UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION 10 LABORATORY
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MEMORANDUM

DATE: October 30, 2006

TO: Rob Pedersen
Dive Team, OEA

FROM: Stephanie Harris, D.V.M., DACVPM
Lead Microbiologist

SUBJECT: Report of Microscopic comments and observations of the samples submitted from Hood Canal Dead Zone.
Project Code: WTR 152A
Account Code: 0708B10P201B42C

The following is a written report of the comments and observations associated with the sediment and grab samples collected on October 25, 2006. The samples were retrieved from cooler 62 at 0730 on October 26, 2006. The samples included: 1 trip blank (not assigned a laboratory number), 3 samples from site 349, 4 samples from site 349B and 2 samples from site 349C. The analyses were performed following laboratory guidelines at the USEPA Region 10 Laboratory, Port Orchard, WA. There is no method number available to site for the microscopy that was performed.

Processing of the samples included:
The trip blank contained approximately 450 ml of clear solution (presumably DI water from the Field Equipment storage Milli Q water system). The contents were poured into 2-250 ml conical centrifuge bottles and centrifuged at 2950 RCF (relative centrifugal force) for 20 minutes. The supernatant was aspirated down to approximately 1ml in each centrifuge bottle. A single 20 ul aliquot from each bottle was examined microscopically. The purpose of this trip blank was to ensure that the field technique and subsequent sample storage did not result in contamination of the samples.

Samples 06434150 – 4152 were processed in the following manner. The sample was allowed to settle until there were three visible interfaces. The top interface was seawater, middle interface was composed of a gelatinous cream-colored layer atop the third interface, a dark brown to black sediment. A portion of the seawater was examined directly by removing a small amount using a sterile syringe and placing it on a microscope slide. The slide was examined using phase contrast microscopy at 200 x magnification. For both the middle and bottom interface, a portion of each interface was removed using a sterile syringe and placed in a Petri dish. A dissecting scope was used to examine the interface and select items of interest. These items were placed on a microscope slide and examined using phase contrast at 200 x magnification. A minimum of two sample bottles for each site were examined to check for consistency within the sampling protocol.

Samples

Trip Blank, 06434150, 06434151 and 06434152

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Trip Blank: The trip blank contained small amounts of minerals and silica; consistent with a Milli Q water system.

Lab number: 06434150 (Source # 349)
Comments and Observations: 3 samples collected at 1210. Two of the samples were examined. The seawater interface contained little in the way of particulates and organisms. The middle interface contained a very diverse population of invertebrates including algae, diatoms, ciliates, amphipods, crustaceans and long chains of filamentous bacteria closely resembling *Beggiatoa albans* some of which contained the sulfur granules associated with this species of bacteria. In addition, the sample contained an amorphous brown material that enclosed the above described ecosystem. This again is consistent with observations of these bacteria. Microscopic examination of this material at 200 magnification provided presumptive identification of *Beggiatoa spp*. Dissecting scope observations included the presence of mud, sticks, white material suspicious of *Beggiatoa spp* in the sedimentary deposits.

Lab number: 06434151 (Source # 349B)
Comments and Observations: Four samples collected from this site, at 1315. Two of the samples were examined. The seawater interface contained little in the way of particulates and organisms. The middle interface was initially examined using the dissecting scope and then selected items were examined using phase contrast microscopy. Again, a diverse marine population of invertebrates was present and included diatoms, algae, crustea and long chains of motile filamentous bacteria that were presumptively identified as *Beggiatoa spp*. The sedimentary deposit was examined and included fine and coarse material, and a diverse population of invertebrates.

Lab number 06434152 (Source # 349C)
Comments and Observations: 2 samples collected at 1515 on October 25, 2006. Two bottles from this sample site was examined. Microscopically, the samples contained a diverse population of marine invertebrates and similar long chains of motile filamentous bacteria consistent with *Beggiatoa spp* in the middle and sedimentary interfaces and minimal material in the seawater interface.

Stephanie Harris, Microbiologist