Totten Inlet baseline studies completed by Aquatic Environmental Sciences in 2002 and 2003

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Executive Summary

This summary briefly describes the results of studies undertaken in 2002, 2003 and 2005 assessing the potential environmental risks associated with the proposed raft culture of 514,670 kg (1,133,363 pounds) of marine mussels per year on the eastern shore of Totten Inlet near its mouth. The 2005 studies were designed specifically to assess Totten Inlet's carrying capacity and is supplemental to the reports of Brooks (2000) and Gardiner *et al.* (2004). Supporting details for the conclusions reached herein are provided in the seven papers provided as attachments to this document. The assessment is documented in this manner because several of the studies are formatted for submission to peer reviewed journals and Brooks (2006) is currently under review by the Canadian Department of Fisheries and Oceans.

Baseline benthic conditions in Totten Inlet. Careful documentation of pre-production baseline conditions is important for understanding the living resources of Totten Inlet that may be put at risk in response to the hazards imposed by the intensive cultivation of mussels. Baseline information also provides a basis for estimating the severity of the response to these hazards and for evaluating changes that may be observed during production. This is particularly important with respect to near field effects that can be measured at points in time. Brooks (2005a) evaluated sediment grain size (SGS), total volatile solids (TVS), total organic carbon (TOC), free sediment sulfides ($S^{=}$) and redox potential (Eh) in 73 samples collected along Totten Inlet's centerline at ca. 200 m intervals from intertidal areas at the south end to and beyond the mouth of the inlet where it enters Pickering and Squaxin Passages. Nine additional samples were evaluated on an orthogonal transect from the eastern to western shores of the inlet at the latitude of Little Skookum Inlet. Twenty four baseline samples were also collected at random locations within an 8.7 hectare (21.5 acre) area around the center of the proposed farm in North Totten Inlet. These samples were evaluated for the same sediment physicochemical variables and in addition, the macrofaunal community was inventoried. An underwater video was recorded on four orthogonal transects at the North Totten site to assess the megafaunal community and to search for rare or commercially important taxa or habitats supporting any life stage of those taxa. Copies of these videos are provided as attachments to the appropriate reports.

General sediment physicochemistry in Totten Inlet. Surveyed subtidal areas south of Little Skookum Inlet were generally dominated by fine-grained sediments containing 70 to 90% silt and clay. Redox potential in the intertidal mudflats at the south end of the inlet were aerobic with positive redox potentials, low sulfides (<100 μM $S^{=}$) and moderately low TVS (6 to 7.5%). Water depths during the survey gradually increased from ca. 3 m MLLW at the south end of the estuary to 15 m at Little Skookum. All surveyed subtidal sediments south of Little Skookum were enriched with organic carbon to concentrations above the upper 90th percentile defined by the Department of Ecology for Puget Sound Reference locations as a function of percent silt and clay. The inlet's enriched condition was reflected in high sulfide concentrations (100 to 580 μM) and zero to -120 mV redox potentials indicative of hypoxic conditions. Excepting the deep channel extending south from the mouth of the inlet, subtidal areas north of Little Skookum Inlet, including most of the North Totten mussel farm site, were generally characterized as a

muddy bowl. In contrast to most of the inlet, the sandy sediments, low sulfide concentrations, and positive redox potentials observed near the inlet's mouth were hypothesized to have been created in a ca. 30 m deep scour-pit located inside the shallow sill surrounding the mouth of Totten Inlet.

Deposition of organic and inorganic solids in Totten Inlet. The source of the organic enrichment observed in Totten Inlet sediments was explored in canister studies at the Deepwater Point mussel farm; at reference locations north of Deepwater Point; and at the proposed mussel farm site in North Totten Inlet. The deposition of volatile suspended solids (TVS) in canisters was significantly higher at the Deepwater Point reference location (47.8 g TVS/m^2-day) than at the North Totten Inlet site (16.38 g TVS/m^2-day) ($t = 4.13$; $p < 0.000$). The rates at either Totten site were higher than reported for any other shellfish producing area of the world. Deposition of TVS was statistically significantly correlated with water column chlorophyll *a* concentrations in North Totten Inlet, but the Pearson Correlation Coefficient was low (0.35) suggesting that the relationship was poor and that factors other than phytoplankton production were contributing to the high TVS observed in the water and sediments. Those other factors were likely associated with terrigenous inputs and the decay and resuspension of macroalgae and other forms of sedimented detritus.

Baseline benthic surveys at the site of the proposed mussel farm. Baseline surveys at the North Totten site included evaluation of 24 sediment samples; monthly collection of replicate water samples at 1.5 m depth for 15 months; monthly evaluation of TSS and TVS deposition rates using canisters for 15 months; video surveys and numerous drifter studies conducted during spring and neap tides. Specific benthic endpoints evaluated, and the range of values observed at the North Totten site, are summarized in Table 1. Detailed results and interpretation of the baseline benthic data are provided in Brooks (2005a).

Table 1. Summary statistics describing baseline studies completed at the site of the proposed mussel farm in North Totten Inlet (Brooks, 2005a).

	Descriptive Statistics (Totten Main Database (restricted taxa) all stations)					
Variable	Valid N	Mean	Confidence -95.000%	Confidence +95.000%	Minimum	Maximum
Depth (feet below MLLW)	24	-55.671	-64.955	-46.387	-83.000	-5.500
Sulfide (micromoles)	24	373.179	232.602	513.756	55.600	1240.000
RPD (cm)	24	7.667	6.798	8.535	5.000	10.000
TVS (proportion)	24	0.026	0.019	0.033	0.009	0.062
TOC (proportion)	24	0.012	0.009	0.015	0.004	0.025
Gravel (percent)	24	0.219	0.074	0.363	0.000	1.400
Sand (percent)	24	78.053	72.218	83.889	46.220	91.040
Silt (percent)	24	12.884	8.863	16.906	4.440	35.490
Clay (percent)	24	8.844	6.945	10.744	4.110	18.290
Fines (percent)	24	21.727	15.853	27.602	8.960	53.780
Abundance (per 0.1m2)	23	217.957	141.128	294.785	54.000	609.000
Taxa (per /0.1 m2)	23	31.783	28.446	35.119	20.000	48.000
Shannon	23	2.713	2.590	2.836	2.064	3.300
Annelids (per 0.1 m2)	23	80.130	41.228	119.033	13.000	336.000
Bivalves (per 0.1 m2)	23	38.348	10.196	66.499	3.000	304.000
Gastropods (per 0.1 m2)	23	38.043	14.804	61.283	3.000	218.000
Arthropoda (per 0.1 m2)	23	24.870	13.597	36.142	2.000	102.000

Water depths under the proposed mussel rafts varied between 35 and 60' MLLW providing a minimum of 11' of water under the mussel lines during a -4.5' MLLW low tide.

Currents in the vicinity of the proposed North Totten Inlet mussel farm. The drifter studies indicated that water from the proposed mussel farm site was flushed out of the inlet at all depths on ebbing spring tides (16.3 to 18.3 foot tidal exchange), but the drifters remained inside the inlet on ebbing neap tides (6.2' tidal exchange). Ebb tide currents on the east side of Totten Inlet were stronger than flood tide currents and the results suggested that mussel farm waste would preferentially be deposited to the north of the rafts rather than to the south. A maximum current speed of 34.2 cm/sec was observed on both flood and ebb tides at depths $\leq$ 7.5 m during the spring tide. The maximum ebb current speed on a neap tide was 27.2 cm/sec at 1.5 m depth, but it was slower (12.3 to 12.7 cm/sec) at 7.5 and 15 m depths. The site appears to be well flushed and the mean observed current speed of 21 cm/sec is similar to that reported by Gardiner *et al.* (2004) at Deepwater Point. The currents were reasonably well modeled using a harmonic temporal profile. Brooks (2006) summarizes the results of ADCP studies conducted by Evans-Hamilton Inc. (EHI, 2006). The North Totten Inlet site is very well flushed with a mean current speed of 16.3 cm/sec oriented parallel to the shoreline. Maximum current speed was 84.3 cm/sec at 3.0 m depth. Perhaps most importantly, currents <3.0 cm/sec represented a minor portion of the total recordings. These results suggest that waste will be well dispersed and the deposition rate of biodeposits from the culture may be < 1.0 kg TVS/m^2-y, in which case the assimilative capacity of the sediments may not be exceeded. Assuming that Taylor Resources continues to intercept mussel fall-off in horizontal nets, these results suggest that any benthic effects would be ephemeral at peak biomass and minor in nature. It is also quite possible that operation of this facility could increase the abundance, diversity and biomass of the macrobenthos.

Physicochemical properties of sediments. The proposed farm lies at a latitude where sediment grain size, sulfide and TVS were found to be changing rapidly during the centerline survey of the inlet. Sediments in the area of the proposed rafts were dominated by sand (78.1 $\pm$ 5.8%) with lesser amounts of silt and clay (21.7 $\pm$ 5.9%) and very little gravel (0.2 $\pm$ 0.1%), which was composed mostly of broken shell. Like other parts of Totten Inlet, sediments near the proposed farm were enriched with organic carbon. Twenty-two (22) of the 24 samples contained concentrations of total organic carbon (TOC) that were higher than the upper 90^{th} percentile values reported by the Department of Ecology for Puget Sound reference stations having the same proportion fines. The organic content of sediments (TVS or TOC) was higher in fine-grained sediments located to the southwest of the proposed farm and lower in the sandy sediments located north and east of the site. Brooks and Mahnken (2003a) observed reduced macrobenthic community richness associated with free sediment sulfide concentrations as low as 100 μM $S^=$. The number of taxa observed in annelid dominated communities was reduced by half at 964 μM $S^=$ and Brooks *et al.* (2004) reported a 50% decrease in species richness in a mollusk dominated community at 447 μM $S^=$. Free sulfide concentrations in sediments from British Columbia reference locations have varied between 0.0 and 486 μM with a mean of 90.7 $\pm$ 15.4 for N = 165 cases. Consistent with the high organic content in Totten sediments, free sulfide concentrations near the proposed mussel farm varied between 55.6 and 1,240 μM with a mean of 373.2 $\pm$ 140.6 μM. However, the reduction-oxidation potential discontinuity (RPD) was relatively deep at 5 to 10 cm (7.7 $\pm$ 0.9 cm) suggesting that while surficial sediments were enriched, they had not become anoxic.

Macrobenthic community inventory. A total of 131 taxa and 4,840 animals were identified in the 23 each 0.1 m^2 modified van Veen samples collected at the North Totten site. Overall, the community was dominated by annelids (38% of total) and mollusks (36% of total) with fewer arthropods (12% of total). The mean abundance of macrofauna at this site (218/0.1 m^2) was lower than documented at Puget Sound Reference Locations (464.2 to 491.4/m^2) sharing similar water depths and proportion fines. Species richness was also generally lower at North Totten (32 $\pm$ 3) than reported for Puget Sound reference stations containing 20 to 50% fines (Mean = 64.4; CV = 31.4). The macrobenthic community's diversity, as measured by Shannon's Index, was higher than reported for WDOE reference locations. However, Shannon's Index values of 1.1 to 1.57 reported in WDOE (1996) are so low as to be questionable in reference conditions. More annelids were observed in fine grained sediments to the south and west in deeper water and more mollusks were found in the coarser nearshore (northeast and southeast) quadrants. Many of the dominant taxa found at the site are characteristic of enriched sediments. These included *Leitoscoloplos pugettensis, Lumbrineris luti, Nephtys cornuta, Paraprionospio pinnata* and *Sigambra tentaculata.* Dominant mollusks included species tolerant of naturally enriched conditions like *Alia gausapata, Alvania compacta, Nassarium perpingis, Macoma nasuta and Psephidia lordi.* However, the macrobenthic community also included taxa that are intolerant of excessive enrichment resulting in high sulfide concentrations like brittle stars (*Ophiuroidea sp.* and *Amphiodia urtica*); arthropods like (*Photis brevipes*); and mollusks such as *Axinopsida serricata, clinocardium nuttallii, Lucina tenuisculpta* and *Macoma secta.* The physicochemical data and macrobenthic community inventory were consistent in describing the area as organically enriched but not yet so eutrophic as to exclude many sulfide intolerant taxa.

Video survey. The benthos at the North Totten site was essentially a shallowly inclined muddy to sandy plain with no critical fish or invertebrate habitats observed. The video survey revealed a megafaunal community containing very few vertebrates (fish) and one that was highly dominated by seawhips (*Stylatula elongata*) – especially in the finer grained sediments found in deepwater to the west and south. These cnidarians are octocorals with a central polyp that is buried in the substrate and that extends 30 to 45 cm above the sediment's surface. Like other members of this class, their lateral polyps are armed with stinging cells (nematocysts) that likely make them unsuitable as spawning habitat for herring or other animals. Cancer crabs (*Cancer gracilis*) were abundant everywhere, but especially so in the intertidal oyster beds. These shallow nearshore oyster beds supported dense macroalgae communities creating diverse habitats that appeared to support an abundant and diverse macrobenthic community. No rare or sensitive species were observed. However, non-commercial quantities (~1.0/10 m^2) of goeducks (*Panopea abrupta*) were observed at depths of 30 to 60 feet.

Baseline water column surveys. Four surface (1.5 m depth) water samples were collected each month at the North Totten site between March 22, 2002 and June 2, 2003. Canisters were also deployed at the Deepwater Point and North Totten reference locations and sampled on each of these days. An additional six water samples (three each during ebb and flood tides on either side of slack water) were collected on July 10, 2002. The samples were analyzed for temperature, salinity, dissolved oxygen, chlorophyll *a*, Total Suspended Solids (TSS), Total Volatile Solids (TVS), PO_4, SiO_4, NO_2, NO_3, NH_4, and turbidity. The results (Brooks, 2005b) suggested that Totten Inlet was a minor consumer of dissolved inorganic nitrogen and a significant producer of chlorophyll *a* (Chl*a*) and phaeopigments (degraded Chl*a*). Bivalves prefer living phytoplankton, but they also consume and grow on detritus of the proper size (3.0 to several hundred μm particle

size). This particulate organic matter (POM) was measured as TVS in both water and canister samples.

Nutrients. Dissolved inorganic nitrogen (DIN) is the nutrient which most frequently limits primary production in marine environments. In general, plant growth is nitrogen limited when DIN concentrations fall below 1.0 μM (0.014 mg/L). Dissolved inorganic nitrogen varied seasonally at the North Totten site from lows of 0.02 mg/L in May, June and July of 2002 to highs of 0.48 mg/L in January 2003. It was not reduced to less than one μM in any sample collected at the North Totten Site and no evidence of nitrogen limitation of phytoplankton production was observed there. It was hypothesized that strong mixing of deep water and nitrogen depleted surface water occurs as water pours over the inlet's sill on flood tides – increasing nitrogen concentrations in the euphotic zone. On July 10, 2002, the mean DIN concentration was significantly (t = *-8.487*, $p < 0.00$) higher (0.11 mg/L) on the flood tide when compared with the ebb tide (0.072 mg/L) suggesting that Totten Inlet may be a net consumer of inorganic nitrogen. However, this observation needs to be confirmed with additional sampling. Nitrogen:phosphorus ratios were less than the Redfield-Richards optimum ratio of 16 N:1 P) for all samples. On July 10, 2002, they were significantly lower (t = *-2.897*, $p = 0.04$) on the ebb tide in comparison with the flood tide (2.172 versus 4.952 respectively). This may have implications for the phytoplankton's community composition; promoting some dinoflagellates that compete better than diatoms at low nitrogen concentrations. Data provided in WDOE (1998) for years between 1980 and 1995 was examined in a search for long-term trends in Totten Inlet DIN concentrations. The slope of a linear regression was positive (indicating increasing DIN concentrations over time), but the coefficient on time was not statistically significant.

Recycling of nutrients from sediments under and near the Deepwater Point mussel farm. On July 10, 2002, Brooks (2003) reported increased DIN concentrations in water sampled at 2.0 (0.103 mg DIN/L) and 20 cm (0.089 mg DIN/L) above the sediment's surface under the Deepwater Point mussel rafts. These concentrations were significantly higher than found at the reference location (0.055 mg/L at 2.0 and 0.47 mg/L at 20.0 cm). DIN concentrations at 50 cm above the sediments under the rafts or at any height above the sediments on the perimeter of the farm or at 30 m downcurrent were not significantly different from reference values. Most of the increased DIN was associated with ammonium (NH_4^+). Brooks (2000, 2005b) reviewed the literature discussing the potential for dampening the amplitude of seasonal changes in dissolved nutrients and primary production associated with the regeneration of sedimented nitrogenous compounds under mussel rafts and the potential reduction in nitrogen budgets associated with nitrification processes.

Particulate Organic Matter (POM) and Chlorophyll a (Chla). Mean Chl*a* concentrations at the North Totten Inlet site (3.74 $\pm$ 0.94 μg/L; Range = 0.54 to 11.84 μg/L) were as high, or higher, than reported for other productive shellfish growing areas around the world. Chlorophyll *a* concentrations were low in March 2002 (1.13 μg/L); peaked at the end of May 2002 (10.58 μg/L); and declined to ca. 2.0 μg/L at the end of August; before rebounding to 8.3 μg/L in October, 2002. Chlorophyll *a* was marginally significantly higher ($p < 0.10$) in ebb tide water when compared with flood tide water on July 10, 2002 and total plant pigments (Chl*a* plus phaeopigments) were significantly higher ($p < 0.05$) in the ebb tide samples. These results suggested that phytoplankton resources in South Puget Sound were not being depleted by the existing bivalve cultures in Totten Inlet and in fact, they suggest that Totten Inlet was a net

producer of plant pigments in comparison with other parts of South Puget Sound. This observation was explored during intensive water column sampling conducted at a fixed station inside the mouth of Totten Inlet between August 24 and December 12, 2005 three hours before and after slack tide at depths of 1.5, 7.5 and 15.0 m. Mean chlorophyll *a* concentrations were higher in ebb tide water during this period (8.30 µg Chla/L) than in flood tide water (8.23 µg/L), but the difference was not statistically significant. The results of this sampling indicated that filter feeding communities, including bivalves, in Totten Inlet are not consuming more phytoplankton than the estuary is producing. Perhaps more importantly, TVS concentrations at the North Totten site (12.58 $\pm$ 2.19 mg/L) were higher than reported for any other shellfish growing region of the world and the study results suggest that filter feeders are sustained by suspended detritus during the winter months. The POM represented by the TVS may be responsible for the continued growth of mussels at the Deepwater Point farm during late fall and winter when primary production is generally light limited in the Pacific Northwest. The chlorophyll *a* profile over the entire period of study from 2002 until December 2005, indicated that phytoplankton in North Totten Inlet are not nutrient limited at any time and that the biomass of phytoplankton remained high until the end of November, 2005, just before peak biomass would be achieved at the new farm. In summary, Totten Inlet is a significant consumer of South Puget Sound nutrients and it is a minor exporter of phytoplankton. The exceptionally high concentrations of particulate detritus explain the very high sediment TVS concentrations reported in these studies and they also explain the steady growth of mussels at the Deepwater Point farm during winter when primary production is light limited in the Pacific Northwest.

*Water column stratification***.** Analysis of data provided in WDOE (1998) found little evidence for either persistent haloclines or thermoclines at Windy Point in Totten Inlet. Additional salinity and temperature data collected at from 0.5 m to 14.5 m at one meter increments of depth from August until December, 2005 gave no indication of significant or persistent stratification. It is hypothesized that the homogeneous distribution of nutrients, chlorophyll *a*, TVS and nutrients inside the mouth of Totten Inlet results from the mixing of South Puget Sound water as it passes over the shallow sill surrounding the mouth of the inlet. This mixing replenishes nutrients in the euphotic zone and distributes suspended mater throughout the water column on each tidal exchange. The increased homogeneity as a function of depth enhances and smoothes out primary productivity and enhances North Totten Inlet's ability to support much higher biomasses of filter feeding organisms – including cultured bivalves.

Correlations between mussel growth increments and water column endpoints. A *daily incremental mussel biomass increase* (DIMBI) was created for the Deepwater Point cultures and compared against water column data collected at the North Totten Inlet site during the same month. Matched water column analyses were available for Deepwater Point and North Totten sites in April and May of 2002. An analysis of variance with site and day as independent variables and Chl*a,* TSS, TVS, Proportion TVS and Turbidity as dependent variables indicated that not all of the cells were equal with respect to Chl*a* ($p < 0.000$), TVS ($p = 0.03$) and Turbidity ($p = 0.015$). Post hoc testing using Tukey's Honest Significant Difference test indicated that the Chl*a* concentration at North Totten on April 27, 2002 (2.23 µg/L) was significantly less than the mean concentrations at Deepwater Point in April (11.97 µg/L) or May (10.58 µg/L) or at North Totten in May (10.58 µg/L). Total volatile solids fell in a rather narrow range of 6.3 to 10.7 mg TVS/L at the two sites. However, TVS was significantly less ($p = 0.018$) at Deepwater Point

(6.3 mg/L) in April in comparison with North Totten (10.7 mg/L) during the same month. Consistent with the Chl*a* results, turbidity was significantly lower at North Totten (1.80 NTU) in April than it was in May (2.38) or at Deepwater Point (2.45 NTU). Turbidity was not significantly different between the two sites in May. These results are consistent with Figures 2 and 3 in Brooks (2005b) and suggest that at least in the spring and early summer of 2002, differences existed between the two sites in April, but not in May. The weight of evidence suggests that the two sites are reasonably similar in terms of food available to filter feeders (Chl*a* and TVS). A better analysis of the correlation between mussel growth and POM can be made when the Pacific Shellfish Institute data for Deepwater Point becomes available. However, using the available data, Table 2 describes relationships between water quality endpoints measured at the North Totten site and daily incremental increases in mussel biomass on the inner row of rafts at the Deepwater Point farm. Biomass increases were well correlated ($r > 0.66$) with temperature, salinity, dissolved oxygen, Chl*a*, turbidity and total plant pigments (Chl*a* plus phaeopigments). Incremental biomass increases were negatively correlated with phaeopigments (degraded Chl*a* associated with senescent or moribund phytoplankton); the ratio of nitrogen:phosphorus (N/P); and secchi disk depth. Mussel growth was not well correlated with the amount of total suspended solids (PIM + POM = TSS) suggesting that metabolic energy was not significantly wasted in filtering inorganic matter out of the sediments. It should be emphasized that several of the independent variables in Table 2 are correlated with each other. For instance, temperature increases in summer along with primary production (Chl*a*) and salinity. The strong positive correlation with Chl*a* (0.91) and reduced correlation with TVS (0.58) suggests a preference by mussels for living phytoplankton as opposed to dead or dying phytoplankton (negative correlation with phaeopigments). The negative correlation with N/P ratios may result because nitrogen is reduced faster than phosphorus during periods of high primary production (i.e. lower N:P ratios when there are high chlorophyll concentrations) leading to faster mussel growth. Mussel growth is fastest when there is a high biomass of living phytoplankton available, resulting in reduced DIN; and therefore lower N:P ratios.

Table 2. Pearson coefficients describing the correlation between environmental factors and the daily incremental increase in mussel biomass at the Deepwater Point farm.

Variable	Correlations (Totten Water Quality Data) Marked correlations are significant at p < .05000 N=12 (Casewise deletion of missing data) Daily incremental increase in mussel biomass
Temperature (deg. C)	0.999
Salinity (parts per thousand)	0.684
Dissolved Oxygen (ppm)	0.763
Chla (mmg/L)	0.905
Phaeopig (mmg/L)	-0.949
TSS (mg/L)	0.312
TVS (mg/L)	0.583
N/P ratio	-0.936
Secchi depth (m)	-0.953
Turbidity (NTU)	0.692
Total Plant Pigments	0.903

Benthic response at the Deepwater Point mussel farm. Sediments under and near the inner row of six rafts at Deepwater Point mussel farm were monitored periodically during the 2002 growout of 138,000 kg of *M. e. galloprovincialis.* Sediments south and under the rafts were dominated by gravel and sand. The grain size distribution changed abruptly to domination by silts and clays at the northern perimeter of the farm. These fine grained sediments extended northward along a downcurrent transect to the reference location placed 1.12 km north of the farm. As noted in the baseline sediment survey, all of Totten Inlet's sediments were organically enriched and that was true of this area as well.

Physicochemical response. Macrofaunal community structure is defined, in large part, by the physicochemical nature of the sediments (SGS, TVS, etc.). At this site, the coarse sediments under, south, and inshore from the farm were dominated by a diverse and abundant megafaunal community including crabs, starfish and anemones. This community was likely enhanced by biodeposits released from the overlying mussel cultures. The environment from the rafts' northern perimeter to the reference location was homogeneous, providing an opportunity to observe subtle physicochemical and biological changes associated with the farm. Canister studies indicated increased deposition of volatile solids under the rafts in July and August 2002 – but not downcurrent from the rafts' perimeter. In October 2002, increased TVS above reference deposition rates were observed to at least 120 m downcurrent. However, this material was quickly assimilated in the sediments and increases in accumulated sediment TVS were not observed until just prior to harvest on November 22, 2002. Free sediment sulfides proved to be the most subtle indicator of physicochemical change. Small, but significant, increases in free sediment sulfides were observed under the rafts in July 2002. The BOD associated with biodeposits from the mussels and their symbiotic community exceeded the sediments' assimilative capacity in November 2002 when free sulfide concentrations of 12,800 to 15,300 μM were observed under the rafts. These concentrations declined exponentially with distance to the north and reached background concentrations at 60 m distance.

Benthic community response. The video record of July 17, 2002 revealed a very abundant and diverse epifaunal community living on the sandy – gravelly sediments under the farm. No living mussels were observed. In part because Taylor Resources collects *fall-off* in horizontal nets suspended under the rafts, and in part because of the large numbers of starfish and crabs observed foraging under the cultures. The infaunal community found in fine-grained sediments from the rafts' perimeter to the north was one adapted to the generally enriched conditions characteristic of most of Totten Inlet. The abundance of macrofauna was significantly reduced under the farm in July 2002 when compared with the reference location. That was likely due to the difference in sediment grain size composition and to the increased sulfide concentrations observed at that time. Many, if not most common infauna found in Totten Inlet would have been excluded from the area under the farm in November 2002 by the high sulfide concentrations observed on that date and subtle, but detectable changes in the macrofaunal community would likely have extended to a distance of 60 m north of the farm. Regression analysis indicated a significant positive cline in the abundance of bivalves with distance to the north of the rafts. This effect appeared to extend to about 60 m in July 2002. Significant clines in macrofaunal community richness or in the abundance of other phyla were not observed at any time. At the species level, taxa that are known to tolerate elevated sulfide concentrations were unaffected near the farm, but the abundance of *Paraprionospio pinnata*, an annelid that is relative intolerant of sulfidic conditions, was reduced in fine-grained sediments at distances less

than between 45 and 60 m north of the rafts. It was concluded that subtle (non-significant) biological effects were apparent at distances less than 60 m north of the farm.

Chemical remediation of sediments. The low sulfide conditions found in sediments under the rafts at the beginning of the 2002 production cycle suggested that chemical remediation was essentially completed during the three month fallow period following the previous harvest.

Epifaunal community sympatric with the cultured mussels. Brooks (2004b) described the growth and structure of the invertebrate community growing sympatrically with the cultured mussels on the Deepwater Point rafts. A total of 14,698 invertebrates and 106 taxa were retrieved from eighteen 30 cm long samples of the cultured mussels. The 24 taxa representing ≥ 1.0% of total abundance accounted for 88.3% of the animals. Eleven of these taxa were annelids; six were arthropods, five were mollusks and the other two were nematodes and anemones. The most abundant mollusks were Olympia oysters (*Ostrea conchaphila*) that set sometime in late July or August and were a dominant part of the community on November 22, 2002. Barnacles and caprelid amphipods were ephemeral; reaching high abundance in spring and summer and dying off later in the year. Other taxa slowly increased in abundance over the coarse of the study. The biomass of symbionts increased exponentially during the production cycle. The following predictive relationship describing the wet tissue weight of symbionts as a function of mussel biomass was developed using non-linear regression analysis.

$$\text{Symbiont biomass in the center of the raft array (kg)} = 248.0 * \exp^{0.0000237 * \text{mussel biomass (kg)}}$$

$$\text{Symbiont biomass on the perimeter of the raft array (kg)} = 456.3 * \exp^{0.0000279 * \text{mussel biomass (kg)}}$$

The biomass of symbionts on the perimeter of the raft array reached 20% of the biomass of mussels in November 2002 just prior to harvest. In the center of the array, symbionts biomass only reached 5.3% of the mussel biomass. Anemones comprised most of the symbionts' biomass at the end of the study. Differences in the waste production by anemones and mussels were not investigated. However, the passive feeding mode of anemones suggests that they may produce less waste than actively feeding bivalves.

Baseline genetic surveys. Consistent with Brooks' (1991) finding that, excepting Penn Cove in Saratoga Passage, mussels throughout Washington State were found primarily in association with freshwater or on floats and piling, few mussels of any species were observed on sediments in Totten Inlet's intertidal areas. Three permanent genetic survey stations were established in Totten Inlet (Brooks, 2005c). Mussels were collected from a predator fence adjacent to the existing Deepwater Point farm; on piling east of the proposed North Totten mussel farm; and on a concrete dike in Little Skookum Inlet. Stratified random sampling designs were used to randomly sample mussels at each of these locations. In addition, non-random samples of mussels morphologically resembling *Mytilus edulis galloprovincialis* were collected to determine the presence – absence of this taxon at each of the sites. The results of these surveys and similar surveys conducted in 1996 and 1997 in Holmes Harbor, Washington indicated that:

- Mussels carrying *M. e. galloprovincialis* alleles were found at all of the sites. Non-random (presence-absence) sampling has repeatedly demonstrated that biologists familiar with the species complex can preferentially identify *M. e. galloprovincialis* and *M. e. trossulus x M. e.*

galloprovincialis hybrids in mixed populations. At Deepwater Point, 18 of 30 non-randomly selected mussels carried *M. e. galloprovincialis* alleles. At North Totten, 14 of 30 mussels had *M. e. galloprovincialis* alleles and in Little Skookum 28 of 30 mussels that were identified as galloprovincialis-like did carry *M. e. galloprovincialis* alleles. This is considered important because it emphasizes the need for strict random sampling study designs if the results are to be used to characterize the genetic make-up of Puget Sound mussel populations.

- Random samples of mussel populations in Holmes Harbor and Totten Inlet have all indicated that between zero and 10% of the individuals carry some *M. e. galloprovincialis* genes. At the three Totten Inlet stations surveyed in 2002, between 0 and 3 percent of the randomly sampled mussels were *M. e. galloprovincialis* and between 0 and 10% of the mussels were hybrids. All of these surveys were conducted in harbors and inlets where *M. e. galloprovincialis* has been intensively cultured using protocols previously approved by the National Marine Fisheries Service, U.S. Army Corps of Engineers and the Washington State Department of Fish and Wildlife. It should be emphasized that the results cannot be applied outside areas where *M. e. galloprovincialis* has been cultured.

Brooks (1991, 2005c) concluded that *M. e. trossulus* is adapted to cold water having reduced salinity whereas *M. e. galloprovincialis* is adapted to areas having fairly high and constant salinity and warmer water temperatures. The low salinity niche occupied by *M. e. trossulus* is likely a response to predation by starfish and crabs, which are abundant throughout most of Puget Sound. These predators are typically restricted from areas around streams where salinity is regularly reduced to a few parts per thousand during low tides and/or during periods of high runoff. Invertebrate predators are also restricted from the uppermost intertidal elevations by desiccation. It is extremely rare to find *M. e. trossulus* or *M. e. galloprovincialis* subtidally in Puget Sound. The absence of mussels from the hard substrates under the Deepwater Point mussel farm, and the presence of large numbers of predators there, is a testament to why *M. trossulus* is generally restricted to low salinity refuges or very high in the intertidal on rocks along the Pacific Coast. Both taxa are found on floats and piling in Puget Sound. Concerns regarding the environmental changes associated with possible warming in the Northeast Pacific, including Puget Sound, were expressed in Sound Waves (2005). Brooks (1991) found that populations of *M. e. trossulus* suffered increased mortality at temperatures above 10 ^{o}C. If the waters of Puget Sound warm, and if summer runoff from snow-pack is reduced, this will likely further diminish populations of this mussel in Puget Sound due to thermal stress and reduced extent of low salinity sanctuaries during summer low freshwater flows. In contrast, *M. e. galloprovincialis* will gain an advantage in Puget Sound if water temperatures increase in winter when this sibling spawns. Brooks (1991) and Wonham (2004) observed *M. e. galloprovincialis* in low numbers at numerous places in Washington State and Heath *et al.* (1995) has described the sibling in several British Columbia locations. At present, *M. e. galloprovincialis* is the dominant mussel in California and *M. e. trossulus* is dominant from Oregon northward. However, the taxa are sympatric throughout their range and *M. e. galloprovincialis* exists throughout the Pacific Northwest. If Northeast Pacific surface waters continue to warm, the latitude defining regions where the two siblings dominate will likely move northward as *M. e. galloprovincialis* gains an advantage. However, it is anticipated that predation by gastropods, starfish, and crabs will continue to control all blue mussel populations in intertidal and subtidal environments of Puget Sound.

Summary. The seven attached reports provide a detailed description of benthic environments in Totten Inlet and their response to the intensive cultivation of mussels on rafts. The focus of these studies has been on benthic and genetic effects. The report describing nutrients and POM at the North Totten site is intended as a supplement to Gardiner *et al.* (2002). Together with the comprehensive literature review and synthesis of historic data provided in Brooks (2000), these documents provide the most comprehensive basis for assessing the environmental risks associated with marine aquaculture (finfish or shellfish) that the author is aware of anywhere in the world. These reports suggest that Totten Inlet will be at approximately ten percent of its *carrying capacity* when the North Totten Inlet mussel farm reaches full production. These results indicate that any negative benthic effects in the immediate vicinity of the proposed mussel farm will be minor and ephemeral. It is quite possible that biodeposits from the farm will actually increase the abundance, diversity and biomass of the macrobenthic community near the farm. Brooks (2006) proposes a monitoring program designed to alert producers and managers when estuaries, like Totten Inlet, approach their *carrying capacity*.

Brooks, K.M. 2000. Literature review describing the environmental effects associated with the intensive culture of mussels (*Mytilus edulis galloprovincialis*). Technical report prepared for Taylor Resources, Southeast 1340 Lynch Road, Shelton, WA 98584. 129 pp. Revised February 16, 2006.

Brooks, K.M. 2003. Measurement of nutrients in bottom water under and adjacent to the Deepwater Point mussel farm in Totten Inlet, Washington. Prepared for the Pacific Shellfish Institute, 120 State Avenue NE #142, Olympia, Washington as part of Department of Commerce Award No. NA16RG1591. 9 pp.

Brooks, K.M. 2004a. The epibenthic community observed in association with the intensive raft culture of *Mytilus galloprovincialis* in Totten Inlet, Washington. Prepared for the Pacific Shellfish Institute, 120 State Avenue NE #142, Olympia, Washington as part of Department of Commerce Award No. NA16RG1591. 19 pp.

Brooks, K.M. 2004b. The frequency of *Mytilus edulis galloprovincialis* alleles in Washington State marine waters where the species is commercially cultivated. Technical report prepared for Taylor Resources, Southeast 1340 Lynch Road, Shelton, WA 98584. 13 pp.

Brooks, K.M. 2005a. Baseline information describing sediment physicochemistry of Totten Inlet and the macrobenthos of the proposed North Totten Inlet mussel farm. Aquatic Environmental Sciences, 644 Old Eaglemount Road, Port Townsend, WA 98368. 64 pp.

Brooks, K.M. 2005b. Benthic response at the Deepwater Point mussel farm in Totten Inlet, Puget Sound, Washington State, U.S.A. Aquatic Environmental Sciences, 644 Old Eaglemount Road, Port Townsend, WA 98368. 41 pp.

Brooks, K.M. 2006. Supplemental study of dissolved nutrients and particulate organic matter in the waters near the proposed mussel farm in North Totten Inlet, Washington State, USA. Aquatic Environmental Sciences, 644 Old Eaglemount Road, Port Townsend, WA 98368. Presented at the Canadian Department of Fisheries and Oceans workshop for the review of working papers on the effects of shellfish aquaculture in the marine environment, held in Moncton, New Brunswick, Canada between February 28 and March 3, 2006. 49 pp.

EHI. 2006. Evans-Hamilton, Incorporated Totten Inlet Circulation Study Report. EHI Job Number: 5514. Evans Hamilton, Inc. 4608 Union Bay Place NE, Seattle, Washington 98105. Prepared for Taylor Resources, SE 130 Lynch Road, Shelton, WA 98584.