

**OUTFALL MONITORING SCIENCE ADVISORY PANEL (OMSAP)
FLOUNDER LESION FOCUS GROUP MEETING
Thursday, March 31, 2005, 10:00 AM - 1:00 PM
Smith Conference Room, WHOI**

MINUTES

ATTENDANCE

Focus Group Members: Andy Solow, WHOI (chair); Sal Frasca, U. Connecticut; Mark Hahn, WHOI; Grace Klein-MacPhee, URI-GSO; Anne McElroy, SUNY Stonybrook; John Stegeman, WHOI; and Scott Weber, New England Aquarium.

Observers: Sandy Baldwin, USGS; Ellen Baptiste Carpenter, Battelle; Bruce Berman, Save the Harbor/Save the Bay (SH/SB); Michael Bothner, USGS; Jeanine Boyle, Battelle; Todd Callaghan, MCZM; David Dow, NMFS; Patty Foley, SH/SB; Maurice Hall, MWRA; Bob Hillman, Battelle; Carlton Hunt, Battelle; Ken Keay, MWRA; Paul Kennedy, MWRA; Matt Liebman, EPA; Megan Lim, SH/SB; Michael Moore, WHOI; Judith Pederson, MITSG; Andrea Rex, MWRA; and Cathy Vakalopoulos, MADEP.

SUMMARY OF FOCUS GROUP RECOMMENDATIONS

The focus group recommends that the following be considered:

High Priority

- Etiology Study
 - To be designed by M. Moore in consultation with S. Frasca and S. Weber and reviewed by the flounder lesion focus group.
- Additional field studies at multiple sites and seasons
 - Focus on January, February, and March at station FF09
- Other suggested diagnostic techniques
 - Co-culture
 - Electronmicroscopy
 - Shotgun sequencing
 - Consensus primers
- Study the progression of lesions in laboratory flounder caught at FF09 in January
 - Morphology – gross and histological through time (early, open, healing, and healed lesions)
 - Immune function – blood and lymph
 - Experimental sediment exposure
 - Experimental transmissibility

Moderate Priority

- Tagging
 - Use spaghetti, not acoustic tags (model after Chesapeake Mycobacteriosis studies), to study healing and migration.
- Field caging study
- Novel contaminants to consider (pharmaceuticals and personal care products)

MINUTES

Welcome and Introductions

A. Solow welcomed everyone and outlined the discussion questions for the meeting. P. Foley introduced herself. She is the president of Save the Harbor/Save the Bay in Boston and chair of the Public Interest Advisory Committee (PIAC). The role of PIAC is to inform the public about the health of the harbor and the bay and to inform the Outfall Monitoring Science Advisory Panel (OMSAP) about the public concerns. She thanked everyone for taking the time and offering their expertise today to discuss this important subject.

The focus group members then introduced themselves. S. Weber is the head veterinarian at the New England Aquarium in Boston. His background is aquatic pathobiology and veterinary medicine. A. McElroy received her degree at WHOI. She worked on flounder aromatic hydrocarbon interactions at U Mass Boston. She is currently at Stonybrook researching flounder and sewage contaminant related interactions. J. Stegeman has worked with M. Moore in examining hepatic neoplasms in winter flounder has also been involved in studies researching chemical effects on fish. M. Hahn was originally trained as a toxicologist. He works in the WHOI biology department and works on comparative biochemistry and molecular biology studying mechanisms of toxicity. G. Klein-MacPhee recently retired from URI but has worked for 30 years on winter flounder. She worked for EPA raising flounder for toxicology studies, has studied flounder exposure to dredge material in Narragansett Bay, and surveyed flounder numbers for RIDEM. They have not seen many lesions off of Rhode Island, but there have not been many flounder either. S. Frasca is an associate professor of pathology at U. Connecticut. He is a veterinary pathologist for the Connecticut Veterinary Medical Diagnostic Laboratory at U. Connecticut. He has an interest in fish pathology. He recently received some of the flounder with lesions to evaluate histologically.

Findings to date

M. Moore summarized what is known to date. He acknowledged the others that have worked on this project: Roxanna Smolowitz (Marine Biological Laboratory), Kevin Uhlinger (MBL), Lisa Lefkovitz (Battelle), John Ziskowski (National Marine Fisheries Service Milford CT), George Sennefelder (NMFS Milford CT), Jeremy King (MA Division of Marine Fisheries), Maurice Hall (Massachusetts Water Resources Authority), Jack Schwartz (MADMF), and David Pierce (MADMF). The work has been funded by the MWRA through a prime contract with Battelle.

MWRA began examining flounder at Deer Island Flats in the mid-1980's. They began routinely surveying five stations every April (Deer Island Flats, new outfall site, Broad Sound, Nantasket Beach, and eastern Cape Cod Bay) in 1992. In 2004, they added additional surveys and sites as part of the flounder lesion investigation. He listed their fishing effort per year at each station and noted that the new outfall went on-line in September 2000. The station at the outfall is actually about a mile away because there is no fishable bottom around the outfall. They try to sample 50 fish, 30 cm or longer, at each station. He then showed photographs of lesions. They mainly began to see the lesions in 2003, although there were some reports of lesions in 2002. All lesions, with one exception, are on the blind side of the flounder and it has been confirmed histologically that this is a classic case of ulcerative dermatitis. He showed photographs of lesions from the June and September 2004 surveys that appear to be in various stages of healing. NMFS data suggest that this is a seasonal occurrence because they had seen ulcers in the spring and not the fall. When M. Moore first saw the lesions a couple of years ago, he had never seen anything like this in winter flounder. He asked other researchers and no else had either. John Ziskowski, Bob Murkolano, and others from NMFS had an extensive dataset from the

1980's in Boston Harbor that summarized visible external pathologies such as fin rot and bent fin and ulcers were statistically absent from the population.

M. Moore said that when he discovered the lesions, he brought samples to Roxanna Smolowitz at MBL for routine histology and microbiology and she came up with no diagnosis. In 2004, MWRA expanded their surveys and worked with NMFS and MADMF so that lesion identification was consistent.

M. Moore then presented MWRA, NMFS, and MADMF data for the spring 2004 survey. There is a fairly broad elevated prevalence in western Mass Bay. NMFS has stations throughout the Gulf of Maine and ulcers were very rare in the rest of GOM (one found off of Portland, Maine and one found off of Yarmouth, Nova Scotia). He then presented a table of prevalence data. He pointed out that there was an absence of fish inshore in the winter and fall and that this was not the case 15 years ago.

A. Rex asked whether the percent healed on the prevalence table is the percent of fish that had healed ulcers, or the percent of ulcers that were healed. M. Moore replied that it's the percent of fish showing a scar from an ulcer. B. Berman said that means a fish with two healing ulcers would count as one. A. Rex asked how a flounder with two healing ulcers and one unhealed ulcer would be counted. M. Moore replied that the fish would be listed on the table twice. S. Frasca asked if there are ulcers of different chronicity on the same animal. M. Moore replied occasionally, yes.

M. Moore said that he was able to advertise the problem to the scientific community and get feedback during his presentation at the NOAA flatfish meeting in Milford in December 2004. The only person that reported lesions was Donald Danila from Connecticut who recently found a small number of lesioned flounder in the Niantic River potentially comparable to what has been seen in Mass Bay. G. Klein-MacPhee added that D. Danila is sampling fish near the nuclear power plant on the Niantic River.

M. Moore then reviewed Roxanna Smolowitz's and Kevin Uhlinger's work in the lab. They grew 21 cold-loving bacterial species from between 1-10 fish per species of bacteria. Many fish had multiple organisms, 7 were isolated from normal skin samples, 19 were isolated from 28 ulcerated samples. He showed a list of the bacterial species found. There is a broad spectrum of diverse microflora on the skin and ulcers. No fungal or viral elements were identified in either routine or specially stained tissue samples. They did see trematodes in the gills and skin and lymphocystis which are common conditions. A. Solow asked if this kind of analysis was also done on fish without ulcers. M. Moore replied yes. There was a broader, more diverse population of bacteria in the ulcers.

A. Solow noted that there is a rarefaction problem here because the sample size is 10 non-ulcerated fish and 28 ulcerated fish. M. Moore explained that in terms of their sample size for controls and ulcerated fish, they had to make a decision as to how much focus they gave to the controls versus trying to isolate a pathogen. A. Solow understood but wanted to point out that the sampling effort is important. They sampled roughly three times as many ulcerated fish and found roughly two times as many species. M. Moore said that the sampling was designed to maximize the chance of encountering the pathogen.

M. Moore then summarized the features of the syndrome. It appears to be seasonal and began in 2002, possibly 2001, but certainly not prior to that. It seems to mostly be limited to western Mass Bay with the highest prevalence near the Mass Bay Disposal Site (MBDS). George Gardner (EPA) did a study on flounder pathology at the MBDS and he will try to find that report. In the last 10 years, there has

been a major source reduction of contaminants as well as improved treatment of the MWRA effluent. The toxic burden has been reduced enormously and that is reflected in the reduction of contaminated-associated liver lesions in the flounder. The treatment plant is currently full primary and 90% secondary treatment. A. Rex added MWRA is using much less chlorine because of the dilution at the discharge and the increased contact time in the disinfection basins. They are also dechlorinating the effluent. A. McElroy asked when MWRA upgraded to secondary. A. Rex replied that one battery of secondary went on-line in 1997 and they ramped up treatment with two additional batteries over the next few years.

M. Moore then discussed flounder migration. The last flounder migration study in this area was conducted by Howe and Coates in 1975. It is his sense from the literature that flounder are driven by an avoidance of 15 degrees Centigrade or higher. Flounder can find cooler water by going into trenches without having to migrate long distances. It was thought that winter flounder don't migrate far in Mass Bay but he is not sure that this is true anymore. This may have changed due to a change in nutrition. Flounder used to feed at Deer Island Flats which was full of polychaete mats. This is no longer the case with the cleaner effluent and outfall relocation. M. Moore speculated that this could have changed the whole migratory pattern of winter flounder.

D. Dow said that NMFS has outlined essential fish habitats for different species including winter flounder. These documents examine changes over time since the 1970's. M. Moore said that NMFS only fishes during the spring and fall, and not during the winter which is an important time, but he will look at their winter flounder report. He pointed out that on 2/5/91 and 3/12/91, the winter flounder in Broad Sound were abundant, but there were none on 3/10/05. M. Liebman asked if there has been a dramatic increase in flounder numbers at the new outfall in the last few years. M. Moore replied yes, but the increase in numbers began before the outfall went on-line.

M. Moore said that he sent S. Frasca three flounder with early ulcers that they found on March 10th. The earlier the lesion is found, the more likely the agent causing them can be found. S. Frasca has a broader diagnostic artillery arsenal than R. Smolowitz so the chances of finding the causative agent are greater this year.

M. Moore pointed out that there are many seasonal and inter-annual factors which co-vary and could hypothetically be associated with the ulcers. A partial listing includes:

- Changes in the fish during spawning.
- Changes in seasonal precipitation.
- Sediment resuspension and transport.
- Inter-annual climate variations – e.g. North Atlantic Oscillation (NAO).
- Undetected changes in effluent chemistry.
- Seasonal pathogen bloom.
- Seasonal predation.

He noted that J. Ziskowski theorized that hagfish could be attacking flounder as they hide in the sediments but hagfish are scavengers so he does not agree with that theory. He showed a lesion that resembled a bite mark and said that hagfish are mainly found in Great South Channel. S. Weber said that hagfish will attack live things as well. G. Klein-MacPhee said that hagfish are opportunistic. M. Bothner asked how hagfish attack. M. Moore replied that hagfish tend to dissolve the tissue of dead fish. They crawl inside the fish and fill the entire cavity like a “bag of worms”. S. Weber added that tend to attack fish in cages where their prey can't get away.

M. Moore showed a diagram of winter/spring bottom temperatures by region. He then listed ulcerogenic infectious agents in fish: *Aeromonas salmonicida*, *Pseudomonas*, *Vibrio*, and *Proteus* and outlined other studies on cod, flounder, and menhaden diseases. He then recommended that they: (1) continue to quantify prevalence and severity of the lesion during April 2005 flounder monitoring studies; (2) continue to cooperate with state and federal fisheries agencies and scientists to evaluate the condition; (3) further evaluate the long-term USGS mooring, sediment trap, and surficial sediment data for seasonal or interannual changes in sediment quality, either natural or anthropogenic, that might be related to the syndrome; and (4) discuss next steps if any regarding diagnosis (pending results from the U. Connecticut lab).

J. Stegeman said that he was struck by the limited instances of ulcers lesions in the literature and wondered if there are more studies that have identified ulcers in fish. M. Moore acknowledged that he has not done an exhaustive review of the literature. M. Hahn added that there has been a lot of work on flatfish in Puget Sound. He asked if anyone knew whether they have seen anything similar out there. M. Moore said that he spoke to someone there two years ago and at the time, the lesions were rare.

S. Weber asked if they have bottom temperature data from the September collection when the animals were healing because temperature is a temporal factor that plays an important role in the activation of the fish's immune system. M. Moore said they do not measure bottom temperatures during the survey work. M. Hall added that MWRA does have temperature data for that particular time and the area.

J. Stegeman asked if there are there other instances of progression of the condition from obvious pathology to healing and scarring in the literature. M. Moore said that he has not seen such studies. S. Weber noted that the ulcers look fairly defined, circumscribed, and they heal. M. Moore replied yes, and he would describe them as unlikely to have been of clinical significance (i.e. the lesions do not seem to affect the health of the fish).

Focus group discussion

Questions for discussion:

1. What kind of study could be conducted to determine whether or not the lesions are attributable to the outfall?
2. Is there a study that would help us definitively understand what is causing this phenomenon?
3. Discuss theories as to the cause of the lesions: e.g. hagfish predation and fish net trauma.
4. Is there agreement that this is a seasonal phenomenon and that the lesions appear to heal over the summer and fall?
5. Is there a risk to human health?
6. Are there alternative laboratory techniques that might help better identify the pathogens in the flounder lesions?

A. Solow began the discussion with question #5, "Is there a risk to human health?" G. Klein-MacPhee asked if people are likely to eat a flounder with a lesion. M. Moore replied yes, because most of the flounder that people eat will not have the skin on it. G. Klein-MacPhee thinks that it's difficult to say whether there is a risk to human health if the cause of the lesions is not known. The rest of the focus group agreed.

A. Solow then moved to question #4, “Is there agreement that this is a seasonal phenomenon and that the lesions appear to heal over the summer and fall?” G. Klein-MacPhee and J. Stegeman thought that based on M. Moore’s and John Ziskowski’s work that this seemed to be the case.

M. Hahn’s only reservation to the conclusion that this is a seasonal phenomenon is that the seasonal incidence data are superimposed on seasonal migration data thus they may not be sampling the same group of fish. It is not clear to him how this is taken into account in interpreting these data. M. Moore noted that the long term data set on hepatic lesions is secure to the extent that they return to each station at the same time each year, however migration is an issue. J. Stegeman thinks that if this is associated with migration, then one population would have many ulcers and another population would have healed ulcers. He finds this less credible than having a seasonal change in the severity of the condition.

S. Frasca thinks it is important to examine fish with healing lesions histologically because the quality of the healing will also speak to the health of the fish. Animals that have a healthy immune system and are on par nutritionally will demonstrate better healing histologically than animals that are in some ways debilitated.

J. Stegeman asked if they are able to get the fish alive on the deck. M. Moore replied yes, but a lot of stress is put on the fish when it is caught and kept. It also takes about three months to reclimate a flounder in a tank. G. Klein-MacPhee agreed and added that if they are caught with lesions, they tend to get worse rather than better.

A. McElroy asked about tagging. S. Weber noted that they are tagging the striped bass in the mycobacteriosis studies in Chesapeake Bay. He suggested offering a reward to fishermen that turn in tagged animals. M. Moore asked if they should use spaghetti tags. A. McElroy said yes, and she thinks that the additional cost of tagging the flounder would be trivial. A. Solow pointed out that the sample size would have to be much larger. M. Moore said that based on the resource available now, they work hard to catch 50 fish at a station. Catching 250 fish at one station could take an entire day of trawling. The resource isn’t that great now. Back in the 1980’s when Deer Island was discharging, the polychaetes were a yard deep there and the boat would be full of fish after only 10 minutes of trawling. A. McElroy did not think that they would need a lot of fish for a tagging study. A. Solow said that they could figure that number out.

C. Hunt asked if the purpose of a tagging study would be to look at migration or healing. The focus group members replied that the purpose would be to see if the lesions are healing. M. Bothner added that it’s also important to find out if the lesions heal but leave scars. The first step of a tagging program would be to prove whether there was a seasonal cycle to the lesions. M. Moore thinks that the lesions heal completely. G. Klein-MacPhee suggested that they could save some of the flounder with healed scars in a tank to see if they completely disappear but if flounder with open ulcers were collected, they would probably die. M. Moore thought that he could keep them alive. J. Stegeman thinks that this kind of study is worth doing with ulcerated/non-ulcerated fish but that seasonality should also be looked at. S. Weber said that if they couldn’t bring them back alive, they could instead collect blood and lymph fairly readily. B. Berman thinks that a tagging study would be useful not only to see if the lesions heal completely, but also to learn about flounder migration which he thinks is a critical issue.

A. Solow noted that the group’s answer to question #4 “Is there agreement that this is a seasonal phenomenon and that the lesions appear to heal over the summer and fall?” is a qualified yes. If there

is a need to get a better answer to that question, then the group has provided some ideas about how to learn whether the lesions heal completely or leave scars. He then moved on to question #6: “Are there alternative laboratory techniques that might help better identify the pathogens in the flounder lesions?”

J. Stegeman asked about pathogens that may be unculturable. S. Frasca said that there are a number of well-known diseases caused by bacteria that are quite difficult to culture such as mycoplasmas and they require specific culture techniques. He thinks that the histopathological work-up that they have done here is certainly thorough. However, there is only so much that can be done conventionally and there are agents that may be unculturable. There are a number of factors that make diagnosis difficult and so alternative diagnostics need to be considered. Perhaps molecular techniques, additional morphologic techniques like electronmicroscopy, or alternative culture techniques (e.g. co-cultures with different agents). These methods are difficult and time consuming and thus would have to be a part of another directed study. Another option is to modify current techniques to optimize for the host in question.

J. Stegeman pointed out that this appears to be a self-limiting condition, i.e. it doesn't spread to cover the whole underside of the fish. So the question is, when is the earliest that they can sample to find the lesions when they are just forming and are the size of a pinprick. M. Moore said that they looked in January and didn't find any lesions. S. Weber said that many fish diseases manifest themselves at different times of the year at certain water temperatures and many viruses have a very narrow temperature range at which they will manifest themselves. He agreed with S. Frasca in that it is difficult to identify many viruses – although a presumptive diagnosis could be made. He agrees that M. Moore's group did an excellent job on their survey work and a thorough job at the pathology. If there were more funding, sampling in different areas and utilizing other laboratory techniques would be useful. He does not know if the genesis of the lesions begins as a pinprick because many of the ulcerative lesions he has seen have not developed like that.

J. Stegeman asked if they thought that based on the appearance of the lesions that this is more likely a viral rather than a bacterial infection. S. Weber replied that a lot more work needs to be done but the lesions do not suggest some of the common bacterial infections that he has seen before. S. Frasca said that usually *Aeromonas* and *Vibrio* are culturable. The fact that there has been difficulty isolating the agent with the conventional methodologies used argues for something that's harder to culture such as viruses. A fundamental question when trying to understand the cause (etiology), is to determine whether or not the ulcer forms from the inside out, or from the outside in. The problem is that the ulcers here have rounded edges and it is difficult to tell whether they formed from the inside or the out. The earliest lesions will give some insight as to how they formed. He thinks that one important study would be one that attempts to determine the pathogenesis of a lesion. He thinks the group should also discuss other factors that could alter the immune function of fish such as exogenous compounds that may affect the endocrine and immune systems. One thing they may want to consider is incubating animals with the lesions along with animals without the lesions to see whether or not transmission occurs.

J. Stegeman agreed that the question of chemicals altering the endocrine and immune systems is interesting. However, looking back to when there was an enormous chemical burden discharged at Deer Island Flats, ulcerative lesions were not detected. He thinks this argues against a chemical involvement in the etiology. A. McElroy agreed and said that no one has seen this at very contaminated sites. She works in an estuary off of New York that gets most of its fresh water from sewage and the fish don't have these lesions. Although the numbers of lesions are certainly higher near the new outfall site, she sees an overall urban signal because the numbers are elevated in the

whole area. She thinks that something has changed in the 2001-2003 time frame that may be affecting their immune system and/or making conditions more favorable for supporting some type of virus.

S. Weber said that there are several confounding factors here. One is that the immune function of fish, which are cold blooded, varies greatly during the winter versus the summer. Just a few degrees change in temperature can activate certain immunological factors but it can take some weeks to for the fish to adapt to the increase in temperature and make all of the proteins that fight off different infections. This means that since it's winter, their immune systems are probably suppressed during this time. The second confounder is nutrition. When working in Alaska, he learned that when sport fishing for halibut, the best place to find them is at the outfalls. Flatfish seek outfalls to feed, and now that the effluent is cleaner, there is no longer this great food source, and poor nutrition can also affect immune function. The third confounder is urban runoff which contains toxins that may influence immune function. This is a multifactorial problem and researching it will take more than one study.

J. Stegeman asked if S. Weber thought that sufficient differences immune function can be detected in the March versus June fish. S. Weber replied that the lab he worked at in Aberdene used flatfish as their models and they canulated (inserted a catheter into a lymph vessel to draw lymph) them. They noticed temporal differences in their immune function. Flatfish are very good models for canulation because lymph can easily be drawn from them. If an infectious agent was involved here, animals could be infected to see how their lymph changes over time. J. Stegeman thinks that it would certainly be worth doing some studies on immune function.

A. Rex asked if, as in this case, it was common to have histopathological work show no evidence of a viral infection. S. Frasca replied yes, this is not uncommon. One coarse way of organizing viruses is DNA-containing viruses and RNA-containing viruses. DNA-containing viruses can leave behind histologic evidence and there are stains that are more readily available to detect them. RNA-containing viruses can also produce histologic evidence, but they are sometimes more difficult to identify with special stains. There are also other types of viruses such as retroviruses that don't leave a histologic legacy of their presence, however, they can be identified molecularly. S. Weber added that it's probably not uncommon with even the best tissue samples to find any agent. This happens at the Aquarium – despite best efforts, sometimes they can't even determine whether the problem is a virus, bacteria, worm, or even trauma. S. Frasca noted that the same situation occurs in humans. For example, there are no etiologic agents for diseases such as Chrohn's Disease yet this disease has been studied for decades.

A. Solow brought up the point that the new outfall was not only a relocation, but also a change in the treatment of the sewage. A. Rex added that these two things did not occur at the same time. MWRA stopped dumping sludge in 1991, the quality of the influent going into Deer Island improved dramatically in the late 1980's. There isn't any change of the quality of the effluent that one can point to at any one point of time. G. Klein-MacPhee asked when the decrease in chlorination occurred. A. Rex replied that the level of chlorination decreased dramatically when the outfall went on-line in September 2000.

M. Hahn asked if there have been microbiological surveys before and after that change in the chlorine to measure the effect on Mass Bay. A. Rex replied yes. They almost never detect sewage indicator bacteria in the area around the outfall. Viral levels were so low both before and after the outfall relocation that they can be considered the same. M. Hahn asked if those surveys were done in the water or the sediments. A. Rex replied that all of the microbial surveys measured sewage indicator bacteria (except for *Clostridium*) in the water column in the direct outfall area. Sediment surveys for

Clostridium perfringens are also conducted and sediment traps are deployed by USGS. S. Weber noted that the New England Aquarium always measures sewage bacteria at their intake in Boston Harbor and it has been a very clean source, at least in the five years that he has been there.

S. Frasca asked if lesions have been seen in other types of fish in the area. M. Moore replied that they routinely catch other bottom fish and they do not see lesions on them. The fishermen say that they only see lesions on winter flounder. B. Berman asked if they took 50 fish a month before the time that the lesions are usually seen from a location with the highest incidence historically and put them in a tank, would they expect the lesions to form on some of them? M. Moore replied that on the basis of the statistics, yes. However, the act of catching them and putting them into the tank may stress them out to the point that they may either die, change their physiology in some way, and/or become immunosuppressed. M. Hahn agrees with a point that S. Frasca brought up – if there was more known about the pathogenicity of the lesions, there would be more by the way of clues to the cause. Trying to define pathogenicity is probably a priority item – to define the pathogenicity, find the earliest evidence of the lesions, and describe them carefully both histologically and microbiologically.

M. Moore said that this work will require a proposal. He thinks S. Frasca and S. Weber could provide laboratory perspectives and he could prepare the field side of the proposal. However, the experimental design will be difficult to develop. The focus group then listed alternative laboratory techniques discussed earlier: alternate culturing techniques including co-cultures, electronmicroscopy inspection of the tissues, molecular shotgun sequencing of the lesions, and consensus primers for certain virus groups.

J. Stegeman then summarized a collection of studies in Marine Ecology Progress conducted in Oslo Fjord in 1986. Animals were sampled in Oslo's Fjord and European flounder were held on chemically characterized sediments collected from various regions within Oslo Fjord. Circumscribed lesions with a depth formed on the animals that were held in captivity on the sediments from the most highly contaminated regions. The focus group discussed these studies and J. Stegeman noted that the 1986 report only provided observations and no conclusions.

A. Rex asked the focus group if the fact that the lesions are forming on the blind side of the flounder has anything to do with the sediments. S. Frasca replied that it's difficult to say. It doesn't necessarily match with what people conventionally think of as a systemic agent. *Salmonicida* infections in fish don't have a side preference. But these fish are bottom dwellers and one side does have a completely different exposure and that could play a significant role. He couldn't answer A. Rex's question but thinks that is an important element to future evaluation of the pathogenesis. M. Moore thinks it's reasonable to assume that they are not going to find one single agent that is causing this problem. Factors that are probably involved are an infectious agent, some type of physical traumatic event, and temperature causing immunosuppression. S. Frasca noted that shell disease in lobster is always seen on the dorsal side.

A. Rex asked if the bottom side of the flounder is tougher. G. Klein-MacPhee replied that the scales are ctenoid (pricklier) on the top and cycloid (smoother) and smaller on the bottom on females. M. Moore added that the males are rough on the bottom. A. Solow asked if there was there any difference in prevalence in males versus females. M. Moore replied that they have not compared males versus females because there are many more females being caught. G. Klein-MacPhee said that they have the same problem off of Rhode Island – it has become much tougher to find flounder, and when they do, there now tends to be many more females than males. M. Hall said that at least 80% of the flounder they catch in Mass Bay are females.

M. Hahn thought that thought this may not be a factor in this case, but that he should at least mention a group of chemicals – pharmaceuticals and personal health care products. These are an emerging group of contaminants from birth control hormones to antibiotics and as far as he knows, there are no data from this area. A. McElroy said that she knows someone that is taking measurements of these types of chemicals and conducted a very small fish study off of New York. The fish were kept on the contaminated sediments for a short period of time. After exposure, they were found to have an elevated protein indicative of exposure to estrogen mimics. Interestingly, three out of four fish also picked up a nematode that was not seen in any of the fish or the sediments.

M. Hahn asked if there are other species besides hagfish that may prey on flounder. He also asked if there have been any new benthic invertebrates detected. K. Keay replied that MWRA has an extensive benthic sampling program and overall, they have not seen any changes in the benthic communities in the four years since the outfall went on-line. However, spatial scales are important and if a predator lives at a density of one per square meter, it probably wouldn't be captured by the sampling program. M. Hahn asked how much time flounder spend off of the bottom. M. Moore replied only in short bursts. G. Klein-MacPhee added that they don't do a lot of swimming in the water column.

J. Stegeman asked about the hydrography in the area of the outfall and whether the effluent is distributed over the same areas where the lesions are found. B. Berman noted that migration should also be taken into account. A. Rex said that the pattern of flounder lesions is not consistent with the presence of the outfall in terms of the gradient of dilution. C. Hunt said that the effluent plume is generally located further inshore and to the south. J. Stegeman concluded that if the lesions are outfall related, it has to be due to a combination of the movement of the water and the movement of the fish. A. Rex also noted that it also depends on the time of the year. During the spring when the lesions are forming, there is no water column stratification and so the effluent is more dilute. During the summer, the effluent is more concentrated on the bottom.

A. McElroy asked if they will continue the surveys and study the seasonality of the lesion formation. A. Rex replied that they have not decided what they will do in addition to their annual spring survey. A. McElroy thinks that they should at least sample at some of the stations in January/February. A. Solow suggested that the focus group ask for M. Moore to outline a proposed plan of research to address the questions discussed today.

T. Callaghan asked if a study similar to the one in Norway could be conducted here. Lesion-free flounder could be placed on sediments collected from where the lesions are prevalent to see if any ulcers form. M. Moore said that he has had problems in the past conducting this kind of study. M. Hahn suggested some type of caging study although he thinks that this would also be very difficult logistically. S. Weber asked about sediment chemistry studies. A. Rex replied that USGS is conducting a large sediment quality study and MWRA has been sampling sediment since 1992. MWRA also monitors the benthos, water quality, and effluent. M. Hahn asked if pharmacological and personal products can be added to the list of chemicals monitored. A. Rex replied that it's possible to test for these chemicals but there are a lot of them, and we need to know what to look for. J. Stegeman asked if MWRA conducts sediment toxicity bioassays. K. Keay replied no. They measure PAHs, PCBs, metals, *Clostridium*, grain size, and TOC. J. Stegeman asked if the results have been relatively static. K. Keay said that there is a rough onshore to offshore gradient from historical sources and little or no change in anything except for a localized increase in the vicinity of the outfall of *Clostridium* since the discharge went on-line.

M. Liebman suggested that the focus group prioritize the list of suggestions (see list on page 1). G. Klein-MacPhee thinks that studying the progression of lesions was a high priority. J. Stegeman suggested that during their next survey, they fix material appropriately for doing sequencing. C. Hunt said that the next survey is in late April. M. Moore said that he could freeze the entire range of ulcer stages that he finds in April. S. Frasca said that they could potentially store tissues for three to six months for future work-up. He suggested that progression studies be conducted to see how the lesion changes under different temperatures and conditions. He noted that his lab does not have the capacity to deal with large projects using complicated diagnostics without having individuals directly associated with the work at U. Connecticut.

A. Solow said that the focus group will present the study proposal to OMSAP who could then recommend to EPA/MADEP that the study be conducted. He understands how complicated these types of problems are and thinks that though this may never be solved, more progress should be made. The focus group agreed. P. Foley said that after having conversations with some of the other PIAC members, PIAC will support OMSAP to recommend fast track funding, because this is an important issue.

Adjourned

Summary prepared by C. Vakalopoulos.