epizootics in the Gulf of Maine and the Gulf of St. Lawrence and from 1991 to 1994, Sweden and Denmark lost about 300 million herring to this disease. Even though there were no mortalities in haddock, the quality of the meat became unacceptable due to this disease. Experimentally, he got 80% mortality in Pacific herring in 30 days at 12^{o}C. The herring prevalence in Puget Sound progresses over time and may limit the age structure of herring in Washington. The annual mortality increased from 20% in the 1970s to 64-87% per year recently and the age structure has gone down. They used to have 5 to 7 year old fish spawning; now they are 2 to 3 year olds.

Hershberger speculated on possible times and places the Yukon Chinook could be getting infected:

- When salmon migrate out of the Bering Sea?
- Perhaps they should look at smaller Bering Sea herring?
- A possible freshwater origin?
- Other host species in the Bering Sea?

He has not personally seen it in whitefish or grayling but the sample size was low. Some people think the pike in the Yukon drainage have it. They detected it in 2 of 6 burbot they examined from the Rapids; they found it in 25% of the surf smelt in the Puget Sound area and saw a 60% infection rate in American shad. But 0/180 Chinook from Puget Sound were infected as were 0/357 yellowtail rockfish a few years ago. Now the infection rate is between 34-69% for yellowtail rockfish.

Relative to dissemination, Hershberger felt that it was possible that *Ichthyophonus* could be actively distributed through the blood and lymph systems, but that it probably actively burrows through the tissues. He wonders whether spores creep along the outside of blood vessels. Relative to temperature, he said that others had found in 1987 that over 15^{o}C, it was pathogenic in rainbow trout. The test was repeated with buffalo sculpins and found the same results. As Kocan had previously said, Hershberger noted that returning Chinook have elevated corticosteroids.

Gene Sandone asked whether Chinook salmon might be getting it all at the same time since the rate appears to be constant over age groups, to which Hershberger replied that yes, perhaps they were getting it as juveniles or just as they were returning. John Hilsinger wanted to know how long the parasite could live after the fish had died, that is, when can you no longer find it? The answer is not known, but Kocan replied that the lethal temperature for *Ichthyophonus* is 24 ^{o}C to 25^{o}C and Hershberger thought that the higher oil content of Yukon River fish might somehow make them more susceptible.

Chris Whipps, Oregon State University, then addressed *Ichthyophonus* molecular studies, an posed the following questions:

- Are there subclinical hosts?
- Are “new” hosts becoming infected?
- Do fish recover?

He has been working with the Polymerase Chain Reaction (PCR) test and pointed out that it was specific and sensitive. Of the samples he tested, PCR for muscle tissue was as good as any other test, but he thought blood apoptosis might have been the reason for failure to find the pathogen in the blood. Muscle biopsies were less destructive than other samples, but the pathogen is less homogenously found there (thus more potential for false negatives). The blood was more likely to have a homogeneous distribution of the pathogen and perhaps special handling might make it more of a suitable sample tissue.

He wants to know the population structure of *Ichthyophonus*, its biology. What is the mode of reproduction? How is it distributed? Its epidemiology is important. What are the possible sources in the ocean or freshwater? Is it panmixic or does it have structured populations? We need cross transmission studies. For example, the *Loma* (another protozoan parasite) of embiotocids (surf perch) is different from that of other species even though it looks the same. There may be several species of *Ichthyophonus*. Some advantages for working with it are that it can be cultured in vitro, it is found in sympatric (same location) species, and there are genetic markers you can look at to determine whether one isolate is the same as another. Again the question was asked how long it could live in freshwater. Hershberger has been able to keep it alive for 3 months, but in the fish Kocan could not find it after 3 days.

Gene Sandone wanted to know the cost per sample for the PCR test to which Whipps replied $5 (just for the reagents) but this could go to $2 in volume. Sandone asked how old a sample could be. Whipps did not know.

Paul Salomone then reported on preliminary results from ADFG's *Ichthyophonus* work this past summer on the Tanana River and its tributaries. First, relative to anesthetizing fish, MS-222 can't be used if the fish are to be released; CO_2 works but is difficult, as is baking soda. The Department's questions are what are the infection rates throughout the river and do they need to increase escapement goals due to *Ichthyophonus*? Though ADFG is still analyzing the data, it does appear that most of the infected fish from the Chena River samples spawned and there was a good correlation between PCR and cultures. Of 109 fish tagged at the Tanana fish wheel, 15 made it to the Chena River or the Salcha River and one fish was sampled at each site. Bill Fliris (the radio tagging took place at his fish wheel) thought radio-tagging there was too stressful for the fish. A punch biopsy seemed okay, but swallowing a radio tag seemed hard. John Hilsinger noted that it was easy to put radio tags in fish at Russian Mission, but they were much fresher from the ocean there. Benedict Jones caught Russian Mission-tagged fish and was told it took 9 days to reach Koyukuk from there.

Simon Jones, the DFO fish pathologist, reported on the surveillance studies they had been doing in British Columbia from their Nanaimo lab. They sampled herring, rockfish, salmonids and invertebrates. They have done laboratory infections of juvenile Chinook and the knowledge gaps he would like to see filled are life history and quantitative diagnostics. For herring, the prevalence may be up to 50% in some British Columbia stocks and there is concern it might be limiting the populations. Different sympatric species of rockfish have different infection rates in different places and can range from 25% to 78% in some of the higher areas. He would like to know whether the isolates from different species of rockfish are different species of *Ichthyophonus*. Sockeye salmon in British Columbia only have 1.6% to 3.3% positive rate for *Ichthyophonus*, but myxosporidian parasites cause them more problems. His lab has looked at more than 3,000 calanoid copepods (a common food of small fish), all were negative and he is

now considering looking at euphausiids (a larger invertebrate food of herring). Thus the information he wants to know is what is the invertebrate hosts for this pathogen? As may be necessary for salmon on the Yukon River, British Columbia now incorporates a "disease factor" in the management of their herring stocks.

The DFO laboratory studies have shown that whether *Ichthyophonus* is injected or fed to fish, it still winds up disseminated throughout their bodies. Jones has seen a "sandpaper" effect in salmon that Hershberger observed in small herring, some of which ultimately get small skin ulcers as a result. Of the stocks experimentally challenged, Jones was able to infect Yukon River Chinook, but not Qualicum (a local stock) Chinook, suggesting the susceptibility of these stocks is different. The knowledge gaps he would like to see filled are:

- Life history - transmission strategies and pathogenicity
- Diagnostics - histology, culture and PCR have variable sensitivity, but limited quantitative capacity.
- Can the impacts of infection be predicted from the severity of the infection?

The ELISA test for *Ichthyophonus*, if developed, could quantitatively detect it. It is a colorimetric assay that uses antibody interactions to measure the level of an infection. For *Kudoa* (another protozoan parasite) the optical density of the ELISA closely predicted the number of spores in the fish. The test would be expensive to start with, but they are working with a British Columbia company that makes pregnancy tests to get the costs down. The test for *Kudoa* cost about $10 per sample now but would be lower with a greater volume of samples. He thinks it would cost about $10,000 to develop it for herring in four to six months and might cost another thousand for salmon. Hershberger noted that it has been a problem trying to gauge the intensity of an infection and ELISA would be great if you could get it to work. He had seen *Ichthyophonus* in recently metamorphosed juveniles. Kocan observed that the spores are about the same size as brine shrimp eggs which are often used to start feeding newly hatched fish. Hershberger noted that in salinity of 32 parts per thousand, *Ichthyophonus* spores are neutrally buoyant while in freshwater, they sink. John Hilsinger wanted to know whether it would be possible to test stomach contents to which Jones replied that the quality of the stomach contents could be a problem.

Potential Funding Sources:

The meeting participants then looked at different funding sources that could possibly be used to fund *Ichthyophonus*-related projects.

Yukon River Panel Restoration and Enhancement (R&E) and Research and Management (R&M) Funds Susan McNeil, ADFG, described these funds. She said that the total for these could be up to $4 million. For the R&E funds, $1.2 million was available for projects related to Canadian stocks and that up to half of this would be for Canadian projects only. Both US and Canadian investigators can apply for the remainder. This year there will be no more radio-telemetry projects so that would create some room for other types of projects. The R&M fund would be for some undetermined smaller amount that Gene Sandone suspects will be around $400,000 thousand or less. These projects are not just for Canada-bound stocks and up to 50% can go to fisheries maintenance or to support the infrastructure of the fisheries. So far, the Yukon River Panel has been making decisions on more than 50% of the fund. Pat Milligan thought that the Yukon River Panel wanted to see more chum salmon projects proposed. The deadline for submitting concept proposals to the R&E funds was Oct. 11, 2004, the day before the meeting.

The deadline for the R&M projects was October 15, 2004, several days after the meeting. The Panel meets again in December and will identify which projects they want to see developed into detailed project descriptions and budgets at that time. Those will be due in January 2005, final decisions on project funding will be made in March and money will be available in April. Funding for a typical project could be around $30,000.

US Fish and Wildlife Service Office of Subsistence Management (USFWS/OSM) Cliff Schleusner, USFWS, explained that the Office of Subsistence Management annually funds about $4.25 million in projects. Last year (2004) there were 167 projects statewide of which 62 were in the Yukon drainage. The deadline for 2005 is past and there are 14 continuation projects and 5 new projects being considered. Two-thirds of these are stock, status and trends projects and one-third are harvest monitoring and traditional ecological knowledge. Because of continuation funding, there is a small budget for Federal Fiscal Year 2006 of about $400,000 (statewide) for new projects. The funding cycle is every three years, thus the next big cycle is in 2007. They advertise in November, proposals are due in January. The technical review committee makes recommendations in May and asks for detailed plans at that time. In June the committee makes recommendation on those plans and the Federal Subsistence Board decides on funding projects in December or January. Right now the Office of Subsistence Management is undergoing a gap analysis for the different regions.

Arctic-Yukon-Kuskokwim Sustainable Salmon Initiative (AYKSSI). Gene Sandone, the ADFG representative on the AYKSSI coalition, addressed this funding source. Currently, there is $13.5 million available which is expected to be spent at a rate of $5 million a year, though eventually they may have $40 million. There is a focused call next year for Norton Sound chum salmon projects. The committee is waiting for a report by the National Research Council (NRC) on their research plan before spending a lot of money. The proposal is a one-shot deal. Sandone said that a call for proposals would go out shortly *(Editor's note: it is now out and the deadline for proposals is January 14, 2005).* There will be a meeting in March 2005 to decide on which projects to fund. The money will be available in April. The NRC will comment on the Research Plan early next year. Members of the AYKSSI include Kawerak, the Tanana Chiefs Conference, the Association of Village Council Presidents, the ADFG Commercial Fisheries and Subsistence Divisions, the Bering Sea Fishermen's Association, the USFWS and the National Marine Fisheries Service. The call will be on the AYKSSI web page (http://www.aykssi.org).

Pacific Salmon. Jill Klein, YRDFA Executive Director, explained that this is money YRDFA has received for the past two years, almost $500,000 a year, and that the YRDFA Board of Directors decides how to spend. This is not currently a formal call for proposals. The Board could consider funding an *Ichthyophonus* project just as they funded this meeting through this funding source. Stan Zuray, a YRDFA Board member, said that he would like to formalize the process rather than simply continue to do it as we have in the past. The previously mentioned funding sources provide good examples of how the process should be.

North Pacific Research Board (NPRB) No one was present at the meeting from the NPRB, but several had looked at it as a potential funding source. Susan McNeil said that it did not have much to do with salmon. She had written a proposal with Larry Merculieff and got $100,000 to put on an elders conference. Perhaps this group could do that. Klein observed that their call for proposals was also very focused and did not see anything that the group could apply for.

Other Sources. Paul Hershberger noted that the USGS for whom he works does a lot of their work for low dollar amounts. They get some from the State of Washington Department of Natural Resources, some from base USGS funding and some from matching funds. Chris Whipps said that Oregon State looks to the National Science Foundation (NSF) or local Oregon institutions for funding. Dick Kocan observed that the NSF will not fund anything related to disease, so don't bother with them. John Hilsinger pointed out that the money ADFG had used to fund Oregon State's work for them was part of the Southeast Sustainable Salmon Initiative. (SSSI). Simon Jones said that most of DFO's work is collaborative. There are some in-house funds, some partnerships. They will be asking the herring fisheries to support their ELISA work but that could be accelerated with some funding from here. The *Kudoa* work was funded through an aquaculture group. Pat Milligan reported collaborations with Kocan and Simon Jones and that there were three separate proposals submitted to R&E for education/outreach, juveniles and adults. Also there are the Northern Funds, but they would be focused on the Stikine, the Taku and a third Gulf of Alaska river. They want to look at the implications of the disease on the spawning grounds just as Paul Salomone's presentation had.

Gap Analysis

Following various presentations and based on them, a round table discussion occurred creating lists of information gaps which follow below by major categories. Neither the categories themselves nor the topics within them are arranged in any kind of priority.

1. Management Implications
    a. Pre-spawning mortality: conservation escapement goals
    b. Undocumented harvest
    c. Stock specific infection rates
    d. Sex-linked prevalence (females)
    e. Annual fluctuation of prevalence rates
        i. Monitoring program
        ii. What causes fluctuation
    f. Spawning success

2. Diagnostics
    a. Quantitative diagnostics linked to survival/performance, stream life
    b. Non-lethal tests

3. Life History
    a. Susceptible stages/physiology
    b. Genetics: different strains of *Ichthyophonus*

4. Epidemiology
    a. Marine reservoirs
    b. Survey other species of freshwater fish
    c. Experimental infections/susceptible species
    d. Environmental factors (temperature, etc.)

5. Fisheries Effects
    a. Infection altering behavior

b. Pre-spawning mortality
c. Impacts on transmission

6. Transmission Studies
    a. Horizontal freshwater transmission
    b. Horizontal marine transmission
    c. Vertical transmission

7. Things We Can Do Now
    a. Education/Outreach
        i. Discard carcasses (considerable discussion on this issue with no resolution as to whether this was a good or bad thing to do)
        ii. Identification of white spots
        iii. Alternate methods to put up fish

8. Other Diseases/Health Issues
    a. *Ichthyophonus* or other disease, how to identify

Priority Topics

The meeting participants were then asked to prioritize these topics by what they considered most important to address first. Each person was asked to give one vote to each of the top five projects they felt should be addressed. The list that follows below are in order of priority unlike the list in the previous section. After this list was created, the group was asked who was interested in pursuing projects related to these topics. It was generally agreed that there were probably natural leaders who should work with all of those involved with each topic to present unified, coordinated project proposals to funding sources.

1. Pre-spawning Mortality USGS (co-leader for laboratory studies), YRDFA (co-leader for laboratory studies), ADFG (leader for field studies), DFO.

2. Monitoring Program YRDFA (leader), ADFG, DFO, TCC, BSFA.

3. Undocumented Harvest DFO (co-leader), ADFG (co-leader, includes Commercial Fisheries and Subsistence Divisions).

4. Education YRDFA (co-leader), DFO (co-leader), MAP, TCC, AVCP

5. Quantitative Diagnostics/Spawning Success/Time-Means-Place of Infection (three-way tie)
    A. Quantitative Diagnostics DFO (leader), BSFA, ADFG.
    B. Spawning Success BSFA (leader), ADFG
    C. Time-Place-Means of Infection OSU (co-leader), BSFA (co-leader), USGS (co-leader)

6. Horizontal Freshwater Transmission DFO (co-leader field studies), YRDFA (co-leader, field studies), USGS (leader, laboratory studies).

Future Collaborative Efforts:

This meeting brought together both those involved in *Ichthyophonus* research and those impacted by its effects on Yukon salmon in a way that should result in more focused, efficient and effective collaborative work on this problem disease. However, it could have been a one-time alliance of these different stakeholders if it was felt that this meeting accomplished all that was necessary and appropriate. The consensus of the group was that unified approach should continue into the foreseeable future. Gene Sandone observed that in the past, the JTC had appointed a subcommittee to advise ADFG on their *Ichthyophonus* project and that if this larger group continued, the JTC *Ichthyophonus* Subcommittee would be redundant and unnecessary, especially since many at this meeting were part of that subcommittee as well. Sullivan noted that the subcommittee had a limited membership just to JTC members and that potential contractors, some of whom were at this meeting, had been excluded in order to (among other reasons) not compromise the State of Alaska procurement procedures. Nevertheless, the project that resulted from that effort is now in progress and the need for an exclusive group such as that has passed. For future work, it was acknowledged that collaborative efforts of all those interested, involved and bringing skills and knowledge to this effort will be the most effective. All present agreed that there should continue to be an *Ichthyophonus* Working Group, that they would like to be part of it, that others such as the Tanana Chiefs Conference, the Council of Athabascan Tribal Governments and representative First Nations groups should be encouraged to participate as well, and that YRDFA should lead the effort. YRDFA accepted the leadership role and will continue to coordinate efforts in the future. The group will have some as yet undefined relationship with the JTC as this and the Yukon River Panel do provide an umbrella for salmon issues, monitoring, research and management for the entire Yukon in both the US and Canada and that subsistence and commercial fishers from both sides of the border are represented on the Yukon River Panel.

Short-Term Actions:

Five *Ichthyophonus*-related project proposals were submitted to the R&E and R&M calls for proposals. Their titles and proponents are:

<u>R&E</u>

- *Ichthyophonus* Diagnostics, Education and Outreach – DFO, YRDFA
- Survey of Juvenile Chinook Salmon for *Ichthyophonus* – DFO, YRDFA
- *Ichthyophonus* Pre-Spawning Mortality Study, Yukon Territory, Canada

<u>R&M</u>

- *Ichthyophonus*-Related Pre-Spawning Mortality of Yukon River Chinook Salmon – YRDFA, USGS
- Middle Yukon *Ichthyophonus* Monitoring – YRDFA, Tanana Fishwheel, ADFG

Long Term Outlook:

The YRDFA Fall Board of Directors meeting, the ADFG Fall Wrap-up, the Joint Technical Committee meeting and the Yukon River Panel meeting will all have an influence on the future direction of the *Ichthyophonus* Working Group. After this series of meetings, Sullivan will

contact all of the October participants and others who have been identified as important future participants and outline some future steps.

Acknowledgements: Thanks to all who participated and offered their knowledge and insights and willingness to go forward in the future with this effort. Especial thanks also to Eva Aigner (YRDFA Office Manager) who coordinated the meeting venue and provided logistical assistance to many of the workshop participants. Thanks also to the Southeast Sustainable Salmon Fund which provided financial support for this meeting through the Alaska Department of Fish and Game and the National Oceanic and Atmospheric Association. None of the opinions expressed in this document necessarily represent official positions of either of these agencies.