

**Mussel Watch Pilot Expansion Study**  
**Technical Meeting Notes**  
**Meeting Date - May 24, 2012**  
**Location - NOAA Sand Point Station, Seattle, WA**

**Executive Summary:**

The Washington Department of Fish and Wildlife has received grant money for a pilot expanded Mussel Watch (MW) study in Washington, to evaluate the geographic extent and magnitude of nearshore contamination in the greater Puget Sound. Several study design elements under consideration were discussed at this technical meeting. These included the timing (season) of mussel sample collection, the feasibility of using caged mussels versus naturally occurring mussels, and the desire to co-locate stations with other nearshore monitoring sites, including for eelgrass and sediment chemistry studies and for Natural Resource Damage Assessment (NRDA) baseline needs. The principle investigators (PIs) introduced the study design elements and there was general agreement that a representative sampling design, as opposed to a random sampling design, was better suited for this study. However, no specific decisions were made regarding station selection criteria and the discussion was cut short to address the main agenda items.

The PIs presented several reasons they believed sampling mussels in the early to mid-winter, when NOAA Mussel Watch traditionally samples in Washington, would best match the needs of this study. After several discussions the group consensus was that the MW Expansion Study should stick to sampling over the winter months.

Next there was a discussion about whether sampling caged mussels instead of naturally occurring mussels would be advantageous for this study. There was general agreement that the use of caged mussels offers many specific advantages for this study, including control of station placement, a known exposure period, and uniform mussel species and starting size/age. A model of a monitoring program that uses caged mussels anchored off shore, in the subtidal zone, was presented. Several attendees felt that the costs involved in deploying/retrieving subtidal cages, and the additional staff needed to supervise volunteers, would be unsustainable and that volunteers would lose a sense of ownership or connection to MW with this model. They also expressed concern that data integrity may be compromised if volunteers participated in laboratory work.

A hybrid approach was proposed. The idea was to strategically use caged mussels by placing them in the intertidal zone (on beaches and/or piers) where they can be co-located with eelgrass and other nearshore monitoring stations when needed, and easily reached by volunteers on foot. There was general agreement that using this approach would provide a good balance of study design elements; it would allow the benefits of caged mussels while ensuring the ability to utilize volunteers to place, and potentially monitor, the cages. The PIs clarified that the MW Expansion Study would provide the equipment and mussels for the cages and train the volunteers in the necessary protocols. The final recommendation was to pursue the hybrid approach described above for the purposes of this pilot Mussel Watch Expansion study.

**In attendance:**

James (Jim) West (WDFW) and Jennifer Lanksbury (WDFW) – PIs for the Mussel Watch Pilot Expansion Study; Steve Quinnell and Laurie Niewolny (WDFW), Kathleen Herrmann and Emily Whitney (Snohomish County), Lincoln Loehr (Snohomish County MRC and SWG), Robert (Bob) Johnston (US Navy, ENVVEST), Alan Mearns (NOAA), Nancy Beckvar (*invited by Mearns*, NOAA), Frank Cox *for Jerry Borchert* (WDOH), Kimberle Stark (King County and SWG), Rich Sheibley *for Tony Paulson* (USGS and SWG)

**Background and Scope:**

The Washington Department of Fish and Wildlife's (WDFW) *Toxics in Biota* team has received grant money for a pilot expansion of Mussel Watch (MW) monitoring in Washington, to be conducted in 2012-2014. This project is funded by the United States Environmental Protection Agency (EPA) through their National Estuary Program (NEP) and granted via WDFW in its capacity as Lead Organization for 'Marine and Nearshore Habitat Restoration and Protection'. The short term goal of this MW Pilot Expansion Study is to evaluate the extent and magnitude of contamination in nearshore biota of the Puget Sound, while the long term goal is to develop a status and trends monitoring program for toxics in Puget Sound nearshore biota.

This pilot study will be designed to evaluate the geographic extent and magnitude of nearshore contamination in the greater Puget Sound, covering a wide range of nearshore land-use conditions including rural, undeveloped, agricultural, urban, and industrial areas ranging over about 60 sites (locations as yet undetermined). Several study design elements, including the season of mussel sampling, use of caged vs. naturally occurring mussels, and placement of stations, are yet to be determined. The first two elements were discussed at this technical meeting, while the later will be addressed at a series of workshops (held to be held on June 28 and July 9 and 13, 2012). The PIs will take into consideration the information, concerns and data gained from these meetings and workshops when making final decisions regarding the design elements for the MW Pilot Expansion Study.

**Purpose of meeting:**

This meeting was intended to gather the few people in Puget Sound who currently use mussels to monitor toxics, to compare notes and discuss technical details regarding use of mussels for this purpose. The list of invitees for this technical meeting included a select group to maximize efficiency and match skills with needs to help the PIs consider specific design element options for this study. Specific issues discussed included the timing of mussel sample collection (i.e. which season to target), the costs/benefits and feasibility of using caged mussels versus naturally occurring mussels for this study, and data compatibility issues that have arisen as the PIs combine mussel contaminant data from past National MW and ENVVEST program sampling.

**Agenda for Mussel Watch Pilot Expansion Study**  
**Technical Workshop, 5/24/2012, Sand Point, WA**

**1:00 – 1:30 Mussel Watch Pilot Study - Scope of Work** (15-20 min)

- Background and brief refresher of study goals/objectives
- Geographic scope
- Station selection criteria

**1:30- 3:45 Study Design Elements**

- Sample timing – when should we sample mussels? (30-45 min)
  - SCMRC seasonal study
  - Seasonality of contaminant inputs
  - Mussel biology
- Naturally Occurring vs. Caged Mussels (1–1.5 hr)
  - What factors are most important to control in this study?
  - Pros/Cons of each method

**3:45-4:15 Washington MW data Summary (2004-2011)** (20-30 min)

- Data compatibility issues - how labs report data (qualifiers, non-detects)
- Procedures for estimating contaminant totals (Total PCBs) and summations (Sum PAHs)
- Analyte lists and what to include in summations (diagnostic analytes?)
- Anticipate comparisons with other media (sediments)?

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**Meeting notes:**

**DOH Summary** - Frank Cox, lead for the Shellfish Biotxin Monitoring Program at the Washington Department of Health (DOH), needed to leave the meeting early, so he was allowed give a history and overview of DOH's use of caged mussels for paralytic shellfish poisoning (PSP) monitoring.

Noteworthy items he expressed included:

- Collecting mussels from the shore has allowed them to monitor for PSP without a boat, this was a great advantage for them
- DOH collects mussels by scraping them off piers or taking them from stocked cages
- Caged mussels are used anywhere the cage can be suspended from a rope
- Theft and/or vandalism can be an issue
- Predation by starfish can be an issue (dependent on location)
- During the summer, cage fouling by algae can be a problem as it can impede water flow

- Sometimes there is a sudden die off in cages, but no chronic pattern of die offs has been evident
- There are inherent differences in species survival and hardiness in cages - he believes survival is highest with *Mytilus galloprovincialis* and goes down for *M. edulis* and *M. trossulus*
- Differences in transplant survival between species may be dependent on local temperatures
- They use an assortment of mussels to stock their cages (no uniform size)
- They only monitor for PSP with their mussels, no other endpoints

**Mussel Watch Pilot Study, Scope of Work** - after Frank departed, Jim introduced the technical meeting agenda. Below is a summary of his introduction:

The scope of the MW Pilot Expansion Study (Expansion Study) is to measure the extent and magnitude of nearshore contaminant conditions. Mussels provide the connection between the land and water. The funding source for this study is a “Crosscut” block grant from the EPA’s NEP program to WDFW/Washington Department of Natural Resources (DNR) to examine contamination in the marine nearshore areas of Puget Sound.

There are multiple benefactors to the Expansion Study. The “Crosscut” funding is a result of expected links between nearshore contamination and upland land use. A related companion study on effects of outfalls on eelgrass is to be conducted, with some stations co-located between the two studies. Past NOAA MW sampling, this Expansion Study, and hopefully future Washington mussel watch studies will continue to inform Puget Sound Ecosystem Monitoring Program (PSEMP) monitoring and will be applied as a tool in the Puget Sound Partnership’s Vital Sign Indicators. Additionally, this study will help inform future MW sampling for stormwater monitoring and subsequent National Pollutant Discharge Elimination System (NPDES) permitting, the process of which is currently being reviewed by the Washington Department of Ecology (Ecology). Also, the MW Expansion Study will provide oil spill baseline data, effectiveness monitoring, status and trends for point sources, and will provide a continuation of the NOAA Mussel Watch work.

Questions, Concerns, Discussion -

*Kathleen* - asked about NOAA Mussel Watch funding. No one knew what the funding status of the National MW Program is like at this point. She suggested sending a letter of support to the NOAA MW Program.

*Lincoln* - raised a concern that if we use mussels from Penn Cove for transplanting into cages, and the initial condition data for polycyclic aromatic hydrocarbons (PAHs) go into Ecology’s Environmental Information Management (EIM) system, it could in theory be used by Ecology, if they back-calculated water quality parameters from that data, to designate Penn Cove as an “impaired water body”. (He noted that Ecology considers the water to be impaired above 0.93 ppb dry weight for each individual

PAH.) He felt that this would not be a proper use of the data and cautioned that certain parties may be harmed in this designation, i.e. could be a risk to the shellfish industry in Penn Cove.

Type of Sampling Design - Jennifer presented some of the major ideas behind the MW Pilot Expansion sample design. Below is a brief summary of her presentation:

As the result of a feasibility study carried out this last summer, funded by the Stormwater Work Group, Jim and Jennifer concluded that using urban growth areas (UGAs) vs. non-UGAs to test for differences in nearshore contamination (using mussels) requires a very high number of sampling stations to gain the statistical power necessary to resolve differences. Since it is not financially feasible to sample to the extent necessary (>200 sites) to resolve differences between these two classes (UGA vs. non-UGA), they suggested using the degree of impervious surface upland from the shoreline to provide potentially better distinctions/classes for resolving statistically significant differences in nearshore contamination. To the extent possible the 60 stations to be sampled in the MW Pilot Expansion Study will be selected to represent three broad classes of impervious surface (low, medium and high) in the upland, with stations also placed to cover as broad a geographic range, within the scope of the study, as possible.

Furthermore, to address the multiple needs of the data, some stations will be selected to represent obvious, large-scale sources of contamination such as cities, large marinas, large outfalls, combined sewer overflows (CSOs), wastewater treatment plants (WWTPs), etc. In addition, the study scope of work requires co-locating some MW stations with eelgrass monitoring sites and will consider the needs of NRDA baseline monitoring locations for oil spill assessment. Since eelgrass monitoring areas often occur along shorelines lacking suitable substrate to support naturally occurring mussels, co-location at those sites may only be possible using caged mussels.

#### Questions, Concerns, Discussion –

*Kathleen* - wondered if the MW Pilot Expansion Study sites would include the traditional NOAA MW sites. It was agreed that the traditional NOAA sites should be included.

*Kathleen* – suggested we consider the level of impervious surface within the watershed or sub-watershed near a MW site. She felt drainage/flow patterns would be better suited for stormwater monitoring, which is NPDES driven.

*Jennifer* – noted we have not yet resolved at what extent/distance near or around a MW site we will consider upland impervious surface. Also, stormwater drainage patterns do not always follow watershed drainage, as stormwater drains can reflect false watersheds (i.e. pipes running underground).

*Bob* – suggested using simpler selection criteria, with fewer elements, to guide station selection. He thought site selection should be done on the basis of management units, using a geographical focus or

basin coverage rather than a set of impervious surface classes. He said since the MW Pilot Expansion Study is relatively small, we should try not to do too much at once.

*Bob* – suggested setting aside a subset of stations to study potential gradients at the eelgrass sites. This separate set of sites could focus on questions relating to the eelgrass study, without worrying about how they map out related to impervious surface.

*All* - there was a general agreement that a representative sampling design, not a random sampling design, was better suited for this study. However, no specific decisions were made as to how to address more specific station selection criteria and meet all the data needs, and the discussion was cut short to address the main agenda items (see below).

At this point Nancy Beckvar joined the meeting, invited by Alan Mearns.

*Further discussion of station location requirements will occur in the MW Pilot Expansion Workshops, to be held on June 28 and July 9 and 13, 2012.*

**Study Design Elements - Sampling Timing** - Jennifer presented the ideas behind sample timing. Below is a brief summary of her presentation:

The Snohomish County Marine Resources Committee (MRC) has collected Mussel Watch samples over several wet and dry seasons in the last 10 years. They found that, generally, mussel contaminant loads were higher during wet seasons. But relative to each other, site contaminant levels were consistent (e.g., site A was always two times higher than site B) no matter the season. This indicated two important points; 1) to get a maximum contaminant signal one should sample in the wet season, and 2) one should consistently sample the same season from year to year.

Data from a study by Anna Kagley et al. (2003) and others indicate that, in Washington, rapid mussel growth begins in early spring, spawning generally occurs sometime between March-May. Losses of up to 50% total wet weight have been reported following mussel spawning. Mussel condition index declines throughout the fall to a minimum in the winter months (December-February), during which the mussels are thought to be reproductively quiescent. To capture a period in time that singles out high contaminant exposure and minimize interference by changing spawning condition and/or variable growth, we believe it is best to sample in the early to mid-winter time period. This sample timing will allow the mussels to capture the increase in stormwater runoff that occurs in the fall, with the rising rainfall, and would allow for continued comparison to previous NOAA Mussel Watch studies (always sampled in the winter).

#### Questions, Concerns, and Discussion –

*Bob* - suggested it is possible to correct for changes in mussel condition index between seasons using lipid correction, i.e. to overcome changes brought on by the reproductive state.

*Jim* - did not feel lipid correction is especially reliable and would like to avoid sampling during the spawning phase during this study. He felt it is much better to eliminate variability associated with spawning condition through sample design, rather than through post hoc correction. Also, larger mussels generally have smaller contaminant concentrations on a per gram basis compared to small mussels, so sampling in the early to mid-winter can control for this variability.

*Jennifer* – said that boating activities probably increase in the late-spring through summer, so contaminant classes related to those activities (PAHs) may increase in the mussels at that time.

*Kim* - mentioned that mussels exposed to freshwater, like in a freshwater lens scenario, temporarily suspend feeding/filtering water. She believes mussels equilibrate to their surroundings in about four weeks, but may take up to 90 days (3 months) to capture the water quality of the vicinity.

*Kathleen* – suggested developing bullet points to compare the pros/cons of moving the sample period up a month from the normal NOAA Mussel Watch winter sampling (generally Dec-March) to justify our logic if we make that decision. She thought it was especially important to maintain comparability with the NOAA MW data set.

*Jennifer and Jim* – noted that if we move to caged mussels that would mean deploying the cages in early October and retrieving in December/January, so that the mussels would be in the water during the (average) yearly precipitation maximum, which likely coincides with the maximum stormwater contaminant input, into the Puget Sound.

*Nancy Beckvar* - asked whether we would be measuring bioeffects (not just contaminant tissue residue) on caged mussels. If so, what kind of end points would we be measuring?

*Jennifer* – said we would measure the growth and weight of mussels over the time period (starting and ending measurements).

*Nancy* – suggested an exposure time period of ~3 months might be too short to capture changes in growth (a latent effect).

*Jennifer* – conceded also that growth might be minimal over the winter months, since mussel condition index appears to go down over those months. She wondered whether this is because the food supply is too low during the winter to sustain growth.

*Jim and Nancy* – discussed the possibility of measuring other bioeffects endpoints, like the levels of some hormones in the mussels.

*Jim* – said his *Toxics in Biota* group measures vitellogenin in fish blood/bile and wondered whether we could measure that or some other contaminant indicator in mussels? He said it would be worth looking into other possibilities.

*Alan* – asked about using Semi-Permeable Membrane Devices (SPMDs) at sites.

*Jim* – replied that they would be most useful to use in tandem with the biota (mussels) vs. by themselves. However, we don't have the budget to support SPMDs.

➤ Ultimately, the consensus was that we should stick to sampling over the winter months.

**Study Design Elements - Naturally Occurring or Caged Mussels** - Jennifer presented several factors important for allowing control of variability in the MW Pilot Expansion Study, and presented some examples of contaminant monitoring studies using caged/transplanted mussels. Below is a brief summary of her presentation:

Several characteristics important to consider when planning a large-scale status and trends monitoring program include, 1) identification of a single species within the habitat of concern that has 2) a ubiquitous distribution (i.e. found in all areas where monitoring is desired) and 3) is easy to collect with 4) relatively simple, affordable sampling protocols. The statistical power of a monitoring study design (i.e. ability to resolve differences, if any occur) is strongly influenced by the variability in the data collected; lower sample variability gives the scientist more power to detect a statistical difference between sites. Some ways to help control variability in a study include sampling organisms exposed to contaminants for a similar period of time, minimizing/avoiding the effects of reproduction, and sampling the same life-stage.

There are mussels at many sites around the Puget Sound, some already sampled by MW, the Snohomish County MRC, ENVVEST, and other programs (DOH). The *Toxics in Biota* group has estimated mussel habitat at most UGA sites around the Sound. However, it is clear that mussels do not occur in *all areas* of the Sound, including some that may be highly desirable for sampling. Also, mussels generally do not co-occur with eelgrass beds, which will make it hard to co-locate sites with the eelgrass study, or in muddy/sandy areas historically monitored for sediment contamination. The match with sediment monitoring sites is not necessarily important for the MW Pilot Expansion study, but may be important if/when mussels are used for NPDES monitoring. There are several different species of mussels living in the Puget Sound, including some hybrids impossible to distinguish without genetic testing. Additionally, naturally occurring mussels vary dramatically in quantity and size at sites between years, sometimes disappearing altogether, as has happened at several national MW program sites that had to be dropped.

The use of caged mussels can solve many of these problems. Perhaps most importantly, caged mussels can be placed anywhere there is need for monitoring. Live mussels can be purchased at any time from local shellfish farms, where a single species of a uniform starting size/age can be selected. Caged mussel can be immersed for a known period of weeks/months, equalizing exposure time between sites.

Jen presented information about a Mediterranean “active bio-monitoring” program called The Mytilos Project. She went over their basic protocol, including a map of their sites, pictures of their cages, size/age of mussels used, depth of placement, and exposure time. She then showed a

mockup of a caged mussel design, based on their design, which was described as a suspended, anchored cage system set at a specific tidal height. The scenario was presented that volunteers could help size and bag mussels at a mussel farm and help deploy mussels (via boat) after an initial resting period at the farm. Mussels would stay on site for a period of months (unspecified) and retrieval may require some snorkeling.

The participants were then asked for feedback and discussion.

#### Questions, Concerns, and Discussion -

*All* - Generally the consensus was that using caged mussels seemed optimal for allowing greater flexibility in site selection and to control variability, but a number of concerns were raised by the participants.

*Kathleen* - was curious how the MW Pilot Expansion Study will be comparable to the historical NOAA MW data, since NOAA samples naturally occurring mussels.

*Kathleen* - felt that the skills necessary to stock, deploy and retrieve caged mussels using this model seemed difficult for volunteers, as compared to the current NOAA MW program. Additionally the volunteers need ownership or connection to the study site/beach they sample, and the more high-tech *Mytilos* model would make that ownership limited. She didn't see how she could mobilize more volunteers for this model. Further, she was concerned that Snohomish County's small MRC budget would not support a *Mytilos*-type model. This is because the additional level of effort, from a county staffing standpoint, that appears to be needed for this caged-mussel model meant she would probably have to reprioritize and drop other County projects.

*Kathleen and Emily* - noted that broad based volunteers should not be used in the lab to shuck mussels or otherwise process them for analysis; however a small group of trained volunteers for this task may be more appropriate. They felt the technical nature of that work and the level of staff effort for training and supervision of lab work would make the use of volunteers cost prohibitive. They also felt that volunteer error could adversely affect the quality of the samples/data.

*Kim, Bob and Laurie* – echoed Kathleen's and Emily's concerns about losing sample/data quality through volunteer errors.

*Jennifer* – conceded that the level of staff time for training/supervision of MW volunteers to help with measurements/shucking might not be cost effective and could threaten data quality. However, she agreed that training a small subset of volunteers, people with some background in laboratory work or science interns, for some of the technical work might still be worthwhile.

*Kim* – felt that relying on wild mussel populations would not be sufficient considering the kind of geographic coverage needed by this study.

*Bob* – said that using caged mussels would be advantageous because it would allow for testing of more hypotheses (e.g., gradient studies). But he felt that the logistics of solely using caged mussels for this study would be overwhelming. He suggested the potential for a combination of wild and caged mussels for this study, to combine surveillance with diagnostics, like testing exposure gradients.

*Alan* – suggested a hybrid approach; using caged mussels, but placing them in the intertidal zone where volunteers could get to them.

*Lincoln* – suggested strapping some cages to pilings too.

*Jennifer* – liked the idea of the hybrid approach because it would combine the main advantages of caging (unlimited site location, controlled exposure period, uniform species and size/age) with the ability to have volunteers participate and have site ownership back.

*Kathleen* - reiterated her concerns about what the volunteers would be expected to do and whether Snohomish County MRC would have to purchase the supplies for the cages.

*Jennifer* – said the MW Pilot Expansion Study would supply the equipment (cages) and mussels, so the costs would be minimal, especially if partners/volunteers didn't need to use boats. She pointed out that sampling of mussels (i.e. the retrieval) would actually be easier than current National MW Program sampling, since it would just involve just taking the mussels out of the cage and sending them in, instead of finding them on rocks and cutting off enough of appropriate size for a sample.

*Alan or Lincoln* – said that volunteers might be very helpful in checking on the cages during the study, to help reduce theft and/or vandalism. Perhaps we could even ask them to put the cages on private property, to reduce likelihood of public access. That would raise the ownership and participation level for the volunteers also.

*Kathleen* – strongly suggested that if we go to a caged mussel model we keep the National MW Program stations.

*Bob* – asked whether there will be need to have a baseline analysis (day 0) if caged mussels are used.

*Jennifer* – said a control/baseline sample would be taken from whatever farm was used, on the day the mussels were transplanted to the study sites. This would be considered the *starting condition* of the mussels.

*Bob* – revisited the idea of station selection/placement, in terms of volunteer involvement, and said we'd need a big marketing component to insure outreach to potential partners, if we plan on putting caged mussels at 60 stations or more.

*Someone* - suggested considering areas outside the current geographical focus as pristine reference site(s). The San Juan Islands were strongly suggested.

- The discussion ended with general agreement that the hybrid approach of placing caged mussels on beaches and/or piers would provide a good balance of study design elements for this MW Pilot Expansion and ensure continued compatibility with volunteer capacity. This included a strong recommendation to maintain sampling at some (if not all) of the NOAA Mussel Watch sites.

**Washington MW Summary (2004-2011) data update** - comparing Snohomish County, ENVVEST, and NOAA Mussel Watch data. Time was running short at this point, so Jim gave a quick rundown of the current data compatibility issues:

Basically, TDI Brooks (the NOAA MW Program lab) and Battelle (ENVVEST program lab) data are different from each other and those differences need to be resolved. The contaminant analysis plan for the MW Pilot Expansion Study is to use NOAA Northwest Fisheries Science Center (Montlake Lab) protocols for lipids and POPs and King County protocols for metals.

*Bob* - mentioned that the cost per sample in the MW Pilot Expansion scope of work seemed low. He said costs should be double that much.

*Jim* - clarified that is because WDFW contracts with the NOAA Montlake lab for contaminant analysis, under an Interagency Agreement. Lipids would be analyzed in depth through a fractioning analysis.

*Bob and Jim* - discussed the merits of why polychlorinated biphenyls (PCBs) were not going to be analyzed via the Aroclor method. They agreed that PAHs should be analyzed for homologues and parent compounds and there should be stable isotope (C and N) analysis.

*Laurie* – said metal analyses need to be planned carefully, to make sure preparation of tissue samples is done in a way that will not limit the contaminant of concern list (which is yet to be determined).

*Jim* - If this process is cost prohibitive samples could be archived until funds are secured for analysis at a later date. However, archiving is less than desirable for more volatile organics and lipid analysis.

Meeting Adjourned - At this point, time was up and participants were thanked for their contributions. It was mentioned that meeting notes will be distributed and that several Workshops will be held in late June or early to mid-July for all interested parties.