# Effects of Phosphatic Clay Dispersal at Two Divergent Sites in Puget Sound, Washington

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#### Abstract

Environmental effects of phosphatic clay dispersed inside net pens and removal efficiencies of phytoplankton were evaluated during non-HAB conditions at a well-flushed salmon farm site and at a poorly flushed, nutrient-sensitive inlet remote from any salmon farms. We planned to evaluate the efficacy and effects of *Heterosigma akashiwo* bloom control in large and small pens at a fish farm site, but few cells occurred near the fish farm during our study years, despite low to moderate seasonal concentrations from 1990 to 1999. Experiments were therefore conducted using the normally occurring phytoplankton community. Impervious vertical perimeter skirts, which were open at the bottom, were used alone or installed around pens prior to the tests to initially retain the clay and facilitate study.

Removal efficiencies of microflagellates ranged from 86% to 99%. Prior and post-treatment cell densities were positively related with a correlation coefficient 0.83. Initial dinoflagellate density and removal were low at the salmon farm site but were both much higher for the poorly flushed inlet site. Diatoms dominated at the salmon farm site and were removed at 48% to 87% of the pretreatment density. No significant overall effects on nitrogen (DIN) or phosphorus (PO<sub>4</sub>) were observed, although orthophosphate increased inside the pens for short periods after clay application. Turbidity at the surface inside the pens exceeded 100 (large pen) to 500 NTU (small pens) at first and slowly declined. Turbidity at greater depths was typically <50 NTU, probably due to accelerated sinking rates of clay/algal particles. A slight subsurface plume was visible from the surface just outside the large pen, but dissipated rapidly with turbidity similar to background, upstream conditions. The large pen application of 54 kg dry wt. clay resulted in no measurable differences in a series of sediment canisters placed on the bottom in the vicinity of the pen. Percent silt and clay in the top 2 cm of seabottom sediments temporarily increased from 1.7% to 4.2% just downstream of the large pen immediately after the experiment, but additional sampling showed that this type of variability occurs naturally and repeated sampling later indicated no measurable differences. No increase in sediment clay content was noted 30 m downstream at any time. Ten adult salmon placed in the large pen displayed coughing activity after treatment, but recovered quickly as the clay settled.

## Introduction

Harmful algal blooms (HABs) are not a major restraint to mariculture worldwide at this time, but they are a continuing problem in some areas (Anderson et al., 2001, Rensel and Whyte 2003). Fish-killing species include a small raphidophyte Heterosigma akashiwo, which produces occasional large scale blooms and losses at salmon farms in both northern and southern hemispheres. In the Pacific Northwest, blooms typically occur during mid-summer or early fall in persistently quiescent and warm weather conditions. The last major bloom sufficiently large to kill mariculture fish in Puget Sound was over 10 years ago, but every year some motile cells are observed in the water column. The exact etiology of fish death remains uncertain, but may involve a variety of causes including damage to gills from reactive oxygen species, mucus buildup leading to blood hypoxia or even brevetoxin-like compounds, although the latter has never been definitively documented.

Salmon farmers use a variety of physical mitigation techniques for HABs including water pumping from depth and towing away from blooms. A wide variety of chemicals and treatments have been tried by others to remove or mitigate HABs (Anderson *et al.*, 2001). Collateral damage to other water column or benthic species can be expected from most of these. The use of certain naturally occurring clays to flocculate blooms appears to be effective and have limited environmental impact in some cases. Such clays are used in the western Pacific in Korea, Japan, and China to protect fisheries resources or mariculture, but until recently, environmental studies have been limited. An important consideration for the use of flocculants such as clays involves the possible transfer of algal toxin from the water column to the sea bottom. However, it is most likely that *H. akashiwo* does not produce a persistent toxin, so use of flocculants may be an acceptable means to treat and remove these blooms.

#### **Materials and Methods**

Three of the four trials occurred near a commercial fish farm site in Deepwater Bay near Cypress Island in North Puget Sound, during the late summer of 2001. The other trial was conducted in July 2002 in inner most Sinclair Inlet, near Bremerton and Central Puget Sound. The fish farm location is naturally replete with macronutrients and well flushed; the inner reaches of Sinclair Inlet are considered nutrient sensitive (Rensel Associates and PTI Environ. Serv. 1991) and have recurring seasonal microflagellate and dinoflagellate blooms including HABs. Experiment one at the fish farm used a relatively large, 144-m<sup>2</sup> surface net-pen assembly, with nylon nets, impervious, vertical-perimeter skirt to 5 m depth and 10 adult Atlantic salmon inside the pen during the clay application. Experiments 2 to 4 at the fish farm and experiment 5 in Sinclair Inlet utilized a small 4.4-m<sup>2</sup> surface area mesocosm pen consisting only of a

|                         | Exp. 1<br>Large Pen<br>Cypress Is. | Exp. 2<br>Small Pen<br>Cypress Is. | Exp. 3<br>Small Pen<br>Cypress Is. | Exp. 4<br>Small Pen<br>Cypress Is | Exp. 5<br>Small Pen<br>Sinclair Inlet |
|-------------------------|------------------------------------|------------------------------------|------------------------------------|-----------------------------------|---------------------------------------|
| Diatoms before          | 320,000                            | 269,000                            | 446,000                            | 232,000                           | 4,000                                 |
| Diatoms after           | 154,000                            | 83,000                             | 106,000                            | 30,000                            | 1,600                                 |
| Removal Efficiency      | 52%                                | 69%                                | 76%                                | 87%                               | 60%                                   |
| Dinoflagellates before  | 16,000                             | 9,000                              | 8,000                              | 5,000                             | 6,163,000                             |
| Dinoflagellates after   | 16,000                             | 9,000                              | 17,000                             | 7,000                             | 2,005,000                             |
| Removal Efficiency      | 0%                                 | 0%                                 | -113%                              | -40%                              | 67%                                   |
| Microflagellates before | 123,000                            | 60,000                             | 93,000                             | 103,000                           | 846,000                               |
| Microflagellates after  | 17,000                             | 5,000                              | 5,000                              | 6,000                             | 400                                   |
| Removal Efficiency      | 86%                                | 92%                                | 95%                                | 94%                               | 99%                                   |

Table 1 Microalgal density in pens (cells/L at 1m) before and after clay treatment and percent removal efficiencies.

nylon perimeter skirt mounted on a frame with 200 g/m<sup>2</sup> loading distributed by a small bilge pump over about 5 minutes. Florida phosphatic clay was hydrated, mixed, screened through 1-mm stainless steel mesh to remove debris and applied via a pressure washer at 375 g/m<sup>2</sup> over 40 min for the large pen trial. Four duplicate sets of 81.1 cm<sup>2</sup> opening area PVC sedimentation collection canisters were placed 5 m upstream and downstream of the large pen at 1, 15 and 30 m distance. Surface and subsurface windowshade drogues and surface floats were deployed during the experiment to confirm the direction and velocity of tidal flow. A petite Ponar grab sampler was used to collect seabottom sediments prior to and after clay treatment and again a week later. The top 2 cm of the undisturbed grab were removed for analysis of sand, silt and clay fractions by screening methods. Sediment samples were not collected in the small pen experiments. Triplicate nutrient, extracted chlorophyll a, grain size and solids measurements were according to USEPAapproved protocols. A Hydrolab Surveyor 3 and H20 sonde were used for experiment one. A Hydrolab 4a sonde/surveyor with WET Labs shuttered turbidity probe, Turner SCUFA fluorometer, and other probes was used inside and outside the pens, prior to and during the other experiments. Cell counts were performed using an inverted microscope, with special care as some clay particles were still present in the post-treatment samples. All experiments were conducted during relatively neap tidal periods for about 4 hours, and the large pen experiment began at predicted slack tide based on local NOAA current tables.

## **Results and Discussion**

For Cypress Island experiments, diatoms were the dominant microalgal group, with density ranging from 2.3 to  $4.5 \times 10^5$  c/L (Table 1). Prevalent species in other experiments included *Rhizosolenia setigera* and *Thalassionema nitzschioides*. In other experiments there, *Pseudo-nitzschia* spp., *Skeletonema costatum* and *Thalassionema nitzschioides* were dominant species in varying quantities. Small, unidentified microflagellates (including a few *H. akashiwo*) were numerically significant too, but dinoflagellate density was approximately an order of magnitude less. In Sinclair Inlet,

dinoflagellates were much more prevalent, averaging  $6.1 \times 10^{\circ}$  c/L prior to treatment and dominated by *Prorocentrum gracile* and *Gymnodinium* spp. Microflagellates were an order of magnitude less abundant, and diatoms were relatively scarce, which is the normal summer condition in this inlet. Microflagellates included unidentified small flagellates, *Platymonas* sp. cf., *Heterosigma akashiwo*, cryptomonads, and choanoflagellates.

Removal efficiencies (RE) of microflagellates after clay use were high, ranging from 86 to 99 percent. The highest efficiency for this group occurred at Sinclair Inlet, which also had the highest initial cell densities. RE of microflagellates was positively correlated to initial cell concentrations for the small pen experiments with a coefficient of 0.83. Lowest RE for this group was in the large pen experiment, which had a protracted period of clay application but higher aerial clay application rate. Diatom RE varied significantly among experiments, from 52% to 87%, with the large pen experiment again having the lowest rate. Dinoflagellate RE was low at Cypress Island (0 to 40%) but much better at Sinclair Inlet where initial densities were 2 orders of magnitudes greater. Collectively, RE was higher for higher initial cell densities, with highest rates for the group of interest, microflagellates. No effect on extracted chlorophyll a was noted in experiment 1 or 2, but initial concentrations were very low, only 1.7 and 1.0 µg/L, respectively. Significant reductions were noted in each of the other smaller cage trials where initial concentrations ranged from 4 (Cypress Island) to 15 µg/L at 1 m. The SCUFA in vivo chlorophyll a results (only collected in Experiments 3-5) generally showed this trend too, but were more variable and suggestive of interferences from the clay despite frequent rinsing of the probe. Water column turbidity prior to treatment at the surface (0.1 to 0.3 m) inside the pens was low to moderate (2 to 5 NTU). Immediately after clay application it rose to >100 NTU for the large pen and ~500 NTU for the small pen, then slowly declined. However, values at depths > 1m did not exceed 50 NTU, probably due to accelerated sinking rates of clay/algal particles at that depth. Turbidity at all depths declined slowly, but remained relatively high near the surface for longer periods. The reason

for this is unknown, but could be related to the generally low initial algal biomass in all but Sinclair Inlet pen. It was possible to see a faint subsurface plume downstream of the large pen, but discrete measurements indicated turbidity levels near background conditions. No plume was seen to escape from the small cages, but tidal currents were minimal.

Sediment grain size nearest the large pen (1 m downstream) showed statistically significant increases of sediment grain size from 1.7% silt/clay to 4.2% silt/clay immediately after the experiment. Subsequent sampling showed naturally large variability in this area and no statistical change from pre-treatment conditions. Current velocity beneath the pens averaged 0.21 m/s during the trial, despite predicted neap tidal conditions. It was unlikely that the clay actually contacted the bottom near the pen, and further downstream (30 m) no significant changes in sediment grain sizes were measured. Sediment collection canister results showed no change from upstream of the large pen to downstream (1, 15 and 30 m). Ten adult salmon placed in the large pen displayed initial coughing symptoms at the outset of that experiment, but recovered quickly as the clay settled. Some of our previous, unpublished work focused on the effects of clay on Atlantic salmon in worst case 5-hour bioassays with constantly resuspended clay treatments of similar loadings. Fish responded by coughing but will cease doing so immediately after removal to clean water. Histopathology of treated fish gills showed no measurable effects from 5 hours of continual exposure.

In summary, these pilot-scale clay treatments demonstrated high removal rates of microflagellates and reasonably good removal of dinoflagellates when initial cell density was great. Careful application of clay treatments will likely not exceed turbidity standards outside permitted mixing zones or result in significant deposition of clay/cell floc immediately downstream of cages. Future studies will report HAB removal results and benthic infauna effects.

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