Benthic response at the Deepwater Point mussel farm inTotten Inlet, Puget Sound, Washington State, U.S.A.

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Abstract. All aggregations of animals result in the discharge of biological waste and cultured mussels are no exception. In general, published reports describe benthic effects that are limited to the immediate vicinity of mussel rafts. Benthic effects were assessed adjacent to the inner row of six mussel rafts at the Deepwater Point mussel farm in Totten Inlet, Washington State in 2002 during production of 86,193 kg of *Mytilus galloprovincialis.* The farm is located in shallow water over relatively coarse sediments (30.2 to 55.8% gravel and 13.6 to 51.4% sand). Sediments become dominated by fines (59.9 to 91.2% silt and clay) downcurrent from the farm and at a local reference station located 1.12 km north of the raft's northern perimeter. Little change was observed in sediment chemistry during the first 186 days of culture. However, total volatile solids collected in near bottom canisters increased, significantly in the near-field during the last five months of growout. Concentrations of free sediment sulfides were the most sensitive indicator of physicochemical effects in these sediments. By the end of the study, just prior to harvest, sulfide concentrations under the rafts were measured at 12,800 to 15,300 μM. They declined exponentially along the downcurrent transect to background concentrations within 60 m of the perimeter. A baseline survey of Totten Inlet found sediments to be significantly organically enriched with TVS concentrations above the upper 90th percentile reported for Puget Sound Reference areas. Common infauna within the inlet, including *Nephtys cornuta, Paraprionospio pinnata, Macoma nasuta, Alvania compacta* and *Sigambra tentaculata*, have previously been shown to be tolerant of naturally enriched conditions. During, July 2002, matched physicochemical and macrofaunal analysis found sulfide concentrations of 1,571 to 2,620 μM under the rafts with an exponential decline along the downcurrent transect reaching reference concentrations at between 15 and 30 m distance. Sediments from the rafts' perimeter to the north were relatively homogeneous with respect to grain size and depth and the free sulfide concentrations declined from 760 μM on the perimeter to between 404 and 416 μM at the reference location. Significant biological effects were not observed in major biological endpoints (species richness, total abundance, Shannon's Index, etc.). However, the abundance of the annelid *Paraprionospio pinnata* was depressed at distances less than 45 to 60 m from the raft's perimeter as was the abundance of bivalves in the genus *Macoma* at distances less than 60 m. It was concluded that physicochemical changes in fine-grained sediments were apparent to 60 m distance just prior to harvest and that midway through the production cycle, subtle effects in the macrofaunal community could be seen inside this same distance. The inner row of rafts at the Deepwater Point farm produced 86,193 kg of mussels in the 2000 – 2001 production cycle and it was fallowed for approximately three months prior to reseeding in December 2001. During the first sampling period, conducted on February 8, 2002, free sulfide concentrations under the center of the row of rafts varied between 443 and 561 μM suggesting that short fallow periods are sufficient for nearly complete chemical remediation to occur.

Keywords: Raft culture; mussels, Benthos; Macrofauna; Sediment chemistry; Washington State

1.0 Background. Idyll (1979) noted that mussel culture produces a greater weight of meat per unit surface area than any other form of farming. Dense populations of organisms, cultured or naturally occurring, result in the discharge of metabolic waste whose fate depends on local environmental conditions such as sunlight, temperature, currents, water depth, other fauna and flora, salinity and a host of other parameters that interact to create the biosphere. Intensive aquaculture is no exception.

Decades of monitoring salmon farms in Washington State and elsewhere have shown that effects on the water column from intensive aquaculture in the Northeast Pacific are minimal and generally do not significantly change background levels of nutrients, dissolved oxygen, turbidity or any other measurable endpoint. This history has been repeated in British Columbia and both governments no longer require water column monitoring in their marine netpen waste management programs. However, particulate waste (feed and feces) are known to accumulate in nearfield sediments at distances less than ca. 185 m from salmon farms creating measurable physicochemical and biological effects that have been extensively studied and reviewed by Brooks (2001a, 20001b), Brooks and Mahnken (2003a, 2003b) and Brooks *et al.* (2002). Brooks (2003) and Brooks *et al.* (2004) have described chemical and biological remediation of sediments and macrofaunal communities at fallow salmon farms.

Unlike the netpen rearing of fish, suspended bivalve cultures do not add nutrients to the ecosystem – they remove phytoplankton and detritus – concentrating the primary and detrital production from large areas of open water into living tissues of the mussels and their commensal communities. These communities are commensal because in the least, the cultured mussels provide habitat and a detrital food base for the multitude of other invertebrates and vertebrates that are associated with intensive bivalve cultures (Brooks, 2004b). Despite the large biomasses of living organisms associated with mussel culture, reviews and modeling by Brooks (2000) and Gardiner *et al.* (2004) concluded that when the North Totten mussel farm is at full production, the inlet will be at approximately 10% of its predicted carrying capacity for suspension feeders. Gardiner *et al.* (2004) quantified the effects of phytoplankton consumption by the cultured mussels at several trophic levels within the inlet and found only minor reductions in the production of any taxa including Pacific salmon and their prey. As discussed by Brooks (2000), mussels are not efficient grazers and only 16 to 20% of the food filtered from the water is incorporated into mussel tissue. The remainder is recycled back into the environment – much of it in a particulate form. Because Totten Inlet is not near its carrying capacity for suspension feeders, it is the nearfield accumulation of waste in sediments that may have the most significant effect on the inlet's biological resources. Benthic effects associated with mussel culture have been found to range from minor by Crawford *et al.* (2003) in Tasmania where mussel production was low to moderate to significant by Dahlback and Gunnarsson (1981) in Sweden. Tenore *et al.* (1982) demonstrated that benthic effects are site specific and that macrofaunal community enhancement can occur at well flushed sites.

1.1. Changes in the physicochemical characteristics of sediments. Kaspar *et al.* (1985) reported that sediments under suspended mussel cultures contained 8.0 to 8.7% TVS in comparison with 7.0 to 7.1% TVS found at reference sites. This relatively small addition of volatile organic material was sufficient to increase the flux of NH_4 from an annual average of 8.6 mmoles/m^2 at the reference site to 32.5 mmoles/m^2 under the mussel rafts. Differences in sediment nitrate (NO_3^-) and nitrite (NO_2^-) were not significantly different. This biological activity had little effect on oxygen consumption from the overlying water. Dame *et al.* (1991) observed that mussels take up seston, including phytoplankton and detritus, and release ammonium (NH_4^+), orthophosphate and silicate. These nutrients are released in a dissolved form and as a component of feces. Nutrients are also released by epibionts from the suspended culture and by microbial processing of sedimented feces and pseudofeces. Dame *et al.* (1991) estimated a nutrient turnover time of one to 38 weeks from sediment underlying suspended mussel culture in the Western Wadden Sea and Eastern Scheldt estuaries of Northern Holland. This slow release of nutrients tends to reduce fluctuations in phytoplankton production by cropping phytoplankton during periods of high production and cycling the nutrients into sediments where they are slowly released to sustain moderate phytoplankton production over longer periods of time. Similar increased ammonium production has been observed by Grant *et al.* (1995). Shaw (1998) evaluated sediment organic content (TVS), redox potential (Eh), sulfides (S^{+}) and infauna in ten estuaries with extensive mussel culture and at ten reference areas around Prince Edward Island, Canada. Seven of the ten estuaries had been in continuous production for 12 or more years. Table (1) summarizes the endpoints evaluated by Shaw (1998). With the exception of redox potential, observed differences between mussel culture areas and reference areas were not statistically significant. Unfortunately, Shaw (1998) did not evaluate sediment grain size and water depth – important parameters for comparing his results with other areas. However, the relatively high organic content in reference area samples suggested that the sediments were likely depositional and therefore dominated by silts and clays. The high organic matter, low redox potential and high sulfide concentrations in reference area sediments suggest that these estuaries were naturally depositional and supported a significantly reduced infaunal community. This work demonstrated that not all natural (anthropogenically undisturbed) benthic environments have ideal physicochemical characteristics and that some do not support diverse and abundant infaunal communities.

Table 1. Mean $\pm$ 1.0 standard deviations of endpoints measured by Shaw (1998) in mussel culture and reference areas adjacent to Prince Edward Island.

Endpoint	Mussel Culture Areas	Reference Areas
Organic matter (%)	10.427 $\pm$ 5.141	9.492 $\pm$ 4.543
Redox potential (mV)	-127 $\pm$ 45	-100 $\pm$ 67
Sulfides (µM)	1320 $\pm$ 879	1121 $\pm$ 682
Infauna abundance (#/0.1 m^2)	135 $\pm$ 152	158 $\pm$ 87
Infauna biomass (g/0.1 m^2)	32.74 $\pm$ 12.45	15.38 $\pm$ 12.45
Percent deposit feeders (%)	36.7 $\pm$ 35.4	45.7 $\pm$ 36.6
Shannon-Wiener Index[1]	0.275 $\pm$ 0.242	0.369 $\pm$ 0.245

[1]The Shannon-Wiener Index (Shannon and Weaver, 1949) is a metric commonly used to describe the diversity of communities of organisms. It is equal to $H' = -\sum p_i \log(p_i)$ where p_i is the proportion of the total abundance attributed to the *i*th taxa. Low values are indicative of populations dominated by a few taxa; whereas, increasing values are associated with more evenly distributed and diverse communities. The maximum value of H' is ln(number species).

Total volatile solids (TVS) as a measure of organic enrichment. Total volatile solids, or the loss in weight of dried material combusted at 450 to 550 ^{o}C, is commonly used to assess organic enrichment in sediments. Volatile solids under suspended mussel culture have been measured at concentrations as high as 25% of the dry sediment weight (Dahlback and Gunnarsson, 1981). The degree and spatial extent of significant TVS increases associated with mussel culture is dependent on the nature of the particulate waste, current vectors and water depths. In general, it appears that significant increases are restricted to the immediate vicinity of the cultures. Mattsson and Linden (1983) observed that high TVS levels of 22% decreased to 15% at 5 m from the cultures and were further reduced to 8% at 15 m.

Sediment cycling of sulfur. Dahlback and Gunnarsson (1981) reported that sulfate reduction in sediments on the west coast of Sweden could be modeled by the following stoichiometric relationship describing the catabolism of organic material via the striping of oxygen from sulfate to reduce this compound to sulfide, some of which combines in the sediments with hydrogen ions to form the toxic compound hydrogen sulfide (H_2S).

$$(CH_2O)_{2n} + nSO_4^{=} \rightarrow 2nCO_2 + 2nH_2O + nS^{=}$$

As oxygen is replenished during remediation, the sulfide is oxidized back to sulfate.

$$H_2S \rightarrow H_2 + S^{=} + 2O_2 \rightarrow SO_4^{=}$$

Dahlback and Gunnarsson (1981) estimated that during May and July, 13% and 24% respectively of the sedimented organic carbon under mussel lines was mineralized through sulfate reduction in the absence of free oxygen. They also calculated oxygen consumption during subsequent oxidation of the sulfide back to sulfate at 1.4 liters O_2/m^2-day. Assuming that the length of the high TVS sediment extends only under the 76.8 m length of the rafts at the proposed mussel farm in North Totten, we can roughly estimate the oxygen lost from the overlying water at 107.5 L/d – equivalent to 75.26 g of O_2 per meter width orthogonal to the prevailing currents. Assuming an average current speed of 5 cm/sec and mixing to a depth of 50 cm as the water flows over the substrate implies that oxygen will be supplied to the sediments from a volume of 2,160,000 liters of water. This leads to a predicted decrease in the dissolved oxygen concentration of 0.03 mg/L. This prediction is consistent with the conclusion of Dahlback and Gunnarsson (1981) that there was no risk of anoxia in the water column at their study site – or that the hydrogen sulfide would affect fauna in the overlying water column. These results suggest that significantly less sulfide is produced in sediments under mussel farms than has been associated with marine fish culture (Holmer and Kristensen, 1992) – even though the levels of TVS were similar. That is likely because fish waste is labile – whereas mussel waste likely contains higher concentrations of inorganic silicate; more refractory cellulose in feces and pseudofeces; and bound protein in the shells of fallen mussels; and is therefore somewhat more refractory.

1.2. Benthic community response to suspended mussel culture. Regardless its source, the accumulation of organic material in the benthos results in increased biological oxygen demand (BOD). The increased biological oxygen demand associated with microbial and infaunal catabolism of labile waste can exceed the diffusion of oxygen into the sediments. Pore water oxygen decreases and anaerobic metabolism replaces aerobic respiration. Anaerobic bacteria strip oxygen from sulfate reducing it to sulfide (Brooks, 2001; Dahlback and Gunnarsson, 1981). *Beggiatoa sp.* bacteria live at the boundary between anaerobic and aerobic environments. When surface sediments become anaerobic, colonies of *Beggiatoa* can be observed on the surface as white filamentous patches. These are natural process that can be observed in every productive estuary. Examples include:

- accumulations of decomposing eelgrass and macroalgae washed up on beaches;
- deposits of terrigenous material (sticks and leaves) at the mouths of small streams where they enter depositional embayments;
- accumulations of dead and decaying salmon in freshwater pools and in the bays receiving water from productive salmon spawning streams;
- under dense communities of marine organisms – like mussel beds in Penn Cove;

Human activity, such as intensive aquaculture in areas with slow currents, can also create conditions where the biodeposits from cultured animals exceed the assimilative capacity of the benthos. The biological community responds to these physicochemical changes in predictable ways. Pearson and Rosenberg (1978) described the macrobenthic succession associated with organic enrichment in marine environments. They found that as the organic content of sediments increased from low levels, species richness first increased in response to the enriched environment. However, when the assimilative capacity of the sediments was exceeded, richness decreased – i.e. fewer species were found. With organic enrichment, the community becomes increasingly dominated by a few organic carbon tolerant opportunists – like the annelids *Capitella capitata, Nephtys cornuta, Schistomeringos sp., Ophyrotrocha sp.,* and arthropods such as *Aoroides sp.* and *Nebalia pugettensis* (Weston, 1990; Brooks, 2001). Benthic ecologists have verified these conditions around the world and identified indicator organisms that are highly tolerant, moderately tolerant, and intolerant, of high levels of organic carbon. Mahnken (1993), Brooks (2001, 2003c), Brooks and Mahnken (2003a), Brooks *et al.* (2004) and Brooks (2004) have identified suites of indicator organisms associated with salmon farming and other forms of organic enrichment in the Pacific Northwest.

The environmental response to intensive aquaculture depends on numerous factors such as the depth of water, local currents (direction and speed), sediment grain size, dissolved oxygen concentrations in the benthic boundary layer, and other currently recognized factors. Aquaculture biodeposits can result in significant long-term enhancement of local benthic communities (Brooks, 1995; Tenore and Dunstan, 1973). They can also result in adverse effects in the benthic community when situated in poorly flushed environments. Mattsson and Linden (1983) noted a shift in the infaunal community from echinoderms and mollusks to opportunistic polychaetes and the amphipod (*Corophium insidiosum*) following several years of suspended mussel culture. Remediation was slow at this Swedish site because currents were weak at only ~3 cm/s. Despite the presence of anaerobic sediments, the authors observed only a small decrease of 3 to 7% saturation in oxygen concentration in the water 10 cm above the mussel farm sediments when compared with a reference area. Like other studies described in this review, Mattson and Linden (1983) observed that fish appeared to flourish around the mussel cultures.

In contrast to the rich macrofaunal communities described in some reports, Tenore and Gonzalez (1975) found an impoverished infaunal community under the rafts in the Ria de Arosa, Spain. *Spiochaetoceras sp.* was the only species observed in abundance under mussel rafts. The presence of the high organic carbon tolerant polychaete species complex (*Capitella capitata*) outside the rafts suggested high organic loading throughout the Ria de Arosa. This hypothesis was supported by Tenore *et al.* (1982) who reported >10% organic matter (TVS) in sediments from both Ria de Arosa and Ria de Muros. Tenore and Dunstan (1973) summarized their investigation as follows. Intensive mussel culture in the Ria de Arosa resulted in:

- an enhancement in the productivity of the Ria by rapid recycling of nutrients and carbon;
- the growth of epifauna on the cultured mussels decreasing biodeposition to the benthos;
- the energy initially contained in the biodeposits of mussels was being utilized to support a detrital food chain characterized by macroinvertebrates that subsequently supported the scavenger and carnivorous trophic levels of crabs and fishes;
- the excretion and subsequent mineralization of soluble organic nitrogen and phosphorus by the epifaunal community, which enhanced nutrient pools available for primary producers.

Tenore *et al.* (1982) revisited this issue in a more detailed study that compared infauna in the Ria de Arosa (high density mussel culture @ 434 g total wet weight/m^2) with those found in the Ria de Muros (low density mussel culture @ ~83 g total wet weight/m^2). Sediments were fine grained and anoxic in both Rias and infaunal biomass decreased from the outer to inner stations. However, they found that in general, the biomass of macrofauna was 5 to 10 times higher in the mouth of the Muros when compared with Arosa where it was uniformly low at 2 to 3 g ash free dry weight (AFDW)/m^2. Macrofaunal biomass was similar in the interior of both Rias. Consistent with Tenore and Gonzales (1975), they found a higher biomass of echinoderms (starfish) and crabs under the rafts in both Rias when compared with other areas. Fish were evenly spread throughout both Rias. The authors concluded that:

- Although the effect of mussel culture on sediment composition was important, its primary influence was upon the food chain pathways in the Arosa. The relative enhancement of megabenthic biomass (fish, crabs, etc.) in raft areas clearly resulted from the high standing crop of the mussels and epifauna;
- Zooplankton standing crops were higher during spring and summer in the area of dense mussel culture (>70 mg AFDW/m^3) than in the low density mussel culture area.
- Much of the organic load produced by the mussels was utilized by the raft epifauna before it reached the benthos;
- The high accumulation rates of biodeposits from the large number of rafts in the Arosa markedly affected sediment composition and benthic infauna;
- The infauna associated with intensive mussel raft culture was a pioneering community with high abundance but low biomass and diversity.

Grant *et al.* (1995) observed only minor benthic impacts associated with suspended shellfish culture on the coast of Nova Scotia. Consistent with other studies, these authors noted a decrease in the sediment's oxidation-reduction potential and an increase in sulfide concentrations. The maximum rates of ammonium released at his study site were twice those observed at the reference station. The benthic community under the mussel site was dominated by gastropods (*Illyanassa sp.*) and deposit feeding bivalves (*Nucula tenuisculpta*). Similar biological responses have been documented in association with mussel culture in other areas of North America (Hargrave *et al.* 1995) and throughout the world (Kaiser *et al.* 1998; Mattson and Linden, 1983; Kusuki, 1977; Kaspar *et al.* 1985).

Crawford *et al.* (2003) found minimal benthic effects at three Tasmanian suspended oyster and mussel farms. The three farms examined had been in production for up to 25 years and produced low 73 mt/yr to moderate 220 mt/yr (equivalent to 484,000 lbs/yr) quantities of oysters and mussels. Sediments at the Eaglehawk Bay farm consisted of fine sands with some shell hash. Redox potential was positive at 1.0 cm depth in all samples. However, the 10 second seek time allowed for the redox probes prior to taking a reading is questionable. Brooks (2001) reported seek times of several minutes in most samples prior to obtaining stable readings using Orion™ probes and meters. This concern is substantiated by the sulfide data for Eaglehawk Bay, which was as high as 740 μM $S^{=}$ under the long-lines - a concentration always associated with negative redox potential in the Pacific Northwest. Significant differences in benthic macrofaunal endpoints and physicochemical variables, including TSS deposition rate, redox potential, sulfides and sediment TOC were not observed between sample stations located under the farm, on its perimeter or at any distance downcurrent. Seagrass was observed during video surveys to be proliferating throughout the Eaglehawk Bay area – including in the area directly under the long-lines. Common macrofauna at all sites included *Mediomastus australiensis* and *Nephtys australiensis.* Both of these genera are associated with moderate levels of organic enrichment.

Similar conditions were documented by Grant *et al.* (1995), Hayakawa *et al.* (2001) and Mitchell (2001). The authors found a higher potential for adverse benthic effects associated with mussels or oysters that were detached from the long-lines and that had settled to the bottom than was caused by biodeposits (feces and pseudofeces) associated with the cultured animals. However, in general they concluded that ". . .shellfish farming is having a minimal effect on the benthic environment" and that, "This, in turn, suggests that extensive monitoring of shellfish farming is not necessary, and that simple measures such as photographic records of the farms collected every 1 or 2 years would be sufficient to address community concerns over negative impacts occurring as a result of shellfish farming activities."

2.0 MATERIALS AND METHODS.

The study reported herein was designed to assess physicochemical and biological responses to the intensive culture of mussels at Deepwater Point, which produced approximately 450 metric tonnes (mt) of *Mytilus galloprovincialis* (live weight) in 2002. The assessment included periodic evaluations of a set of sediment physicochemical variables between March and October 2002 and a macrobenthic community inventory in July 2002. Deposition of total suspended solids (TSS) and total volatile solids (TVS) was evaluated using a series of canisters located at the Deepwater Point farm between March and October 2002. A physicochemical sediment survey was also completed on the dominantly downcurrent transect at the Gallagher Cove farm in Totten Inlet. Triplicate samples were collected at local reference stations near each of these farms on each sample day. The following methods were used.

2.1. Experimental design. A regression approach to sampling was taken at Deepwater Point and Gallagher Cove. Brooks (2001) provides a review of the benefits of this approach for evaluating aquaculture effects. Sediment samples were collected at distances of 0.0, 15, 30, 45, 60, 80, 100 and 125 meters on a downcurrent (northern) transect at Deepwater Point in March, April, May, July and October of 2002 to assess sediment physicochemical conditions as a function of season and cultured mussel biomass. A concurrent marcrofaunal, sediment physicochemical and video survey was completed on three orthogonal transects at Deepwater Point in July 2002. Sediment samples were collected at distances of -30 m (triplicate samples at the raft's center), 0, 15, 30, 45, 60, 75, 90, 105, 125, 145 and 165 m on the northern (downcurrent) transect and at distances of 0, 15, 30, 45 and 60 m on the southerly (upcurrent) transect. Samples were also collected on an inshore (westerly) transect at distances of 15, 30 and 45 m. Sediments became too coarse for sampling (large gravel and cobble) at stations closer to shore. The offshore transect, which is orthogonal to the currents, was not sampled because of interference by the adjoining row of mussel rafts. Sediments were sampled for physicochemical characteristics in October 2002 using a similar regression approach at distances of -30 m, 0, 15,30, 45, 60, 75, 90, 105 and 125 m on a downcurrent transect bearing 350 ^{o}M. Three samples were collected approximately 30 m apart at a local reference station located >1,120 m north of the rafts on most sample dates. However, single reference station samples were collected at Deepwater Point in March 2002 and at Gallagher Cove in October 2002.

2.2. Methods.

Station positioning. Station positioning at the Deepwater Point and Gallagher Cove mussel farms was accomplished by attaching a ½" polypropylene line with a stainless steel clip near the center of each side of the farm's structure. The premarked line was run through a nylon insert in the vessel's bow pulpit and tied off at a cleat adjacent to the sampling davit. The vessel was then maneuvered in reverse to maintain the line fairly tight on an orthogonal bearing from the raft. For each sample, the davit operator gave an audible mark when the grab reached the bottom and the vessel's latitude and longitude was logged using a Magellan™ differential GPS navigation system with an advertised precision of $\pm$ 3.0 m in three dimensions. The accuracy of the GPS was verified prior to each survey using a monument located at AES. For purposes of this monitoring, no requirement to correct for or record hydrowire angle was imposed.

Sample dates. The dates on which samples were collected are summarized in Table 6.

Table 6. Summary of sample dates and types of samples collected at the Deepwater Point and Gallagher Cover mussel farms in Totten Inlet during 2002. The numbers in each cell refer to the number of samples collected and analyzed.

Date	Location	Physicochemistry	Macrofauna	Canister	Video
3/22/2002	Deepwater Point Rafts	12			
4/26/2002	Deepwater Point Rafts	12		6	
5/29/2002	Deepwater Point Rafts	15		6	
6/20/2002	Deepwater Point Rafts			6	
7/8/2002	Deepwater Point Rafts	26	26	6	Four transects
8/27/2002	Deepwater Point Rafts			6	
10/9/2002	Deepwater Point Rafts			6	
10/31/2002	Deepwater Point Rafts	19			
11/20/2002	Deepwater Point Rafts			6	
11/22/2002	Gallagher Cove	11			

Grab sampling. A 0.0225 m^2 stainless steel Petite Ponar grab was used for physicochemical sampling. Samples for macrofaunal inventories were collected using a stainless steel modified van Veen grab with a 0.1 m^2 footprint. Both grabs were deployed to the bottom with a maximum vertical speed of 30 cm/sec. Upon retrieval, overlying water in acceptable samples was siphoned through 500 μm sieves and the contents of the sieve returned to the grab. Acceptable samples met the requirements of PSEP (1996) with a minimum penetration depth of 6 cm and an intact, relatively horizontal sediment surface containing overlying water. Water depth and position of each sample were recorded on the datasheet along with an organoleptic evaluation of hydrogen sulfide and/or hydrocarbons. The penetration depth and the depth of the redox potential discontinuity (RPD) were also recorded along with comments related to sample quality such as leakage, winnowing or undue disturbance. Each sample was photographed in the grab to record the following features:

- Texture and color
- Biological structures (shells, tubes, macrophytes)
- Presence of debris (wood chips, wood fibers, trash, etc.)
- Presence of bacterial mats, oily sheens, etc.

The top two cm of sediment were subsampled for physicochemical analyses. Subsamples were placed in a stainless steel bowl and gently stirred prior to filling new 125 ml urine specimen cups whose tops and sides were pre-labeled. Approximately 100 ml of sediment were placed in the cups, which were then sealed and placed on shaved ice in a cooler. Subsamples of 0.1 m^2 grabs intended for macrofaunal analyses covered an area that was $\leq$ 5.0% of the grab's footprint in accordance with PSEP (1996). The 100 ml sample was sufficient for all physicochemical analyses, including triplicate analyses when required (1 per set or 1 per 20 samples). Infaunal samples were then washed into five gallon plastic buckets with overlying water and stored on deck until sieved on 1.0 mm stainless steel screens and fixed in 10% formalin on the day of collection. All water coming into contact with the macrofaunal samples was filtered at 100 μM in the field. The grabs and sampling tools were washed with ambient seawater between samples. Samples were analyzed using the following procedures.

Total Volatile Solids. Samples were stored at 4^o C until analyzed within 14 days. Standard Method 2540.E or EPA Method 160.4 was used for this analysis. Total Volatile Solids analysis was accomplished on ca. 35-gram surficial sediment samples. Samples were dried at 103 $\pm$ 2^o C in aluminum boats that had been pre-cleaned by combusting at 550 ^{o}C for one hour and tarred. Drying was continued until no further weight reduction was observed. The samples were then combusted at 550 ^{o}C for one hour or until no further weight loss was recorded. Total Volatile Solids were calculated as the percent difference between the dried and combusted weights. Quality assurance required triplicate analyses on one of every 20 samples or on one sample per batch if fewer than 20 samples were analyzed. A maximum of 20 percent Relative Percent Difference was established as the Data Qualification Control Limit.

Sediment Grain Size. These analyses were accomplished on approximately 50 grams of surficial sediment. The sediments were subsampled and weighed in tared aluminum boats. They were then wet sieved on a 0.064 mm sieve. The fraction retained on the sieve was dried in an oven at 92 ^{o}C and processed using the dry sieve and pipette method of Plumb (1981). The sieves used for the analysis had mesh openings of 2.0, 0.89, 0.25 and 0.064 mm. Particles passing the 0.064 mm sieve during wet sieving were analyzed by sinking rates in a column of water (pipette analysis).

Total Organic Carbon. Samples were iced immediately in the field. Upon return to Aquatic Environmental Sciences, samples identified for TOC analysis were subsampled into glass vials and frozen the same day. The samples were then shipped on frozen phase change gel packs in coolers to the University of Washington Oceanographic Laboratory via an overnight deliver service. The samples were dried, homogenized by grinding, subsampled and weighed, acid-fumed, redried, and analyzed on a Leeman Labs Model CEC440 Elemental Analyzer. Caffeine and acetanilide standards and triplicate analyses were analyzed with each batch of ten samples.

Free Sediment Sulfides. Samples were analyzed using an Orion™ advanced portable ISE/pH/mV/ORP/temperature meter model 290A with a Model 9616 BNC *Ionplus* Silver/Sulfide electrode. The meter has a concentration range of 0.000 to 19,900 μM $S^{=}$ and a relative accuracy of $\pm$ 0.5% of the reading.

<u>Calibration of the total sulfides field probe</u>. A sulfide antioxidant buffer solution (SAOB) was prepared in 250-ml HDPE screw-top jars by adding 20.00 g of NaOH and 17.90 g

EDTA ($Na_2C_{10}O_8N_2.2H_2O$) and diluting to 250 ml with distilled water. The 2 M NaOH and 0.2 M Na_2EDTA solution was stored in a refrigerator or cooler at 4^o C. Immediately before sample analysis, 8.75 grams of L-ascorbic acid (pre-weighed in vials) was added to each 250 ml of the NaOH-EDTA solution. The SAOB buffer solution is stable for up to 4.0 hours after addition of L-ascorbic acid. As 5 ml of this solution was used for each sample analyzed (described below), 250 ml was sufficient for 50 samples. Stock and diluted $S^=$ solutions used as electrode calibration standards were kept cool (~4 ^{o}C) and diluted just before use. Diluted standards are stable for up to 3 hours and were made up in the morning and at mid-day on the day of sample analysis. The $S^=$ electrode was calibrated before and after each batch of not more than 12 samples. Three $S^=$ standards (100, 1000 and 10,000 μM) were used for a three-point electrode calibration. A stock $S^=$ solution of 0.01 M Na_2S was prepared by weighing 0.2402 g $Na_2S.9H_2O$ into a Biotight jar and diluting to 100 ml with distilled water. The stock was made fresh every 48 hours and stored at 4^o C in a dark bottle. A 1000 μM $S^=$ standard (10^{-3} M) was prepared by transferring 10 ml of the 0.01 M Na_2S stock solution (10,000 μM) into an amber jar and diluting to 100 ml with distilled water. A 100 μM $S^=$ standard (10^{-4} M) was prepared by transferring 10 ml of the 1000 μM standard to an amber jar and diluting to 100 ml with distilled water. Dilution standards were mixed thoroughly before use. A three-point calibration procedure was performed following the meter's instruction manual. Just before calibration of the $S^=$ electrode, 25 ml of each standard was transferred to a dark bottle and 25 ml of SAOB (containing ascorbic acid) added. The combined solution was kept tightly capped until used for standardizing the probe.

Measurement of sediment total sulfides. The sample was gently homogenized using a stainless steel spatula. Five ml of SAOB buffer was pipetted into a 30 ml graduated beaker and sediment added with a spatula to the 10 ml mark. A flat-tip stainless steel spatula was used to gently mix and homogenize the sediment sample with the SAOB buffer. Following this, the $S^=$ electrode was used to further stir the sediment. The $S^=$ electrode reading usually stabilized in two to four minutes. Sediment was gently wiped from the electrode after recording the reading and placing the probe into the next sample. The probes were standardized every 2 hours or after completing 12 analyses.

Quality assurance for sediment total sulfide analyses. Triplicate analyses were conducted on one of every 20 samples or on one per batch when fewer than 20 samples were analyzed. The Data Qualification Control Limit was set at 20% Relative Percent Difference (RPD). Fresh standards were made up daily. The analytical balance was inspected and calibrated at least once per week. The probes were checked at three points at the end of each set of samples and the probe's response recorded. However, no corrections to the data were appropriate based on these checks. In general, the probes have been found to drift no more than 10 to 15 percent in two hours.

Redox potential. This analysis should only be conducted in the field using the following, or similar, procedures. Aquatic Environmental Sciences uses an Orion™ advanced portable ISE/pH/mV/ORP/temperature meter model 290A with a Model 9678BN Epoxy Sure-Flow Combination Redox/ORP probe. The meter's accuracy in the ORP mode is ± 0.2 mV or ± 0.05% of the reading, whichever is greater.

Calibration of the Redox Electrode. Calibration reagents were prepared 12 to 24 hours before use and held refrigerated. Redox Standard A (0.1 M potassium ferrocyanide and 0.05 M potassium ferricyanide) was prepared by weighing 4.22 g $K_4Fe(CN)_6.3H_2O$ and 1.65 g

$K_3Fe(CN)_6$ into a 100-ml volumetric flask. Approximately 50 ml of distilled water was added with swirling to dissolve the solids. The solution was then diluted to volume (100 ml) with distilled water. Standard B (0.01 M potassium ferrocyanide, 0.05 M potassium ferricyanide, and 0.36 M potassium fluoride) was prepared by weighing 0.42 g $K_4Fe(CN)_6.3H_2O$, 1.65 g $K_3Fe(CN)_6$, and 3.39 g $KF.2H_2O$ into a 100 ml volumetric flask. 50 ml of distilled water was added to dissolve the solids, and the solution was then diluted to 100 ml with distilled water. A KCl filling solution for the internal reference electrode was prepared by placing 2.5 ml of 4 M KCl in 50 ml of distilled water to give a 0.2 M KCl solution. Redox standards were used to calibrate electrodes at ambient temperature (15 to 20^{o} C) at the start and end of each batch of samples. Standard A was transferred to a 150-ml beaker, and the electrode placed in the solution until the reading stabilized with stirring (1 to 2 minutes). The potential of Standard A is approximately +147 $\pm$ 9 mV. The electrode was rinsed with distilled water and the measurement repeated with Standard B (potential of +216 $\pm$ 9 mV). The potential in Standard A is approximately + 69 mV greater than in Standard B. The actual readings were recorded on the field datasheet. The potential of the reference electrode, which is a function of temperature, (+224 – 1.0*Temperature (^{o}C)) was added to the potential measured in the field during analysis.

Measurement of sediment redox potential. The Eh electrode was inserted into the sediment sample and a mV reading recorded after one to two minutes. The electrode was then removed, gently wiped free of sediment prior to being used to measure the next sample. The probe was recalibrated at least once every two hours. Probes were rinsed in distilled water and stored in pH 7.0 buffer between batches of samples.

Quality assurance procedures for the measurement of redox potential. Triplicate analyses were conducted on one of every 20 samples, or on one sample per batch if fewer than 20 samples were analyzed. The calibration values were recorded. No Data Qualification Control Limit was established for this test.

Macrofauna. Overlying water in the van Veen grab was siphoned off through a 500 µm stainless steel screen. Following removal of the subsample for physicochemical analyses, the contents of the grab were washed into a five-gallon bucket using 100 µm filtered seawater. Samples were then sieved on 1.0 mm stainless steel screens at a shore station using 100 µm filtered seawater. The retained material was placed in 1.0 or 2.0 liter HDPE bottles and fixed using 10% buffered formalin in seawater. Each sample container had matching inside, outside and cap labels. Fixed samples were washed with filtered fresh water and preserved in 70% isopropyl alcohol within 5 days of fixing. The sieved and preserved infaunal samples were sorted and identified at Aquatic Environmental Sciences. Infaunal organisms were sorted from the background matrix under 10x magnification using Leica M3Z and M6Z dissecting microscopes. A second technician repicked 20% of each sample. Quality assurance guidelines required a picking efficiency of $\geq$95%. Any sample failing this QA benchmark was completely repicked. Infauna were identified to the lowest level practicable – generally to species. Scientific names used in the database are those given by Banse and Hobson (1974) and Hobson and Banse (1981) for annelids; Kozloff (1987) and Hart (1982) for crustaceans and Kozloff (1987), Keen and Coan (1974) and Abbott (1974) for mollusks. The Excel™ database was imported into Statistica™ software for analysis. All species were compared with verified specimens in Aquatic Environmental Sciences' reference collection. A study reference collection, containing representative specimens of the most common taxa, was prepared.

Canister Studies. The canister design is described in Figure 1. The design was influenced by several reports. The Canadian Department of Fisheries and Oceans (DFO 1997) has criticized the placement of canisters on the bottom because of the potential to sample resuspended sediments. For this study, the canisters were suspended three meters above the bottom. The 9:1 aspect ratio for these canisters was chosen based on the work of Roff and Hopcroft (1987) who found minimum loss of heavy dyes placed in traps of this aspect ratio. These authors also noted that baffled inserts placed in traps encouraged the loss of both dye and particles. Therefore gratings were not inserted into the mouth of these canisters. Roff and Hopcroff (1987) also found that the use of saline solutions in traps greatly increased the retention of dyes. Gardner (1979) recommended simple cylindrical canisters with an aspect ratio >2.0. Similarly Bloesch and Burns (1980) and Blomqvist and Hakanson (1981) recommended simple cylindrical canisters with a diameter of 5 to 20 cm having an aspect ratio greater than 5:1 in calm waters or >10:1 in turbulent waters. Both of these reports found that all kinds of accessories (lattices, baffles, etc.) were not necessary and did not improve trap efficiency. These authors also discouraged surface mooring of canisters and therefore this design incorporates a subsurface mooring buoy located about two meters above the canister. They also observed that undisturbed samples can have the supernatant water drawn off before sampling the contents (as was done in this study). Organic matter was preserved in the canisters during the ca. 30 day deployments by adding 100 ml of heavy seawater mixed with formalin to the canister. The mixture was created by added 100 ml of 37% buffered formalin to 500 ml of 60 o/oo seawater (60 grams of NaCl in one liter of water). The canisters were lowered slowly to the bottom so as not to displace the heavy formalin

Canisters were deployed throughout the study at six stations located under the center of the line of rafts; on the perimeter of the northernmost raft at the Deepwater Point mussel farm; and at distances of 30, 60 and 120 m downcurrent from the farm. A sixth canister was deployed at the Deepwater Point reference station and a seventh at the buoy marking the center of the proposed North Totten mussel farm. Upon retrieval, Overlying water was siphoned off through a 500 µm Nytex™ screen leaving approximately 100 ml of overlying water in the canister. The contents were then stirred to create a slurry, which was poured into 2.0 liter HDPE bottles. The canister walls were rinsed with successive aliquots of 100 µM filtered seawater. This rinsate was added to the sample. The canister's contents were further fixed by adding 5% of 37% buffered formalin. The bottles were tightly capped and inverted five times to mix the formalin. The canister's contents were allowed to settle for five days and the supernatant siphoned off. Approximately 0.1 ml of the contents was examined under 100x magnification to determine their composition. The slurry was then dried in pre-tared aluminum foil boats. Normal TSS (drying at 103 $\pm$ 2 ^oC) and TVS (combustion at 550 $\pm$ 10 ^oC) procedures were used to determine the amount of PIM and POM collected in the canisters during deployment.

Photographic record. A color digital photograph was taken of each sediment sample prior to subsampling using a Sony MVC-CD1000 digital camera that records to a 3.5" CD providing a permanent record of the image. The number of each photograph was recorded on the field datasheets.

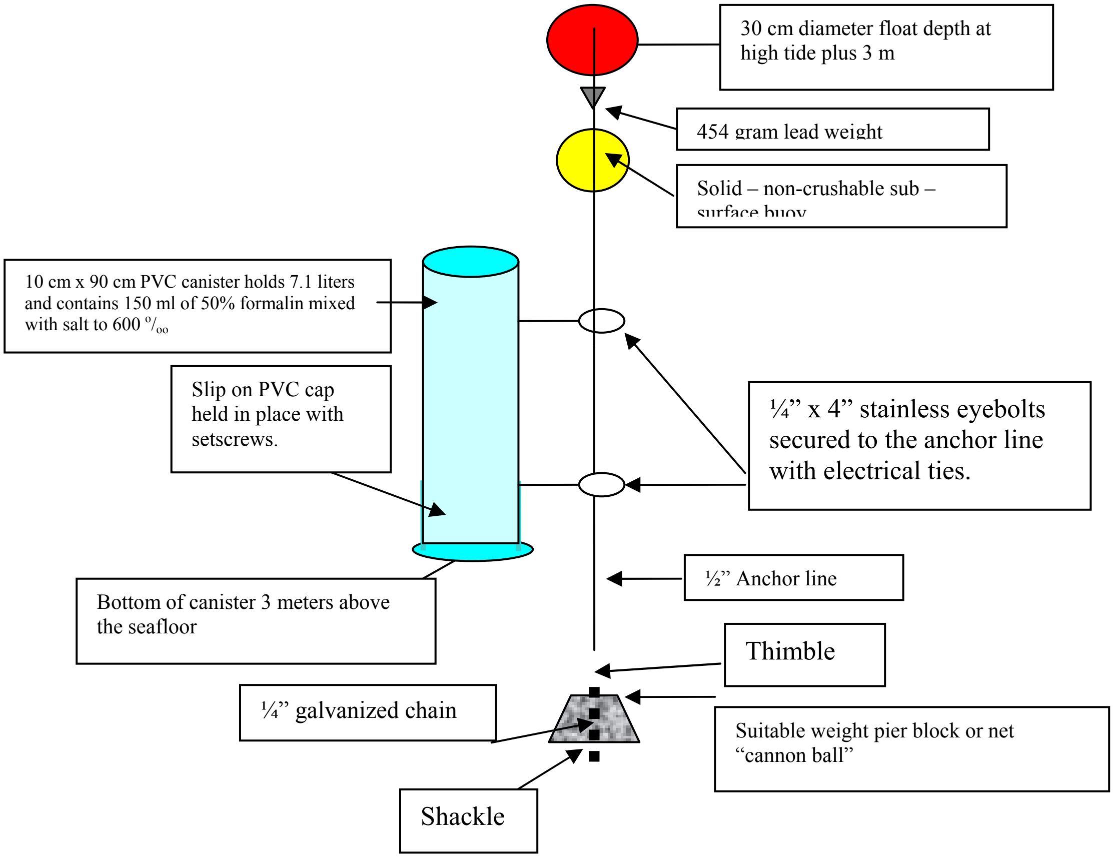


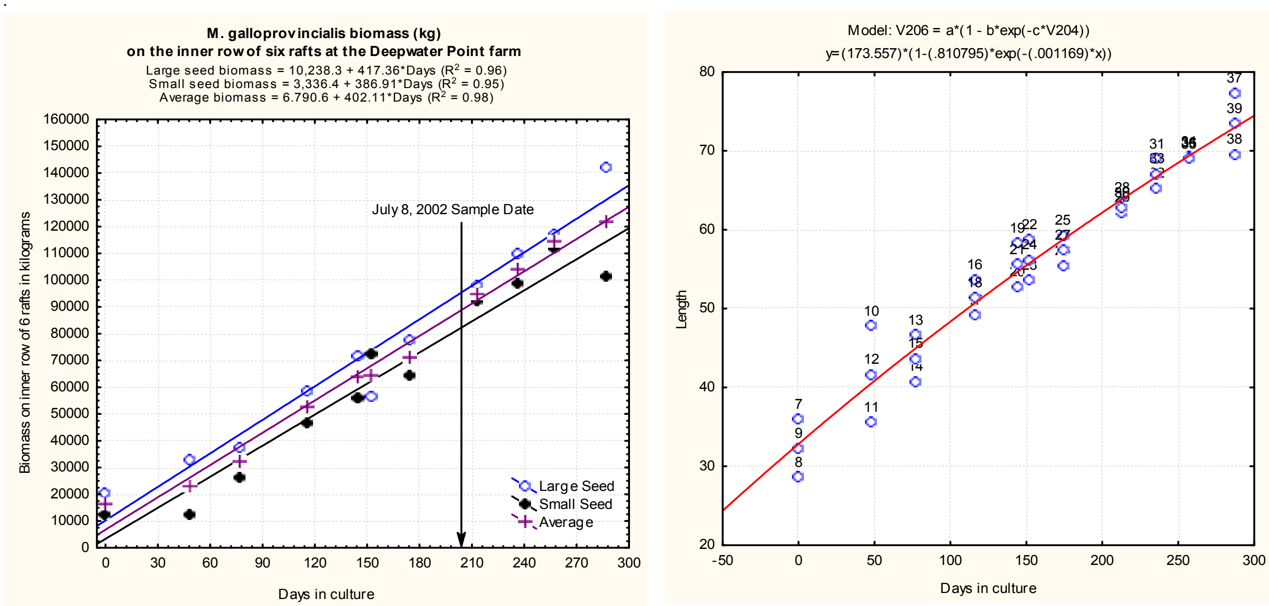
Figure 1. Canister design.

Video record. A cabled Outland Technology™, UWC-160 camera, equipped with a UWL-22 (200 watt) 115 volt light was used to produce the video used in this assessment. The camera and light source were equipped with a 100 meter cable. Figure (2) describes the fixture used in this survey. The distance between the markings on the crossbar was 7.5 cm. The camera was maneuvered at < 30 cm/sec along the transects and differential GPS locations annotated with real and tape time on the field datasheets. The beginning of each transect was annotated by photographing a placard with the site and transect indicated. Real time was converted to tape time keyed to the placard to establish the location of specific segments of video.

Figure 2. Camera fixture used to video benthic conditions in Totten Inlet.

Data analysis. Data was entered into Excel™ spreadsheets for calculation of various indices. Data analysis was accomplished in Statistica™ Version 6. For inferential analyses, proportional data was transformed using Arcsin(Sqrt(proportion)) and count data was normalized using a Ln(N+1) transformation. Unless specifically stated, significance was assessed at $\alpha = 0.05$, which is the probability of rejecting a null hypothesis when it is true.

3.0. Results. The inner row of rafts at Deepwater Point produced 86,193 kg (189,624 pounds) live weight of *Mytilus edulis galloprovincialis* during the 2000 – 2001 production cycle. These mussels were harvested between May 31 and September 5, 2001. Hatchery reared *M. e. galloprovincialis* seed was seeded on the evaluated row of rafts during December 2002 following a three month fallow period (Taylor resources production records). Two size classes were seeded (average valve lengths = 28.7 and 36 mm). They were first measured on February 8, 2002 and last measured on November 22, 2002 just prior to harvest when their average length exceeded 70 mm. Their growth is documented in Figures 3a and 3b, which were constructed using unpublished data provided by the Pacific Shellfish Institute. Shellfish typically grow in a sigmoidal pattern. Slow growth was observed between the first two sample days in late winter 2002. After that, the culture appears to have remained in the exponential growth phase during the entire production cycle suggesting that food was not limiting their growth – even in the late fall just prior to harvest. Note that approximately 90,000 kg of mussels were present on the rafts during the July 8, 2002 survey and there were 138,000 kg present on November 22, 2002. Figure 4 describes the length versus weight of the cultured mussels. Spawning in the population would have been indicated by a marked reduction in individual mussel weights and there is no indication of either spawning or other physiological stress in this data. These data indicate that the population grew rapidly during the entire production cycle – suggesting an adequate food supply – even during the winter months when chlorophyll *a* concentrations were reduced from summer concentrations..



a) Biomass of mussels on Row 1

b) Length versus days in culture on Row 1

Figure 3. Growth of *Mytilus edulis galloprovincialis* at the Deepwater Point farm in Totten Inlet, Washington State.

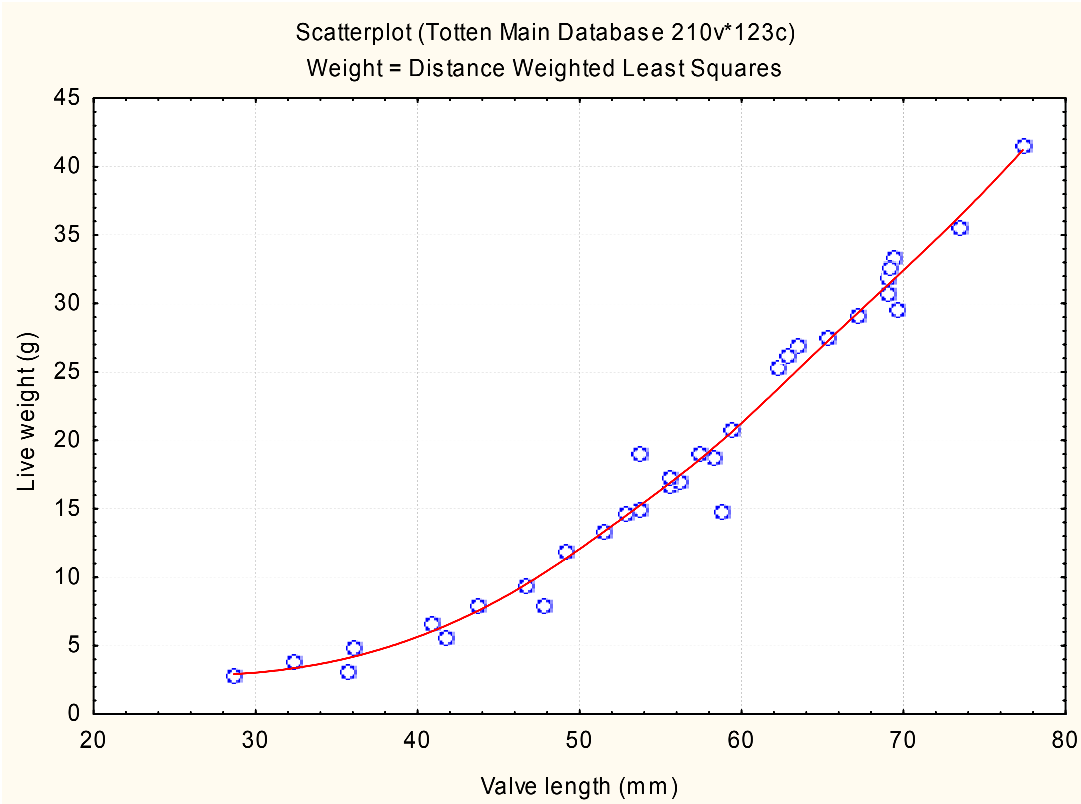


Figure 4. Mean lengths (mm) and weights (g) of cultured mussels at the Deepwater Point mussel farm in Totten Inlet, Washington State.

3.1. Sample station location. Figure 5 describes the location of the Totten Inlet mussel farm and sample stations recorded using dGPS on July 8, 2002. The reference location is 1,120 m to the north of the mussel farm on the western shore of Totten Inlet in water of similar depth. The latitude and longitude of each sample station is provided in Appendix (1).

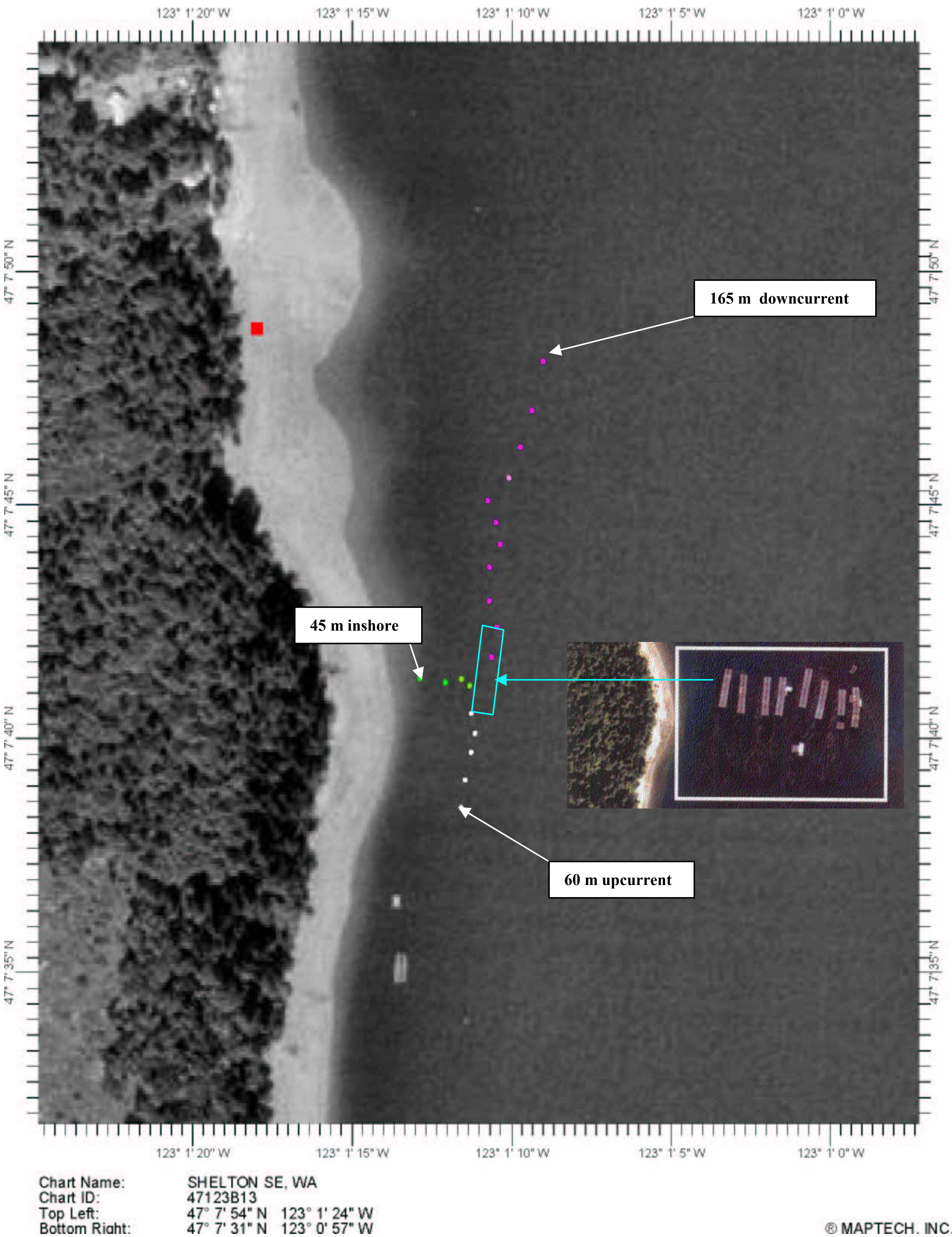


Figure 5. Location of sample stations recorded using dGPS during the July 8, 2002 survey at Deepwater Point, Totten Inlet, Washington State.

3.2. Sediment physicochemistry at the Deepwater Point mussel farm.

Sediment grain size distribution (SGS). The results of the July 8, 2002 survey are provided in Figure 6. The proportion fines were relatively low (25.4 to 38.6% under and on the perimeter of the rafts. Sediments were more coarse at shallower depths inshore and upcurrent from the farm. Sediments on the inshore and upcurrent transects were dominated by gravel (30.2 to 55.8% gravel) and sand (13.6 to 51.4%). Most of the sampling was conducted on the northerly (downcurrent) transect where sediments were dominated by silt and clay, which represented 59.9 to 91.2% of the sediment matrix. The reference station was chosen to be representative of the proportion fines on the downcurrent transect. Sediment under the rafts were significantly coarser than those found at the reference station (t = -11.4. p = 0.0003) but stations on the downcurrent (030 ^{o}M) transect were not significantly different (t = -1.5, p = 0.145). The sediment grain size indicates that the Deepwater Point farm is located in a transitional area with fine-grained sediment from the rafts' northern perimeter to the north and coarser grained sediments under the farm and to the south or inshore. Differences in this habitat metric are large enough to influence resident macrobenthic communities. However, the fine-grained habitat appears relatively homogeneous on the dominantly downcurrent transect, which was the focus of this study.

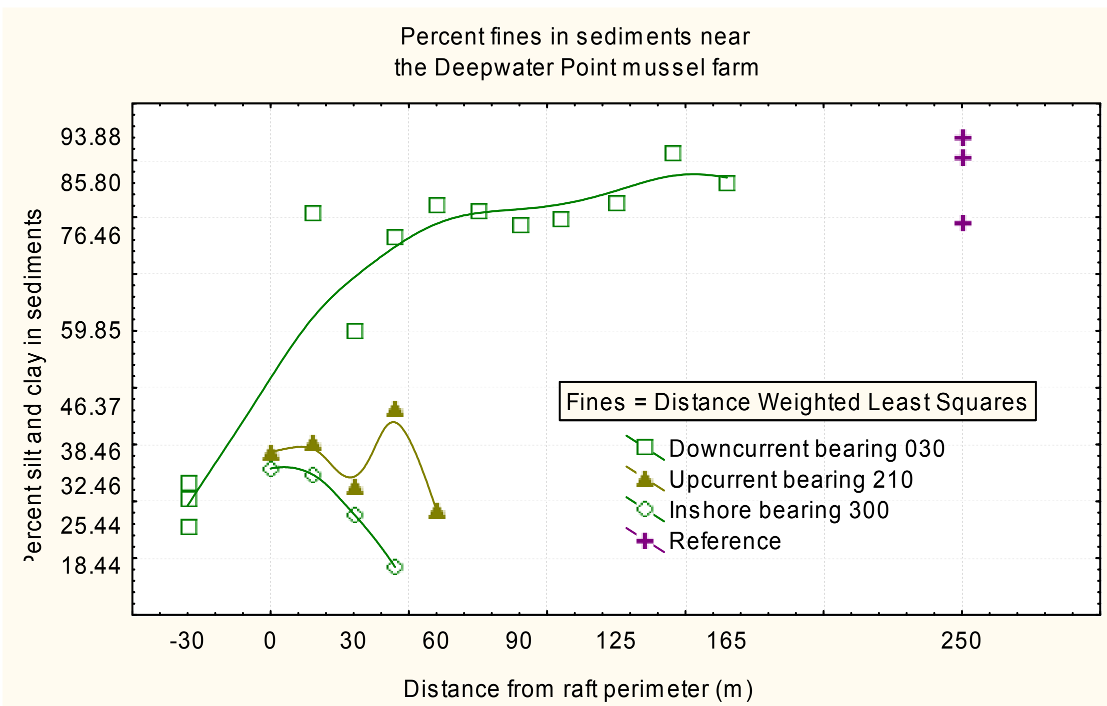


Figure 6. Percent fines (silt and clay) in sediments as a function of distance from the Deepwater Point mussel farm raft perimeters and at the center of the rafts.

Canister study results. Canisters were located at -30 m (raft center) and at distances of 0 (perimeter), 60, 120 and 180 m on the dominantly downcurrent northern transect. The reference canister was located 1,120 m north of the farm. For ease of display, the reference location is displayed at 250 m distance in the following graphs and tables. The results are summarized in Table 2. Reference location deposition rates were subtracted from the treatment rates to construct Figures 6 for TSS and Figure 7 for TVS.

Table 2. Total suspended and total volatile solids deposition rates determined using canisters at the North Totten (NT) and Deepwater Point (DW) sites between March 27, 2002 and June 2, 2003.

Date In	Location	Distance (m)	Treatment Treatment or Control	Days Deployed	Total Solids ($g\ TSS/m^2$-d)	Percent TVS	TVS Deposition ($g\ TVS/m^2$-d)
3/22/2002	DW	-30	T	36	240.59	0.116	27.98
3/22/2002	DW	0	T	36	196.90	0.110	21.66
3/22/2002	DW	60	T	36	195.20	0.108	21.04
3/22/2002	DW	120	T	36	206.03	0.112	23.08
3/22/2002	DW	180	T	36	212.04	0.111	23.54
3/22/2002	DW	250	C	36	135.80	0.115	15.56
3/22/2002	NT	250	C	36	87.02	0.118	10.22
4/27/2002	DW	-30	T	32	331.40	0.110	36.59
4/27/2002	DW	0	T	32	259.18	0.110	28.61
4/27/2002	DW	60	T	32	268.92	0.127	34.02
4/27/2002	DW	180	T	32	309.49	0.117	36.06
4/27/2002	NT	250	C	32	214.18	0.114	24.39
5/29/2002	DW	-30	T	22	391.58	0.149	58.38
5/29/2002	DW	0	T	22	329.82	0.129	42.38
5/29/2002	DW	60	T	22	349.44	0.124	43.33
5/29/2002	DW	120	T	22	258.05	0.114	29.39
5/29/2002	DW	180	T	22	307.41	0.155	47.71
5/29/2002	DW	250	C	22	321.15	0.124	39.92
5/29/2002	NT	250	C	22	127.14	0.148	18.77
6/20/2002	DW	-30	T	32	406.29	0.117	47.66
6/20/2002	DW	0	T	32	344.00	0.112	38.39
6/20/2002	DW	60	T	32	349.68	0.110	38.47
6/20/2002	DW	120	T	32	354.31	0.100	35.29
6/20/2002	DW	180	T	32	239.81	0.128	30.70
6/20/2002	DW	250	C	32	464.49	0.126	58.43
6/20/2002	NT	250	C	32	134.35	0.122	16.40
7/22/2002	DW	-30	T	36	778.25	0.167	129.58
7/22/2002	DW	0	T	36	698.82	0.124	86.44
7/22/2002	DW	60	T	36	605.66	0.141	85.40
7/22/2002	DW	120	T	36	642.58	0.111	71.39
7/22/2002	DW	180	T	36	520.81	0.141	73.49
7/22/2002	DW	250	C	36	516.37	0.141	73.02
7/22/2002	NT	250	C	36	300.92	0.105	31.51
8/27/2002	DW	-30	T	43	599.30	0.112	67.18
8/27/2002	DW	0	T	43	590.86	0.111	65.41
8/27/2002	DW	60	T	43	248.37	0.101	25.06
8/27/2002	DW	120	T	43	356.60	0.114	40.62
8/27/2002	DW	180	T	43	335.75	0.134	44.82
8/27/2002	DW	250	C	43	330.40	0.148	48.80
8/27/2002	NT	250	C	43	166.76	0.137	22.80
10/9/2002	DW	-30	T	42	658.92	0.162	106.48
10/9/2002	DW	0	T	42	417.43	0.168	70.04
10/9/2002	DW	60	T	42	368.06	0.138	50.72
10/9/2002	DW	120	T	42	438.72	0.091	39.84
10/9/2002	DW	180	T	42	250.82	0.117	29.30
10/9/2002	DW	250	C	42	262.29	0.098	25.63
10/9/2002	NT	250	C	42	117.07	0.108	12.69
11/20/2002	NT	250	C	55	67.34	0.083	5.60
1/14/2003	NT	250	C	50	48.86	0.091	4.44
3/5/2003	NT	250	C	36	85.70	0.161	13.82
4/10/2003	NT	250	C	53	191.67	0.102	19.63

Deposition of TSS. Differences in the deposition of total suspended solids (TSS) between farm and reference stations are provided in Figure 7. The zero (no difference line is highlighted in blue. Large increases in solids deposition above those observed at the reference location were not recorded until Day 218 of the production cycle. Rates under the rafts on and after that date were higher than reference rates by 261.9 to 396.6 g/m^2-day. Increased TVS was apparent to a distance of 120 m during the last five months of the production cycle. The total solids includes undigested calcium carbonate and silicon dioxide tests from phytoplankton and particulate inorganic matter filtered from the water and rejected as pseudofeces at the labial palps. The large negative deposition rate on Day 186 is unexplained, but was likely due to an unusual localized high deposition rate at the reference location.

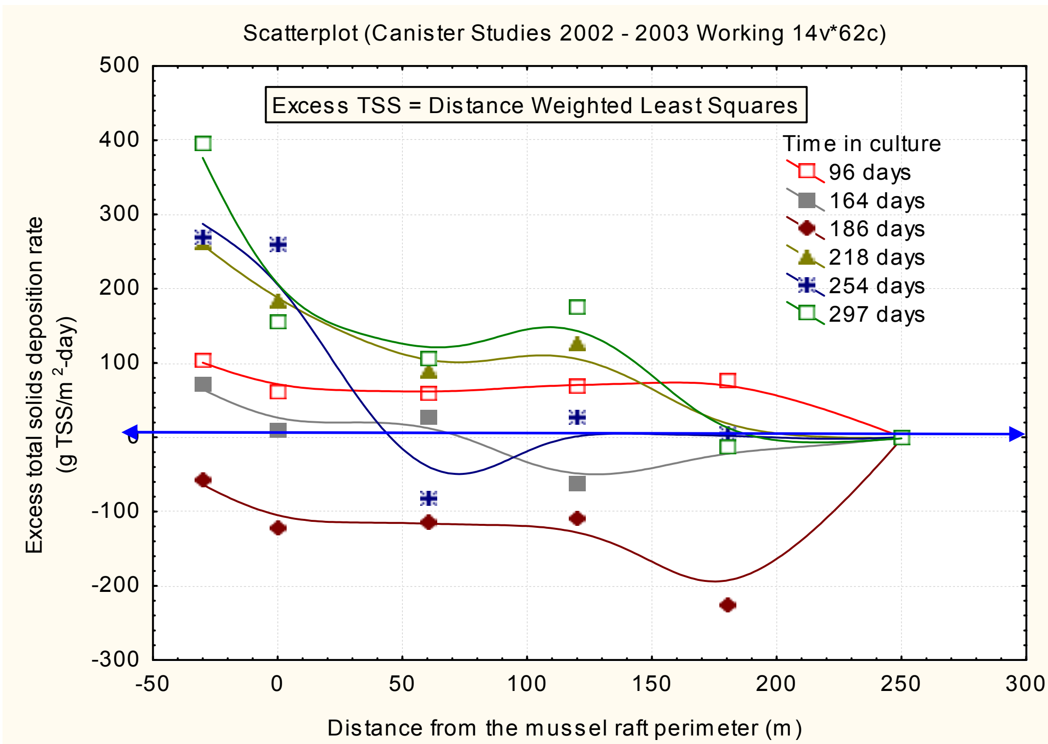


Figure 7. Total suspended solids deposition rates (mg TSS/m^2-day) above (or below) reference deposition rates determined using canisters located downcurrent from the Deepwater Point mussel farm in 2002. The reference location is 1.12 km from the farm, but was plotted at 250 m to avoid presenting the ordinate on a logarithmic scale.

Deposition of TVS (organic) matter. The negative values seen in Figure 7 for TSS at nearfield stations prior to day 218 were also present in the TVS data. They were likely associated with higher deposition rates at the reference station located 1.12 km from the farm in comparison with the near field stations. To correct for this, the data was normalized to 180 m distance. This is reasonable in that the literature review found that benthic effects are generally restricted to the area immediately around raft cultured mussels and significantly elevated TVS would not be expected at a distance of 180 m. To facilitate the regression analysis, the zero station was established at the center of the row of rafts rather than on the perimeter of the downcurrent raft. The results are provided in Figure 8. On and after 218 days of culture, when the mussel biomass reached 98,331 kg, there is evidence of increased TSS and TVS deposition associated with the culture. Deposition rates peaked at the maximum biomass of 154,581 pounds recorded on October 9 2002 following 297 days in culture. Excess deposition rates were recorded to distances of between 60 and 120 m during the 218 day sample and to approximately 180 m on day 297.

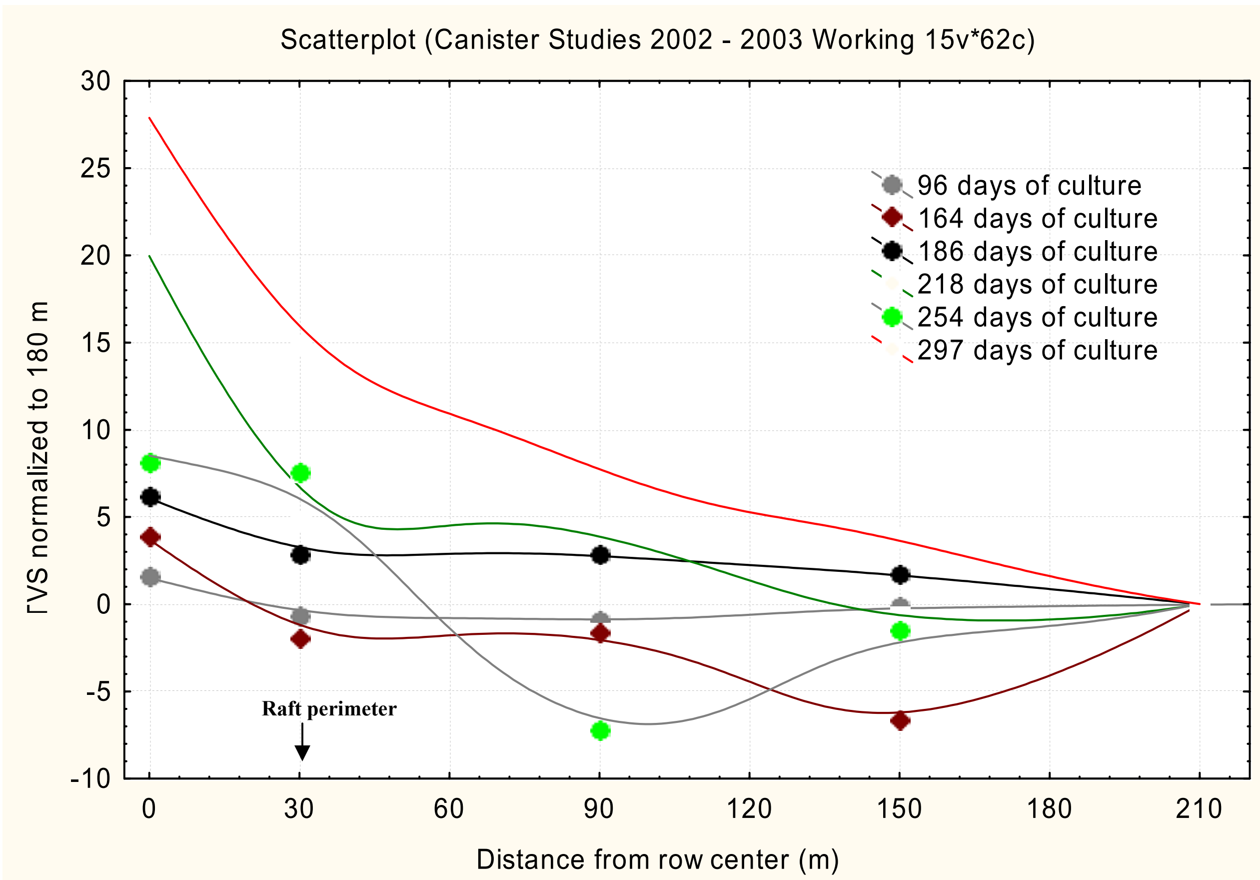
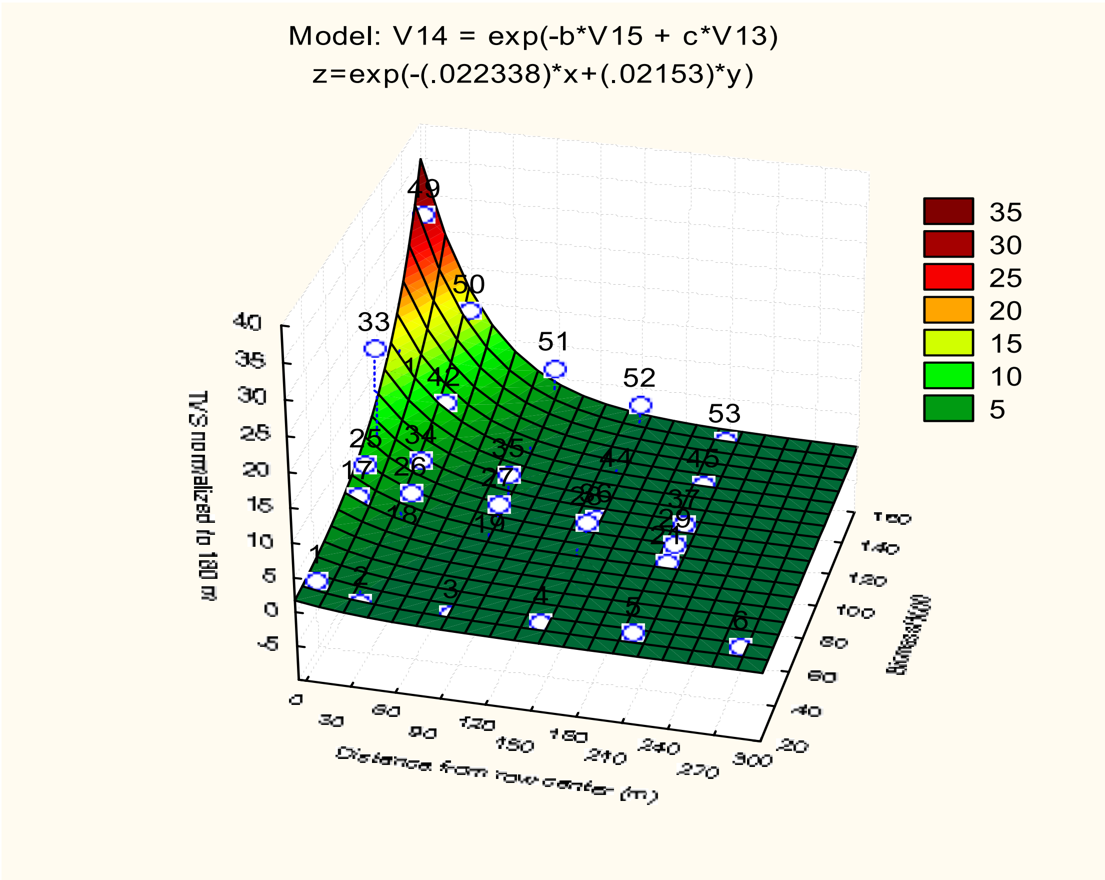


Figure 8. Excess TVS above that recorded at the Deepwater Point 180 m station by date and distance from the mussel farm's center on the downcurrent transect in 2002.

Multi-dimensional non-linear regression analysis was used to describe the 180 m normalized canister results with distance from the center of the row of rafts and mussel biomass as independent variables. The results of an exponential model explaining 74% of the variation in the database are summarized in Figure 9. The coefficients for distance (V15) and mussel biomass (V13) were both significant. The results indicate that TVS deposition rates were not elevated during the early stages of the growout, but that they increased exponentially with the mussel's biomass. They were highest near the farm and decreased exponentially with distance. However, at peak biomass, elevated TVS was observed to 150 m distance from the center of the rafts (120 m from the downcurrent perimeter). The regression model is valid only under the culture and hydrodynamic conditions observed in 2002 at the Deepwater Point farm.

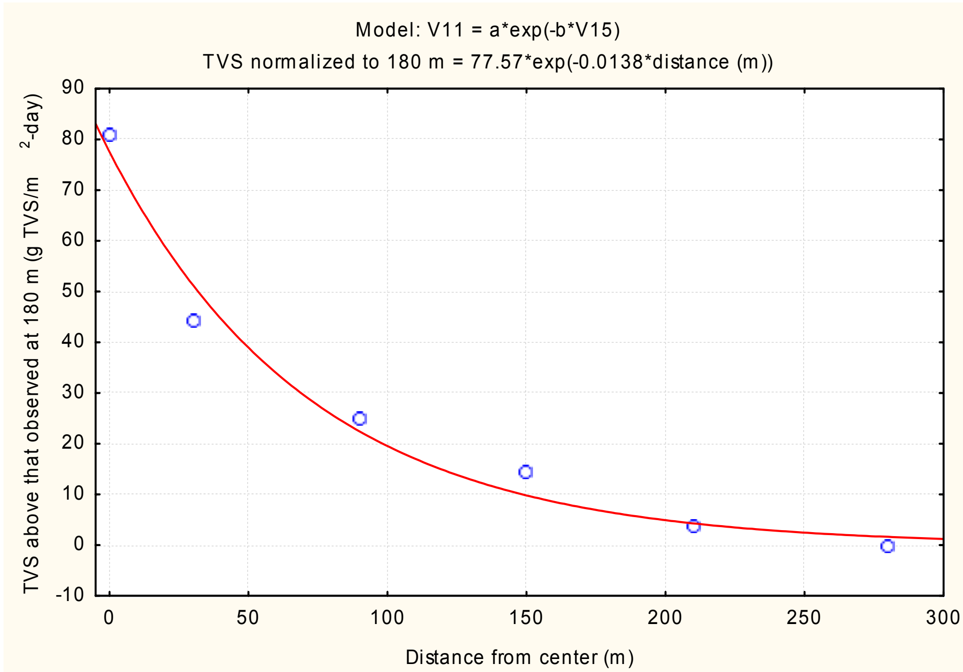
$$\text{Excess TVS (g TVS/m}^2\text{-day)} = \exp^{(-0.0223*\text{distance (m)} + 0.0215*\text{biomass of cultured mussels})}$$



	Model is: V14 = exp(-b*V15 + c*V13) (Canister Studies 2002 - 2003 Working) Dep. Var. : TVS normalized to 180 m Level of confidence: 95.0% (alpha=0.050) R^2_a = 0.74					
	Estimate	Standard error	t-value df = 29	p-level	Lo. Conf Limit	Up. Conf Limit
b	0.022	0.006	3.735	0.001	0.010	0.035
c	0.022	0.001	28.621	0.000	0.020	0.023

Figure 9. Non-linear regression model describing TVS normalized to the values observed at a distance of 180 m from the Deepwater Point raft's downcurrent perimeter during 2002.

The sensitivity achievable using this canister system is seen in Figure 10 describing TVS excess deposition above reference conditions as a function of distance from the center of the row of evaluated rafts at Deepwater Point. These data were collected between October 9 and November 23, 2002 just prior to commencing harvest of the inner row of cultured mussels. The data has been normalized to the TVS deposition rate observed at the mid Totten Inlet reference location during the same period of time. The exponential model described 98.1% of the variation in the data during that period and describes a deposition rate of 77.6 g TVS/m^2-day in the center of the rafts. The model predicts a rate of 51.3 g TVS/m^2-day on the downcurrent perimeter of the farm with further exponential declines to 4.3 g TVS/m^2-day at 180 m distance from the raft's perimeter (210 m from the center of the row of rafts). Thus, while previous studies have concluded that benthic effects associated with mussel farming are restricted to the area beneath the rafts, these canister results suggest that a peak biomass, excess TVS deposition rates occur to distances of 150 to 180 m from the raft's perimeter.



	Model is: V11 = a*exp(-b*V15) (Canister Studies 2002 - 2003 Working) Dep. Var. : Excess TVS Level of confidence: 95.0% (alpha=0.050)					
	Estimate	Standard error	t-value df = 4	p-level	Lo. Conf Limit	Up. Conf Limit
a	77.573	4.297	18.052	0.000	65.643	89.504
b	0.014	0.002	8.021	0.001	0.009	0.019

Figure 9. Excess TVS above that observed in the 180 m canister at the Deepwater Point mussel farm. Distances are measured from the center of the line of rafts. The 30 m station is on the downcurrent perimeter of the row. R^2_a for the regression equaled 0.981.

Accumulation of TVS in Deepwater Point sediments. Sediment TVS as a function of distance from the raft's perimeter on the downcurrent transect and days of culture for the last four sampling days is summarized in Figure 10. Increases in sediment TVS are apparent only on the last sampling day when the percent TVS is sediments was above 8 percent in the near field – even in the erosional environment under the mussel rafts. Changes in TVS at the reference location are most likely associated with season. The lowest percent sediment TVS was recorded in May, 2002 (Day 165) and the highest in July, 2002 (Day 205). Large changes in sediment TVS as a function of culture day were not observed within 205 meters of the farm except at the end of the study (Day 320) when the mussels were about to be harvested.

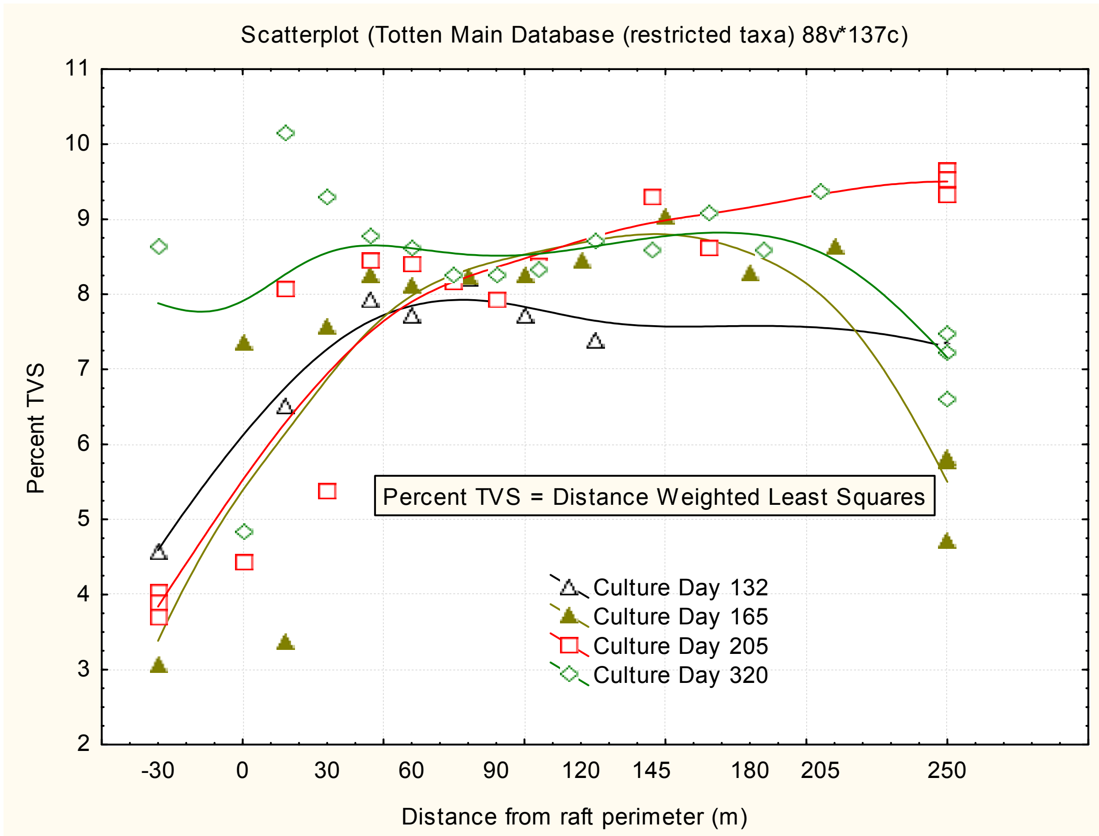


Figure 10. Percent TVS in sediments downcurrent from the northern perimeter of the Deepwater Point mussel rafts as a function of sample day.

Free sediment sulfides near the Deepwater Point mussel farm. The data in Figure 11 is consistent with the TVS results in demonstrating greatest sediment chemical change occurring in the last two sample periods when the mussels were near harvest. Subtle increases in free sediment sulfides were first apparent on Day 165. Moderate increases were observed at distances less than 15 m on July 8, 2002 and on October 31, 2002, large increases in sulfide were observed under the rafts and these increases extended to a distance between 60 and 75 m. The sulfide concentrations observed under the rafts on July 8 (1,571 to 2,620 μM) would have excluded many sensitive taxa and the three replicates collected under the center of the raft on October 31, 2002 (12,800, 13,300 and 15,300 μM) were sufficiently high to exclude most infauna (Brooks, 2001a). Their effects on epifauna attached to, but respiring above, the sediment surface are unknown.

Summary of physicochemical data at the Deepwater Point mussel farm. The area upcurrent (to the south) and under the mussel rafts was erosional and as will be seen in the video section of this report, it contained large amounts of gravel and cobble. Sediments quickly became dominated by fines (silt and clay) from the raft's perimeter to the north along the dominantly downcurrent transect. This appears to be a natural feature of Deepwater Point. Otherwise, fine grained sediments would be found on both the upcurrent and downcurrent transects with only the area under the rafts scoured. However, the relatively shallow water under this inner row of mussel rafts may create a Venturi effect resulting in increased current speeds and erosion of the bottom. Little change in sediment TVS was observed during most of the production cycle. However, observable increases were detected during the last sample period just prior to harvesting the crop of mussels. Sediment concentrations of free sulfides responded quickly and reached concentrations at which community effects could be anticipated within 60 m of the rafts on and after 165 days of culture.

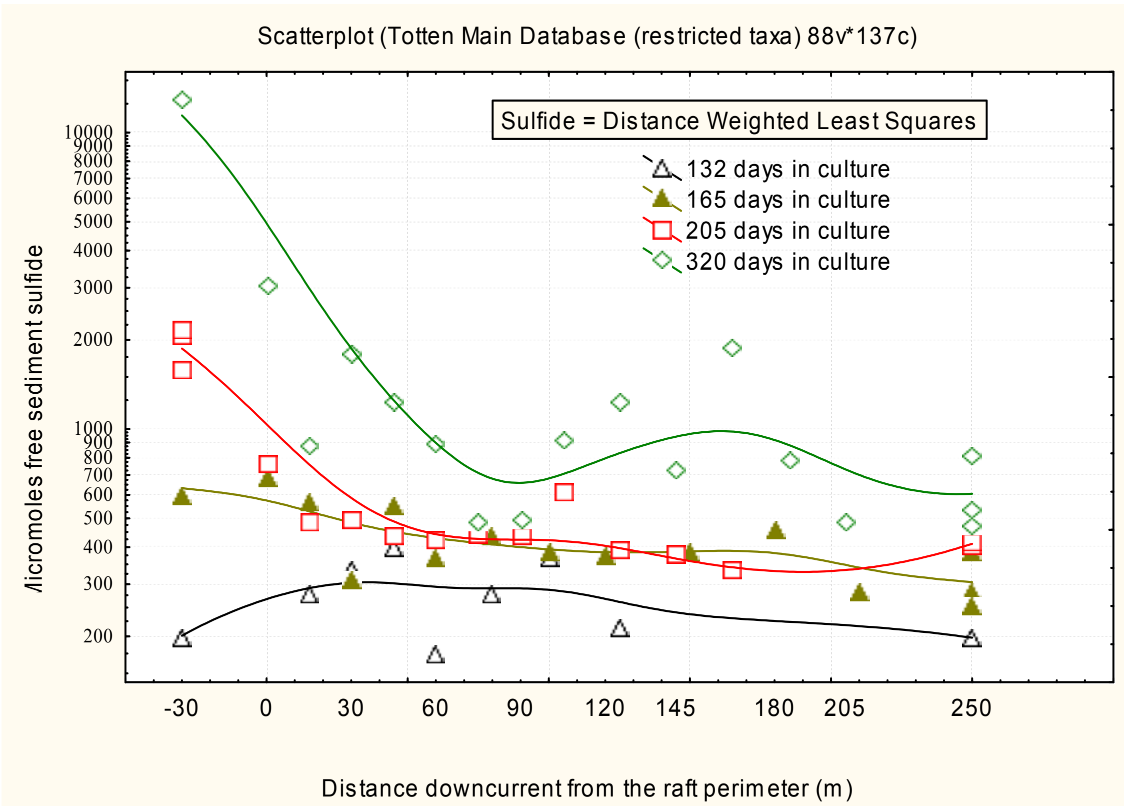


Figure 10. Free sediment sulfides (µM) in sediments located downcurrent (north) of the Deepwater Point mussel farm as a function of sample day.

3.3. Physicochemical characteristics at the Gallagher Cove mussel farm. A total of 225,644 kg of mussels were harvest from this farm in January, February and March of 2003, beginning approximately one month after this survey. A single set of samples was collected from the center of the downcurrent raft and at distances of 0, 15, 30, 45, 60, 75, 90, 105 and at 125 m on the downcurrent (250 ^{o}M) transect and at a local reference location on November 22, 2002. Water depths at this shallow site varied between 26.5 and 32.0 feet at the time of collecting the samples. The sediment grain size distribution was dominated by sand (20.8 to 61.03%) and fines (36.5 to 75.6 percent). Sediment TVS was 7.6% under the center of the mussel rafts. It declined to 5.8% on the perimeter of the rafts and remained in a range of 4.8 to 6.3% to the end of the downcurrent transect. Reference location TVS was 6.2%. Sulfide concentrations declined from 414 µM under the center of the rafts to 252 µM at 45 m downcurrent. Sediment sulfide concentrations from there to the reference location varied between 112 and 211 µM. Physicochemical effects in the sediments were minor and no significant effects in the macrobenthic community could be predicted as a result of this farm.

3.4. Macrofaunal community response.

Reference location community characterization. Twenty-one (21) taxa were observed at the Deepwater Point reference station in three replicate grabs. The mean number of taxa per sample was 20 and the mean abundance was 181 animals/0.1 m^2 grab or 1,810/m^2. Both of these values were within the range reported in WDOE (2004) for PSAMP LT 102R and MSMPNOAA 10-2-234. Brooks (2005a) reported enriched sediment conditions in this area of Totten Inlet with TVS ranging between 8.5 and 10% and free sulfide concentrations (measured at the end of October, 2002) of 100 to 250 μM. These enriched conditions are reflected in the reference area macrobenthic community, which is summarized in Table 3. These 9 taxa were found in an abundance $\geq$ 1.0% of the total reference abundance and they represented 61% of the animals retrieved from reference station sediments. Shannon's index was moderately high in two of the samples (2.318 and 2.340) and low in the third sample (1.335), which was dominated by *Nephtys cornuta* (66%) and unidentified *Nephtys sp.* (prostomia only) represented another 13%. All of the reference station samples were dominated by annelids (67 to 85% of total) and bivalves (12 to 21%). Few gastropods, arthropods or other phyla were observed in the samples. The annelids *Nephtys cornuta, Paraprionospio pinnata* and *Sigambra tentaculata* are all associated with enriched sediments as are the mollusks *Macoma nasuta* and *Alvania compacta.* The physicochemical data and the macrobenthic community at the Deepwater Point reference location (Brooks, 2005a) is consistent with the evidence presented in this report indicating that sediment in the inner reaches of Totten Inlet are organically enriched to the point where the benthic community was affected.

Table 3. Taxa representing $\geq$ 1.0% of the total abundance of macrobenthic organisms collected in three 0.1 m^2 grabs at the Deepwater Point reference location on July 8, 2002. Abundance of each taxon is the mean of three grab samples.

Taxon	Mean abundance	Percent of total abundance
Nephtys cornuta	79	30.4
Nephtys species	21	8.1
Paraprionospio pinnata	16	6.2
Psephidia lordi	15	5.8
Macoma nasuta	10	3.9
Laonice pugettensis	6	2.3
Alvania compacta	5	1.9
Sigambra tentaculata	3	1.2
Tharyx secundus	3	1.2

Benthic community under the mussel rafts. Thirty-two (32) taxa were identified under the rafts. The three replicate samples yielded a mean of 16 taxa and 74.7 animals/0.1 m^2. Twelve taxa were found in an abundance $\geq$ 1.0% of the total and they accounted for 84.8% of the animals retrieved in the samples. These are listed in order of decreasing abundance in Table 4 along with the percent they represented of the total. The most abundant animals were sea anemones (*Thuiaria cf. thuja*) that are filter feeders attached to hard substrates and that feed above the sediments. The infaunal annelids *Dorvillea annulata* and *Nephtys cornuta* are tolerant of sulfides as are the surface feeding gastropods *Nassarius perpingis and Alia gausapata. Dorvillea annulata* has been observed proliferating in sediments containing as much as 15,800 μM $S^{=}$. The barnacle, *Chthamalus dalli* is a surface filter feeder found attached to shells and rocks under the farm. Unless water in the benthic boundary layer is depleted of oxygen or contains toxic levels of either hydrogen sulfide or ammonia, the dominant surface filter feeding animals would not be affected by the apparent enrichment. Shannon's index was moderate to high under the rafts (1.84 to 2.41) and not significantly different from that observed at the reference location (t = 0.41; p = 0.70). The mean number of taxa (16/sample) was not significantly different from that found at the reference location (t = -1.11; p = 0.328). However, the mean abundance (74.7.3/0.1 m^2) was significantly less (t = -3.74; p = 0.020) than observed at the reference location (181/0.1 m^2). Thus, significant differences were not found in the number of taxa or Shannon's Index when comparing the reference location with those stations located under the farm. However, there were fewer macrofauna under the farm in comparison with the reference community. The video record, to be described in a separate section of this report, revealed an enhanced megafaunal community under the rafts in comparison with any of the areas documented north of the raft's perimeter or at the reference location.

Table 4. Taxa representing ≥ 1.0% of the total abundance of macrobenthic organisms collected in three 0.1 m^2 grabs from the center of the northernmost inshore raft in the Deepwater Point mussel farm on July 8, 2002. Abundance of each taxon is the mean of three grab samples.

Taxon	*Mean abundance*	*Percent of total*
Thuiaria cf. thuja	19	25.9
Chthamalus dalli	14	18.8
Nassarius perpingis	6	8.0
Goniada maculate	5	6.6
Armandia brevis	4	5.4
Foraminifera	4	5.4
Megalopae or zoea	3	4.0
Dorvillea annulata	2	2.7
Paleanotus cf. occidentale	2	2.7
Modiolus or *Mytilus sp. juv.*	2	2.7
Alia gausapata	1	1.3
Nephtys cornuta	1	1.3

Benthic community inshore and upcurrent from the farm. The macrobenthic community observed upstream and inshore, in areas containing coarser sediments, was similar but more diverse (28 to 51 taxa/0.1 m^2) and more abundant (317 animals/0.1 m^2) than found under the farm or at the reference location. Shannon's Index was high (2.4 to 3.4) for all of these stations. The 17 taxa listed in Table 5 comprised 93.7% of the total. The macrobenthic community was dominated by filter feeders (*Chthamalus dalli* and *Modiolus or Mytilus juveniles*) and sulfide tolerant annelids and gastropods. *Nephtys cornuta* appears to be ubiquitous throughout much of the central regions of Totten Inlet and it is indicative of enriched conditions. However, note that this annelid was found in much lower abundance in the coarse inshore and upcurrent sediments (40/0.1 m^2) than it was at the reference location (79/0.1 m^2).

Table 5. Abundance of taxa representing ≥ 1.0% of the total abundance of macrobenthic organisms collected along the inshore (northwest) and upcurrent (southeast) transects at the Deepwater Point mussel farm on July 8, 2002. Data are abundance/0.1 m^2 grab.

Taxon	*Abundance/0.1 m^2*	*Percent of total abundance*
Chthamalus dalli	49	18.54
Nephtys cornuta	40	15.23
Alvania compacta	36	13.72
Nassarius perpingis	23	8.72
Modiolus or Mytilus juv.	9	3.44
Armandia brevis	8	3.15
Amphicteis macronata	8	3.02
Capitella capitata complex	8	2.89
Dorvillea annulata	7	2.85
Paleanotus cf. occidentale	7	2.73
Nephtys sp.	7	2.68
Sigambra tentaculata	6	2.35
Megalopae or zoea	6	2.31
Glycinde picta or armigera	6	2.14
Polydora sp.	5	2.06
Paramoera bousfieldi	5	1.93
Juvenile bivalves	5	1.93
Goniada maculate	5	1.76
Tharyx secundus	3	1.13
Macoma nasuta	3	1.13
Alia gaussapata	3	1.13

Macrofaunal community characterization along the northern (downcurrent) transect. This 165 m long transect has fairly homogeneous and consisted of mostly fine-grained (85 to 92% silt and clay) sediments beyond the northern perimeter of the mussel rafts (Figure 6). The increased sulfide concentrations observed in a continuous gradient along this transect were of sufficient magnitude within the farm's footprint to influence the macrobenthic community (Figure 10). A total 2,487 macrofaunal organisms and 64 taxa were identified in the 11 samples collected along the downcurrent transect. The 14 taxa found in abundance ≥ 1.0% of the total abundance at these 11 stations (see Figure 5) are summarized in Table 6 and represented 92.3% of the total community abundance. Table 6 also includes a summary by major taxonomic group. Fifty-three percent of the organisms were annelids and 25.4 percent were taxa from phyla other than Annelida, Molluska or Arthropoda. The "Other" category at Deepwater Point was dominated by hydroid polyps in the genus *Garveia.* Few mollusks or arthropods were observed in this enriched environment. *Metacaprella anomala* is an epibenthic amphipod found in high abundance on many aquaculture structures during the spring months. It is found only on muddy benthic environments as allochthonous input and can be used as an indication of the distance to which small organic material may be dispersed. In this case 88 *M. anomala* were found in a single sample located 105 m from the northern perimeter of the rafts.

Table 6. Taxa representing ≥ 1.0% of the total abundance of macrobenthic organisms collected in 0.1 m^2 grabs along the northern (downcurrent) transect at the Deepwater Point mussel farm on July 8, 2002. Abundance is the mean for 11 grabs.

Taxon	*Mean abundance/0.1 m^2*	*Percent of total abundance*
Nephtys cornuta	746	30.0
Hydroid polyps (*cf. Garveia sp.*)	600	24.1
Paraprionospio pinnata	226	9.1
Megalopae or zoea	193	7.8
Nephtys sp.	133	5.3
Metacaprella anomala	88	3.5
Psephidia lordi	67	2.7
Goniada maculate	46	1.8
Cirratulidae sp.	43	1.7
Nassarius perpingis	40	1.6
Tharyx secundus	34	1.4
Macoma sp. (pairs of siphons)	32	1.3
Macoma nasuta	24	1.0
Sigambra tentaculata	23	0.9
Annelids	1,318	53.0
Bivalves	147	5.9
Gastropods	70	2.8
Arthropods	320	12.9
Other	632	25.4
Total	2,487	100.0

The most dominant taxon was *Nephtys cornuta*, which is an indicator of enriched benthic environments that has been observed throughout the inner portions of Totten Inlet. *Paraprionospio pinnata* is frequently found in sediments enriched with natural, semi-refractory, organic matter such as macroalgae detritus. It has not been observed proliferating in high sulfide environments and its presence suggests that these downcurrent sediments remain relatively healthy. All of the other annelids and the bivalves and gastropods found here are tolerant of low to moderate sulfide concentrations of at least a few hundred micromoles, but have not been observed in enriched sediments near salmon farms at the peak of production (Brooks 2001a).

Macrofaunal community abundance and species richness. Figure 11 describes the abundance and species richness observed along a northerly transect from the Deepwater Point mussel farm. The reference station, located 1.12 km north of the farm is displayed at 250 m distance to facilitate viewing. Macrofaunal abundance was low under the rafts. The results of *t-tests* comparing the under raft (-30 m) stations with the reference location are provided in Table 7, which indicates that macrofaunal abundance was lower under the rafts ($t = -3.74$; p = 0.020. There was an apparent increase in abundance above reference values at distances between 45 and 145 m. However, the community was patchy; no statistically significant clines were observed; and all of the values were within the reference ranges reported by WDOE (1996). The number of taxa was steady along the transect ranging between 13 and 23. Linear regression analysis indicated that only the intercept (mean) was significant with respect to species richness supporting the lack of an effect on this endpoint.

Shannon's Index is also displayed in Figure 12. Excepting the small values of 1.48) at 30 m and 1.33 at the reference location, the range was small (1.84 to 2.52) near the farm. Both of the low Shannon's Index values were associated with domination by the annelid *Nephtys sp.*, which represented 131 of the 165 (79%) of the total abundance at the reference location and 128 of 220 (58%) at the 30 m station.

Abundance of annelids and bivalves. The abundance of this phylum and class is described in Figure 13 as a function of distance from the northern raft perimeter. Table 7 indicates that significantly fewer annelids or bivalves were observed under the rafts than were observed at the reference location ($t = -5.909$; p = 0.004; and $t = -3.476$; p = 0.025 respectively). For those stations located on or beyond the perimeter of the northern raft, the coefficient on distance (Table 8) was significant only for bivalves. None of the other coefficients on distance or any of the physicochemical endpoints (independent variables) were significant ($\alpha = 0.05$) using either simple or multiple regression analyses. Therefore, there was a significant increase in the number of bivalves with distance from the farm. But the increase in annelid abundance was not significant.

Abundance of gastropods arthropods and other taxa. The abundance of these taxonomic groupings is summarized in Figure 14. The distribution of gastropods and arthropods was fairly regular, with one high count of *Metacaprella anomala* (88/0.1 m^2) at 105 m distance. In general, there were higher abundances of these taxa near the farm than were retrieved from the reference location. However, regression analysis revealed no significant trends in the data.

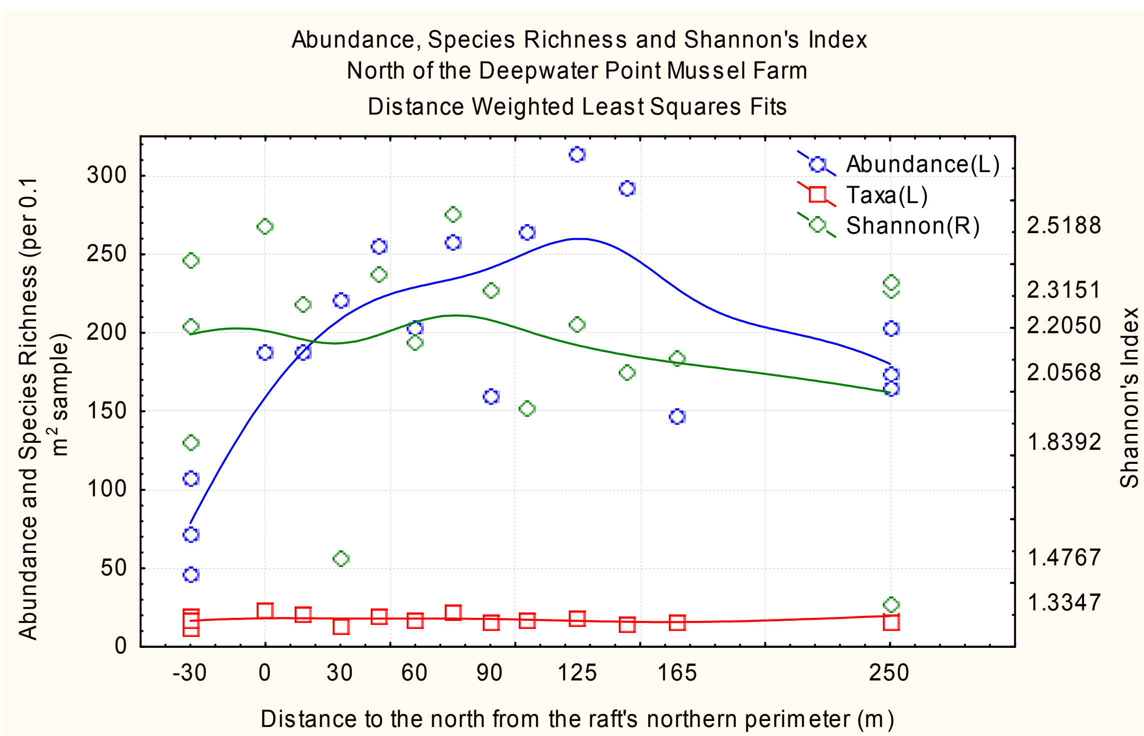


Figure 12. Macrofaunal abundance, species richness and Shannon's Index observed in 0.1 m^2 modified van Veen grab samples collected on the perimeter and north of the Deepwater Point mussel farm on July 8, 2002.

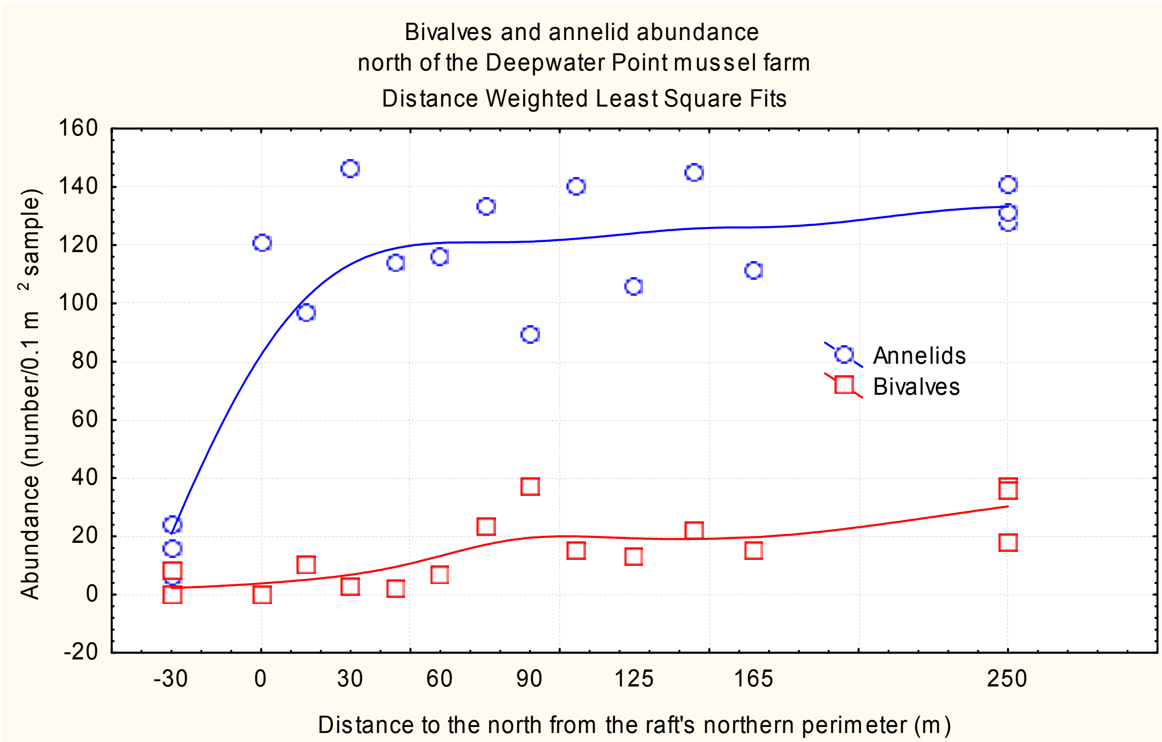


Figure 13. Abundance of annelids and bivalves observed in 0.1 m^2 modified van Veen grab samples collected on the perimeter and north of the Deepwater Point mussel farm on July 8, 2002.

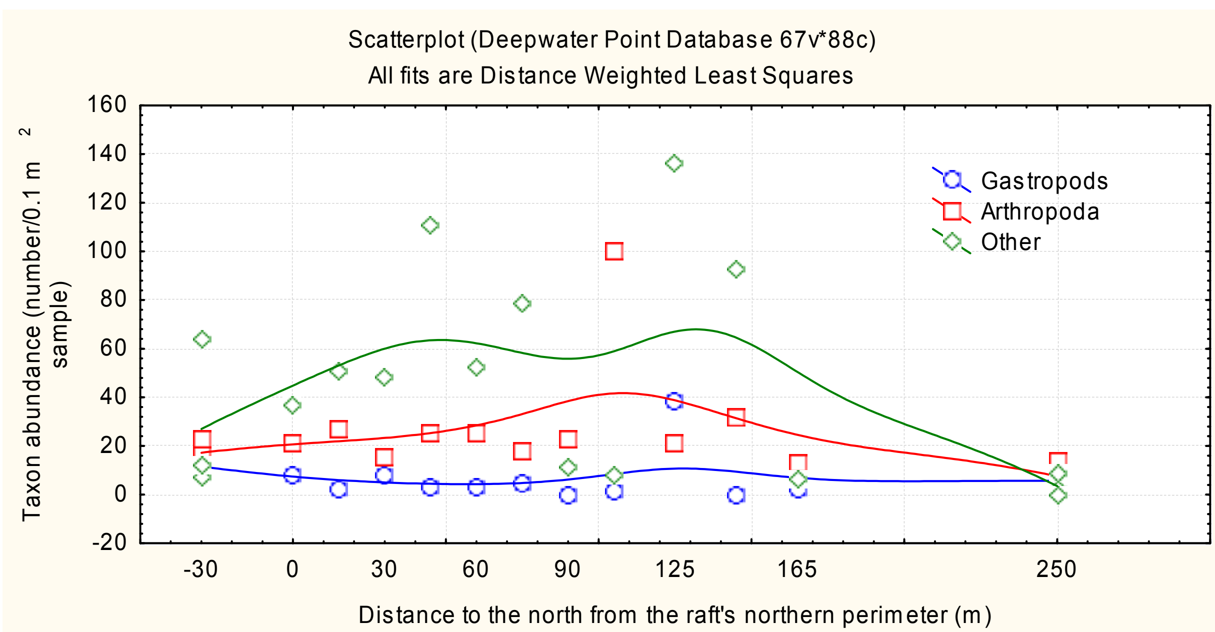


Figure 14. Abundance of gastropods, arthropods and other taxa observed in 0.1 m^2 modified van Veen grab samples collected on the perimeter and north of the Deepwater Point mussel farm on July 8, 2002.

Benthic video recorded on July 17, 2002. Four benthic video transects were recorded on July 17, 2002 (Figure 15). The downcurrent (north) track was 126 m long; the offshore track was terminated at 15 m because of interference with the next row of rafts. The inshore track was 45 m long and the southern (upcurrent) track 75 m in length. A twenty minute video summary is provided in Appendix 2.

Downcurrent transect. Megafauna on the northern perimeter of the rafts were dominated by several species of anemones and *Cancer sp.* crabs. Sediments were predominantly silt and clay at distances beyond 15 meters from the raft's perimeter. The sediments became increasingly soft to the north and were very soft at the reference location (1,120 m north of the rafts). Few megafauna were observed beyond 15 meters. However, significant bioturbation of the sediment's surface was apparent at and beyond 45 m. Low densities of benthic diatoms were apparent at most locations. Marine snow (bacterial colonies digesting particulate organic matter or POM) was dense throughout. A small bloom of unidentified scyphozoan medusae, covering perhaps half a hectare at densities of 5 to 10/m^3 was observed in the southern part of Totten Inlet in July and several, apparently moribund, medusae were observed lying on the bottom along the northern transect.

Offshore transect. This transect was firm with numerous large bivalve shells (clams not mussels) together with gravel and cobble. A composting predator net was observed on the bottom covered with anemones, crabs and a large number of sea stars (*Pisaster, Evasterias* and *Pycnopodia.*

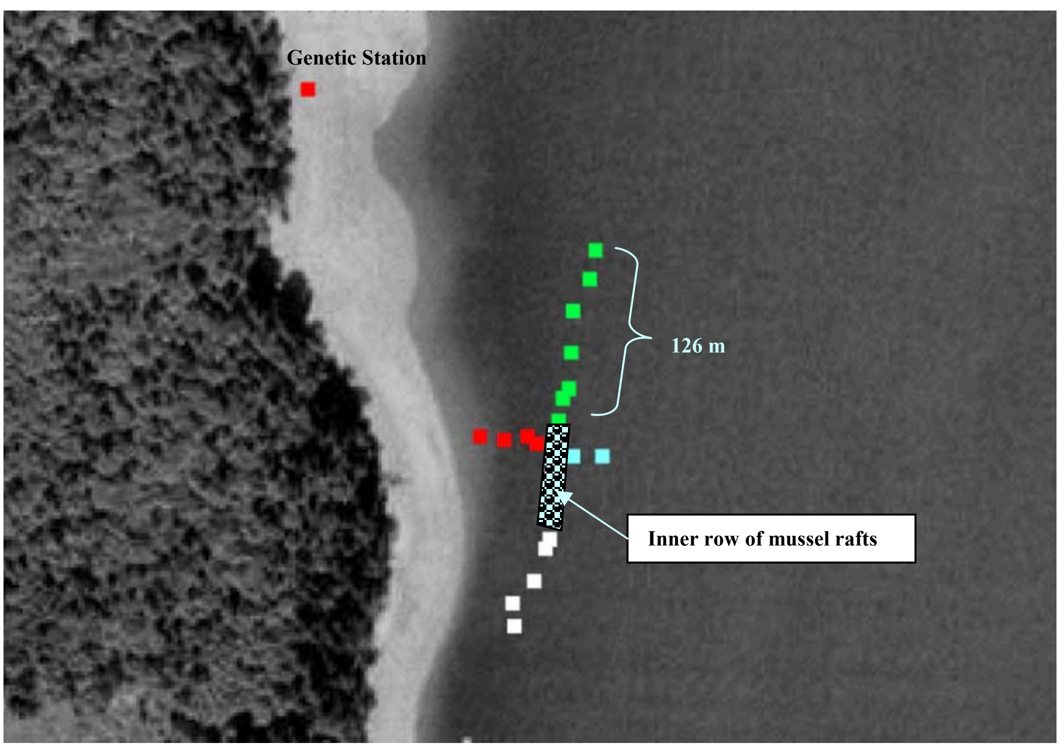


Figure 15. Video tracks describing benthic conditions recorded on July 7, 2002 at the Deepwater Point mussel farm.

Upcurrent. Crabs and anemones were the dominant megafauna on this transect. The substrate remained firm with numerous empty clamshells observed on the surface. There was no evidence of accumulations of mussel feces or pseudofeces and surface features (clam shells, cobble and rock) were not smothered by biodeposits or detached mussels from the farm. The sediments appeared bright, with no evidence of anaerobic conditions on the surface such as *Beggiatoa* or dark surficial sediments having low chroma.

Inshore. The inshore transect contained healthy populations of anemones. However, few starfish were observed and the substrate, while covered with clamshells, appeared too firm for extensive bivalve populations. If mussels were to subtidally colonize in Totten Inlet, they would likely be found on these firm substrates at Deepwater Point. No living mussels were observed on the bottom. Dense colonies of benthic diatoms were evident in shallow water at depths less than 6 m along this transect.

Video summary. Filter feeding anemones, particularly the plumose anemone (*Metridium senile*) are frequently observed throughout the Northeast Pacific near aquaculture facilities where they colonize firm substrates. The area under the Deepwater Point farm was no exception and high densities of several species of anemones were observed. The large numbers of starfish and *Cancer sp.* crabs were likely attracted to the area by biodeposits released from the culture. While shell debris is common near naturally occurring or cultured mussels, there was no evidence of any accumulation of shell under the Deepwater Point farm. Attached macroalgae, including nitrophilous *Ulva lactuca* and *U. fenestrata* were not dominant at Deepwater Point – even at shallow depths along the inshore transect. The deepest water surveyed during these recordings was 42.5 feet on the southern perimeter of the line of rafts. However, most of the video was recorded in 30 to 35 feet of water (10 meters). The high turbidity associated with dense algal blooms and high TSS at Deepwater Point appears to have resulted in an extinction coefficient sufficient to limit primary production in as little 5 to 6 m water depth. The result is that benthic diatom production was sparse everywhere except on the inshore transect in water < 6 m deep. As discussed by Brooks (2005b) this observation has implications for the cycling of nitrogen from the sediments. No significant subtidal bivalve populations were observed – nor was any subtidal spawning habitat for herring, etc. observed. Sea whips (*Stylatula spp.* and *Vrigularia spp.*) that were common at the North Totten site in deep water, were absent at the shallow depths found near the Deepwater Point farm.

Inferential tests of biological endpoints. Abundance data was transformed ($Log_{10}(N + 1)$ as were proportional data (Arcsin(Sqrt(proportion))) to reduce expected heteroscedasticity for all inferential tests. *T-tests* were used to assess the significance of differences in macrofaunal community endpoints between the center of raft location (N = 3) and the reference location (N = 3). The results are provided in Table 7. The null hypotheses that the endpoints were equal at the two locations was rejected ($\alpha = 0.05$) for total abundance and the abundance of annelids and bivalves. In each case, higher abundance was observed at the reference location than under the center of the raft complex indicating statistically significant effects on the community's structure.

Table 7. Summary of *t-tests* assessing differences between the abundance of major taxa and species richness observed in triplicate samples collected under the center of the raft array (-30 m) and at the Deepwater Point Reference Location.

Variable	T-tests; Grouping: Distance (Deepwater Point Database) Group 1: -30 Group 2: 250										
	Mean -30	Mean 250	t-value	df	p	Valid N -30	Valid N 250	Std.Dev. -30	Std.Dev. 250	F-ratio Variances	p Variances
Log Abundance	1.854	2.258	-3.744	4	0.020	3.000	3.000	0.181	0.047	15.097	0.124
Log Taxa	1.223	1.309	-1.114	4	0.328	3.000	3.000	0.098	0.091	1.149	0.931
Log10 Annelids	1.158	2.128	-5.909	4	0.004	3.000	3.000	0.283	0.022	169.528	0.012
Log10 Bivalves	0.318	1.476	-3.476	4	0.025	3.000	3.000	0.551	0.171	10.434	0.175
Log10 Gastropods	1.082	0.683	1.506	4	0.207	3.000	3.000	0.166	0.428	6.664	0.261
Log 10 Arthropods	1.246	0.874	1.973	4	0.120	3.000	3.000	0.153	0.288	3.539	0.441
Log 10 Other	1.277	0.492	1.967	4	0.121	3.000	3.000	0.476	0.500	1.103	0.951

The results of simple linear regression for each of the major macrofaunal endpoints as a function of distance from the northern perimeter of the northern raft is summarized in Table 8. The coefficient on distance was significant only for bivalves. Multiple linear regression was then completed using those cases from 0 to 165 m distance with bivalve abundance as the dependent variable and proportion fines, TVS and sulfides as predictors. The intercept was significant, but none of the coefficients on physicochemical variables were significant. Therefore, it is reasonable to conclude that the clines were not a significant result of these physicochemical variables or their interactions. The decreased abundance of bivalves under the rafts may have been associated with increased starfish, crab and gastropod predation near the farm where high densities of these predators were observed, or the coarse sediments under the rafts may have been inimical to these taxa. However, no cause and effect relationships were investigated or determined.

Table 8. Simple linear regression evaluating clines in abundance of major taxonomic metrics from the northern perimeter of the rafts to 165 m north as a function of distance from the raft's perimeter.

Dependnt Variable	Test of SS Whole Model vs. SS Residual (Deepwater Point Database)										
	Multiple R	Multiple R^2	Adjusted R^2	SS Model	df Model	MS Model	SS Residual	df Residual	MS Residual	F	p
Shannon	0.149	0.022	-0.086	0.020	1.000	0.020	0.871	9.000	0.097	0.205	0.662
Log Abundance	0.156	0.024	-0.084	0.003	1.000	0.003	0.111	9.000	0.012	0.226	0.646
Log Taxa	0.490	0.241	0.156	0.013	1.000	0.013	0.041	9.000	0.005	2.851	0.126
Log10 Annelids	0.068	0.005	-0.106	0.000	1.000	0.000	0.049	9.000	0.005	0.041	0.843
Log10 Bivalves	0.695	0.483	0.426	1.043	1.000	1.043	1.115	9.000	0.124	8.414	0.018
Log10 Gastropods	0.231	0.054	-0.052	0.113	1.000	0.113	1.990	9.000	0.221	0.509	0.493
Log 10 Arthropods	0.081	0.007	-0.104	0.003	1.000	0.003	0.502	9.000	0.056	0.059	0.813
Log 10 Other	0.278	0.077	-0.025	0.156	1.000	0.156	1.860	9.000	0.207	0.754	0.408

Table 9 provides a matrix plot of Pearson correlation coefficients with biological endpoints represented in columns and physicochemical variables in rows for all July 8, 2002 Deepwater Point sample stations. The relationships described in the table between biological and physicochemical variables are largely dependent on the environment and the nature of the organic matter incorporated into the sediments. It should be emphasized that grab samples evaluate only the relatively sessile infauna and epifauna. The video record described earlier indicated much higher abundance of megafauna, particularly starfish, crabs and anemones under the farm, whereas the macrobenthic data, upon which Table 9 was based, represents only a portion of the benthic community. These coefficients indicate that annelids increased with distance from the rafts; with depth; with the proportion fines; and with the amount of organic matter in the sediments (TVS or TOC). They were negatively correlated with the proportion sand and/or gravel in the sediments and with concentrations of free sulfides. Sulfide concentrations under the raft on July 8, 2002 were moderately high (1,571 to 2,160 μM $S^{=}$). These are concentrations that would not have excluded many taxa that were common at the reference area, such as *Nephtys cornuta, Macoma nasuta, Alvania compacta* and *Sigambra tentaculata*. One hypothesis explaining the negative correlation with sulfide is that these taxa are generally found in fine-grained environments and those sediments under the farm, where moderately high sulfide concentrations were found, were too coarse for them.

Table 9. Pearson correlation coefficients describing the covariance of physicochemical and major biological endpoints for all stations sampled at the Deepwater Point mussel farm on July 8, 2002 and at the mid-Totten reference location.

	Correlations (Deepwater Point Database) Marked correlations are significant at p < .05000 N=26 (Casewise deletion of missing data)					
Variable	Distance	Sulfide	ARCSINSQRT TVS	T Gravel	T Sand	T Fines
Distance	1.00	-0.55	0.70	-0.76	-0.57	0.76
Sulfide	-0.55	1.00	-0.55	0.62	0.33	-0.60
Log Abundance	0.10	-0.26	0.01	-0.14	0.05	0.11
Log Taxa	-0.26	0.16	-0.48	0.53	0.37	-0.56
Log10 Annelids	0.42	-0.60	0.25	-0.35	-0.20	0.36
Log10 Bivalves	0.48	-0.23	0.30	-0.34	-0.19	0.31
Log10 Gastropods	-0.44	0.44	-0.59	0.69	0.43	-0.70
Log 10 Arthropods	-0.28	0.08	-0.35	0.05	0.50	-0.20
Log 10 Other	-0.52	-0.04	-0.15	0.16	0.08	-0.12

Consistent with the sediment grain size determinations and the video record, Gravel and sand decreased with distance to the north and fines (silt and clay) increased. Note that sulfides decreased with distance – even though the benthos became more depositional to the north and would have accumulated natural detritus at higher rates than would have occurred at the more erosional environment near Deepwater Point. As expected, the abundance of arthropods increased in coarser sediments. The results presented in Table 9 suggest that the coarser sediments at Deepwater Point likely had a more significant effect on the macrobenthos than did the physicochemical changes created by biodeposits from the farm.

Multidimensional analysis. Fine-grained sediments throughout Totten Inlet were found by Brooks (2005a) to be organically enriched and to contain higher than expected concentrations of free sediment sulfides. The macrobenthic communities at the Deepwater Point reference location and in fine-grained sediments at the site of the proposed mussel farm in North Totten Inlet were dominated by taxa that have been shown to be tolerant of these enriched conditions. The benthos under, inshore and upcurrent from the Deepwater Point mussel farm contained large proportions of gravel and sand and were uncharacteristically coarse in comparison with most of Totten Inlet. Sediments under the farm contained sulfide concentrations that were high enough to adversely affect several of the dominant taxa found in Totten Inlet. However, these taxa, while tolerant of elevated sulfides, are not adapted to the coarse sediments at this site. Therefore, these two dominant physicochemical factors (coarse sediments and high sulfides under the farm) were both inimical to these dominants. This complicates any attempt to understand the macrofaunal community's response solely to biodeposits from the farm. The dominant taxa and physicochemical data for all Deepwater Point stations was analyzed using Factor Analysis with a Varimax normalized principal factors extraction. Factor loadings for a two factor analysis are provided in Table 10. Factor loadings greater than 0.70 are highlighted. The two factor analysis explained 52% of the variance whereas a three factor analysis explained 61%. The main difference between the two is that distance was positively loaded on Factor 3 in the three factor analysis and it was negative loaded on Factor 1 in the two factor analysis. The two factor analysis is presented to simplify graphical presentations. However, it is worth noting that two bivalves, *Psephidia lordi* and *Macoma nasuta*, were positively loaded with distance in the three factor analysis – likely because both of these mollusks prefer fine grained sediments, which increased with increasing distance to the north of the farm.

The two orthogonal factors are plotted in Figure 16. Four groups are recognizable. Group 1 includes macrofaunal abundance, bivalves and annelids indicating that bivalves and annelids are the two phyla responsible for most of the observed abundance. Group two appears to represent taxa responsible for increased diversity. These include all of the gastropods, arthropods and a single annelid (*Glycinde picta*). All of these taxa are moderately tolerant of sulfide but have not been shown to proliferate in enriched environments. No physicochemical endpoints are associated with either Group 1 or Group 2. Group three contains gravel, sulfide and sand, which are physicochemical endpoints observed in higher proportion or concentration under the farm rather than further away. *Dorvillea annulata* is known to be highly tolerant of free sulfides. The group of taxa labeled *Other* was dominated by hydroid polyps and barnacles (*Chthamalus dalli*). These animals require a firm substrate (rocks, gravel and shell) upon which to settle and suitable environments were found only under, upstream and inshore from the farm. Note that Group 3 is negatively loaded on Factor 1 and that *Distance* is positively loaded on this Factor. Group 4 contains physicochemical variables associated with the depositional area north of the farm including *Distance, TVS,* and *Fines*, which are all indicators of a depositional environment. The taxa associated with

Variable	Factor Loadings (Varimax normalized) Extraction: Principal components (Marked loadings are >.700000) Factor 1	Factor 2
Distance	-0.831	-0.196
Sulfide	0.727	0.186
Paraprionospio pinnata	-0.661	-0.310
N. cornuta + sp.	-0.703	0.035
Goniada maculata	0.257	0.276
Metacaprella anomala	-0.183	-0.062
Psephidia lordi	-0.519	-0.110
Nassarius perpingis	0.272	0.731
Tharyx secundus	-0.293	-0.094
Macoma nasuta	-0.552	0.198
Sigambra tentaculata	-0.156	0.234
Alvania compacta	0.079	0.736
Armandia brevis	0.333	0.709
Dorvillea annulata	0.313	-0.039
Glycinde picta	-0.013	0.888
Alia gaussapata	0.185	0.741
Thuiaria thuja	0.487	-0.155
ARCSINSQRT TVS	-0.701	-0.430
Log Abundance	-0.441	0.742
Log Taxa	0.128	0.821
Log10 Annelids	-0.701	0.463
Log10 Bivalves	-0.632	0.500
Log10 Gastropods	0.430	0.740
Log 10 Arthropods	0.012	0.572
Log 10 Other	0.202	0.158
T Gravel	0.814	0.350
T Sand	0.506	0.445
T Fines	-0.811	-0.427
Expl.Var	6.888	6.581
Prp.Totl	0.246	0.235

Table 10. Factor loadings resulting from a two-factor principal components analysis with Varimax normalization of the Deepwater Point database.

this area include the annelids *Nephtys cornuta, Paraprionospio pinnata and Tharyx secundus* plus the bivalves *Macoma nasuta* and *Psephidia lordi*, all of which are typically found in fine grained sediments. *Metacaprella anomala* represents allochthonous input from the farm. Its association with *Distance* suggests that currents carried this nearly neutrally buoyant amphipod a significant distance (105 m in this case) during its settlement to the benthos.

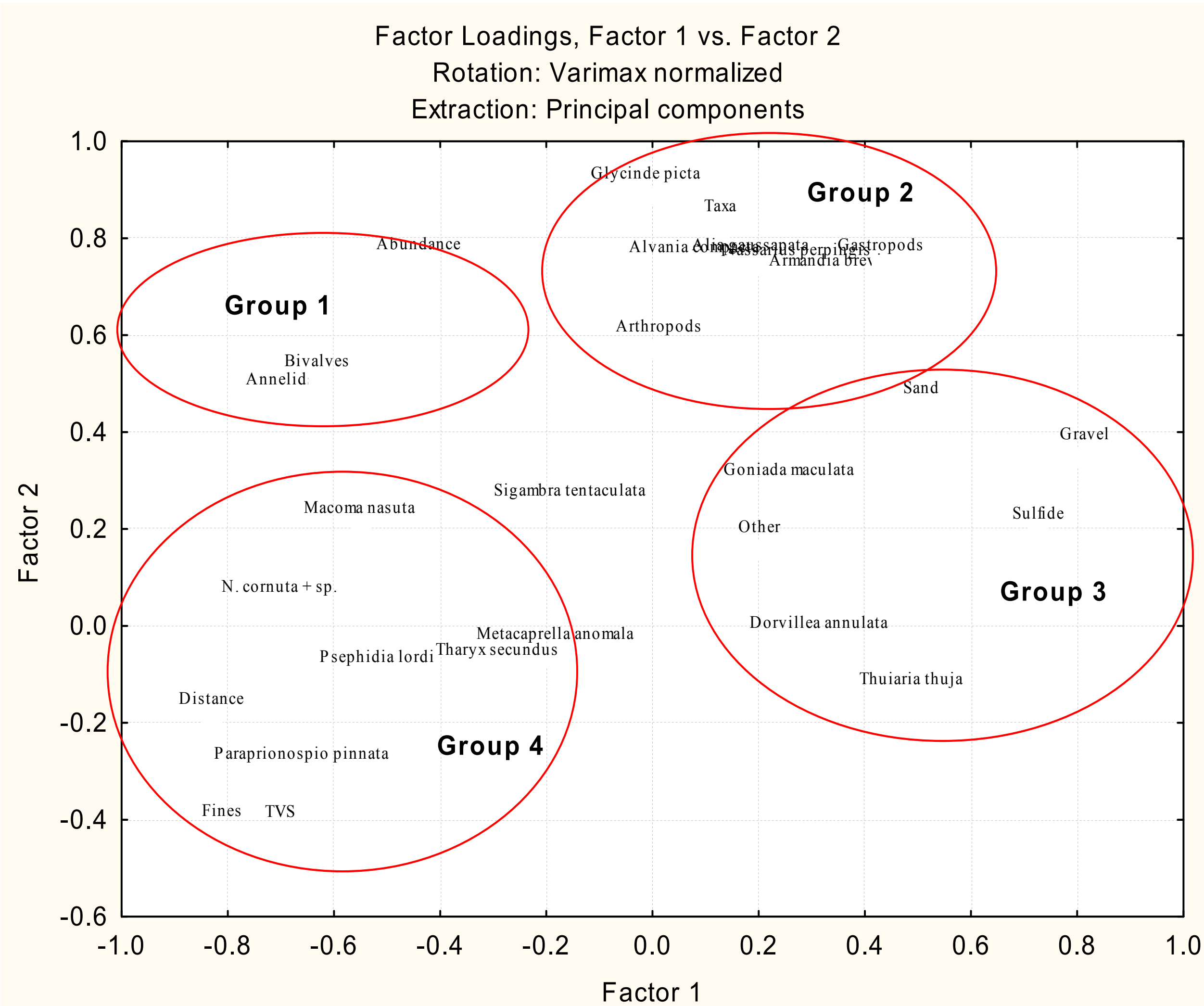


Figure 16. Cartesian plot describing the relationship between variables loaded on Factor 1 (the ordinate) and Factor 2 (the abscissa). Data are for all samples collected at the Deepwater Point mussel farm on July 8, 2002.

4.0. Discussion. The sediment grain size data and video record indicate that this mussel farm is located on the northern edge of an erosional area associated with Deepwater Point. The benthos downcurrent (north) of the farm was characterized as a nearly flat muddy plain dominated by silts and clays containing significant amounts of organic matter (TVS). The increased TVS downcurrent from the Deepwater Point farm was observed early in this study. Several hypotheses could be invoked to explain the increase, including one that biodeposits originating with the cultured mussels and their epifaunal communities were increasing TVS from the farm to the reference station located 1.12 km to the north. These higher than expected TVS concentrations were the basis for conducting a baseline survey along the entire centerline of Totten Inlet from tide-flats on the south end through its mouth on Dana Passage. Brooks (2005a) found that the proportion TVS (or TOC) in Totten Inlet sediments was positively and significantly correlated with percent fines and that all surveyed areas of the inner inlet's sediments contained higher concentrations of organic matter than are typically found in Puget Sound. The baseline survey also found that macrofauna in the fine grained sediments at the North Totten Inlet site were dominated by deposit feeding organisms characteristic of enriched environments. The Totten Inlet baseline survey determined that in Totten Inlet, the Percent TVS = 1.1193 + 0.0899* percent fines ($r^2 = 0.89$, $p < 0.000$). Because the sediment grain size changes significantly under and near the mussel rafts, this equation was used to further examine TVS concentrations near the Deepwater Point mussel farm (Figure 17). Higher TVS than found in other parts of Totten Inlet would be represented by datapoints significantly above the fitted regression line. Large excursions of observed from predicted TVS are not observed in Figure 16 and the slope of the line is essentially one indicating that in the database, predicted and observed TVS were generally equal. There are eight points lying above the 95% confidence interval suggesting higher than expected TVS. These eight cases were for downcurrent distances of 60 to 105 m, inshore 15 m from the raft and one at the reference location. There were also five datapoints below the lower limit identifying samples in which the observed TVS at Deepwater Point was less than predicted for Totten Inlet in the baseline survey. The results presented in Figure 16 suggest that the Deepwater Point mussel farm was not significantly increasing sediment TVS downcurrent from the farm – at least not above conditions existing in most of the already enriched inlet.

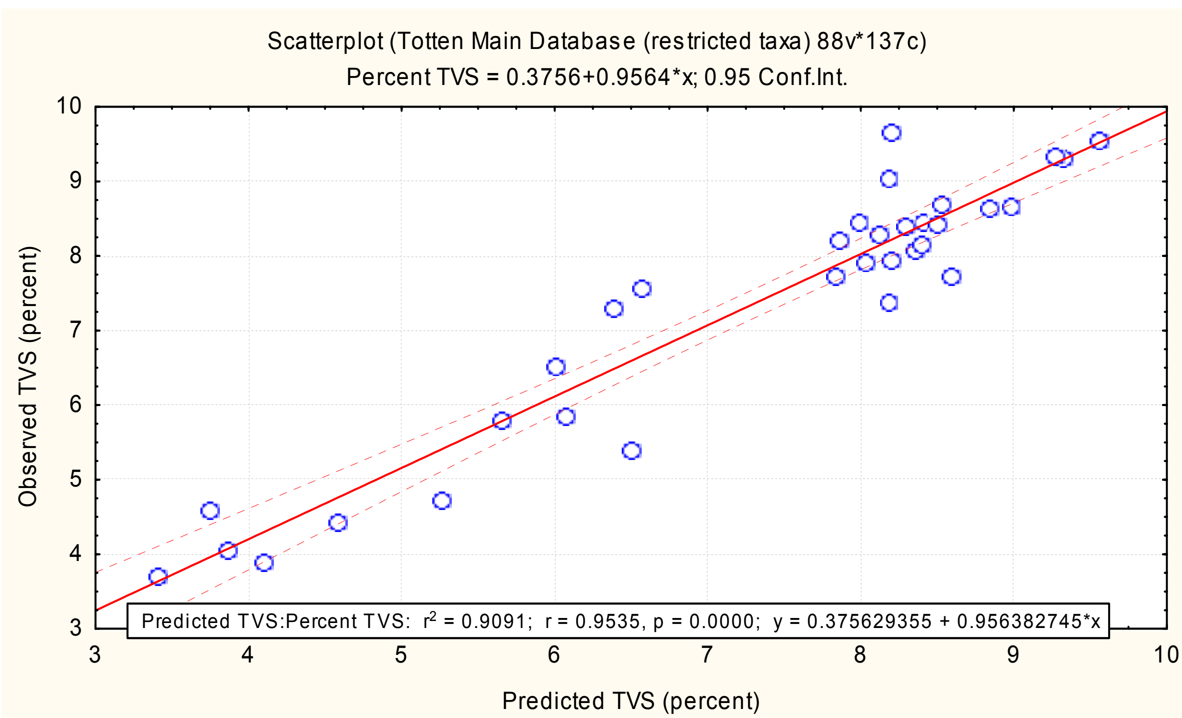


Figure 17. Observed (abscissa) TVS along the downcurrent transect at the Deepwater Point mussel farm as a function of TVS predicted based on the proportion fines (silt and clay) observed throughout Totten Inlet in Brooks (2005). 95% confidence intervals are provided.

Sulfide response to enrichment. Free sediment sulfides are a metabolic byproduct of the anaerobic catabolism of labile organic material by *Desulfovibrio sp.* and other sulfur reducing bacteria. Sulfides are frequently the most sensitive indicator of chemical change in sediments near intensive aquaculture facilities and they appear to be the most sensitive physicochemical indicator at the Deepwater Point mussel farm. For instance, increased TVS was not apparent in July 2002 (Figure 9), but a large and significant ($t = 8.39$; $p < 0.00$) increase in $S^{=}$ was recorded under the rafts in comparison with the reference location on that date. Both TVS ($t = -3.81$; $p < 0.00$) and $S^{=}$ ($t = 17.11$, $p < 0.000$) were good indicators of significant carbon deposition under and on the perimeter of the mussel rafts at the time of harvest. Sulfide concentrations were found to be elevated above 200 µM throughout much of Totten Inlet by Brooks (2005a) and concentrations as high as 809 µM $S^{=}$ were observed at the Deepwater Point reference location during the October 31, 2002 survey. Sulfide concentrations this high will exclude many arthropods, echinoderms and sensitive annelids and mollusks. Brooks and Mahnken (2003a) found that in annelid dominated macrobenthic communities, half of the taxa were excluded from an area when free sediment sulfides were greater than 960 µM and Brooks *et al.* (2004) reported a 50% reduction in the richness of a mollusk dominated macrobenthic community at 447 µM $S^{=}$.

Moderate to minor effects appear to have been limited to less than 145 m from the farm at the peak of production. However, the 12,800 to 15,300 µM of sulfide observed under the farm's footprint just before the mussels were harvested was sufficiently high to exclude most infaunal organisms. The effect on epifauna, including crabs, shrimp, anemones, and predatory gastropods is unknown. Brooks (unpublished) has video recordings of *Pandalus platyceros* and *Cancer magister* foraging on top of mats of *Beggiatoa sp.* bacteria covering anaerobic sediments where free sediment sulfide concentrations were >20,000 µM $S^{=}$, suggesting that surface dwelling megafauna is not necessarily excluded from these areas. Based on the sulfide history at the Deepwater Point mussel farm, significant adverse effects in the infaunal community were likely restricted to the area directly under the farm's footprint.

Mussel waste, including shell, had not accumulated to any recognizable depth under the farm when the video was recorded on July 17, 2002. Taylor Resources maintains suspended nets under their cultures to collect mussels that fall off the lines. The shell is periodically pumped into barges and used to harden soft intertidal clam and oyster beds. This management practice appears to have eliminated any accumulation of shell under the farm and it is likely in part responsible for the minor macrobenthic effects seen at this site.

Extent of sediment physicochemical changes. The distances at which benthic effects occur in association with raft cultured mussels are a function of the size, shape and density of the discharged particles, water depths below the point of discharge and current speeds. Within the narrow range of salinities and temperatures found in Totten Inlet, water density will not have an appreciable effect on settling rates. Haamer (1996) reported that the settling speed of feces from harvest size mussels varied between 2 and 10 cm/sec. If one conservatively assumes that mussel feces and pseudofeces settle with an average vertical velocity of 3 cm/sec; that the mean water depth under the mussel lines at the Deepwater Point mussel farm is 7.8 m; and that the mean current speed is 16.0 cm/sec (Gardiner *et al.*, 2004) then the average fecal pellet is expected to be carried 41.6 m downcurrent from the perimeter of the farm. This is an average distance and because these tides are harmonically driven, higher deposition is expected under the rafts and lower deposition rates would occur further than 41.6 m from the rafts. Significant increases in free sediment sulfides were observed only directly under the Deepwater Point rafts until the end of the production cycle on Day 320 (Figure 10). On that day, sulfides were significantly increased at distances between 45 and 60 m from the downcurrent perimeter suggesting that an average fecal pellet settling velocity of 3 cm/sec gives reasonable estimates for predicting the extent of physicochemical effects in the inlet.

Macrobenthic community response to enrichment. On July 8, 2002, the total abundance of macrofauna measured in grab samples was significantly less under the Deepwater Point farm's footprint than was observed at the reference location. Significant differences in other biological endpoints were not observed. The differences (see Figure 15) appear to have been more associated with the coarse grained sediment found under the farm, which excluded common surface and subsurface deposit feeding annelids, than with the sulfide concentrations observed on July 8, 2002. In contrast to the macrofaunal data, the video record described a healthy megafaunal community at that time under the rafts where mussel and epibiont fall-off were being consumed by large numbers of crabs and starfish whereas the megafaunal community at the reference location was nearly depauperate. The high sulfide concentrations observed in November 2002 just prior to harvest would likely have excluded most infauna from the area immediately under the rafts. Cause and effect relationships were not established in this study. However, it appears that the macrobenthic community under the farm was determined primarily by the coarse sediment grain size, which excluded most sub-surface deposit feeders at all times, and by short-term high sulfide concentrations observed just before the culture was ready for harvest.

Extent of biological effects. Nephtys cornuta is found primarily in fine-grained sediments and is tolerant of moderately enriched conditions ($\leq$ 3,600 μM $S^{=}$; see Brooks, 2001). In July 2002, this annelid was absent from the coarse sediments under the rafts, but was found in an abundance equal to or greater than observed at the reference station at all other distances, including the raft's perimeter. In contrast, *Paraprionospio pinnata,* is an annelid abundant in naturally enriched (refractory TVS) conditions, but which is excluded from sediments containing $\geq$ 1,750 μM $S^{=}$ (Brooks, 2001a). This annelid had reduced abundance in fine-grained Deepwater Point sediments to a distance of between 45 and 60 m downcurrent. Similarly, bivalves (particularly *Macoma sp.*) were found in reduced abundance closer than 60 m from the raft's perimeter but not at 75 m and beyond. Thus, it appears that biological effects, although not statistically significant, were apparent to a distance of ca. 60 m from the culture.

Chemical and biological remediation. To the author's knowledge, chemical and/or biological remediation of sediments at fallow mussel farms has not been reported in the literature. However, Brooks (2003) and Brooks *et al.* (2004) have studied remediation at several salmon farms in British Columbia. Those results suggest that chemical remediation would be complete in a few months at Deepwater Point followed by biological remediation during the next spring – summer macrofaunal recruiting season. New cohorts of mussels are seeded at the Deepwater Point farm within a few months of harvesting the previous crop. In this instance, the inner row of rafts was fallowed from the end of August 2001 until the rafts were reseeded in December 2001 (~ 3 months). The hypothetically fast remediation times is supported by the low free sediment sulfides observed under the rafts on March 22, 2002 at the beginning of this production cycle (443 to 561 μM $S^{=}$).

5.0. Summary and conclusions. Approximately 138,000 kg of mussels were cultured on the inner row of six rafts at Deepwater Point in 2002. These results demonstrate minor benthic effects associated with the intensive culture of these mussels. The term minor is used in consideration of the observations of TVS deposition rates of 105 g/m^2-day and sulfide concentrations as high as 7,400 µM associated with the epifaunal community (dominated by mussels) resident on creosote treated piling in Sooke Basin British Columbia (Goyette and Brooks, 1998 and 2000) If sediments under the rafts had contained higher concentrations of fine material (silts and clays), the moderately high sulfide concentrations observed in July would have excluded sensitive infauna and the very high sulfide concentrations observed in November 2002 would have excluded all but a few opportunistic annelids. However, sediments under the rafts were dominated by gravel and sand; an environment not conducive to surface and subsurface deposit feeding annelids common in Totten Inlet's enriched sediments. Instead, the benthic community under the rafts was dominated by surface living megafauna including starfish, crabs, anemones and predatory gastropods. The megafaunal community was likely enhanced by biodeposits (food) released from the overlying mussel cultures and their symbiotic community. This community was diverse and abundant when the July 17, 2002 video recordings were made. Sediments from the raft's northern perimeter to the reference location contained significant quantities of silt and clay, representing an environmentally homogeneous environment. Elevated concentrations of free sediment sulfides were observed to 45 m from the raft's perimeter in July and 60 m from the perimeter in late November. The biological results presented in this report indicated subtle infaunal community effects extending to a distance of between 45 and 75 m downcurrent from the farm; but not beyond that distance. These rafts were reseeded approximately 3 months following harvest of the last crop. The low sulfide and TVS concentrations observed during the first sample period in March 22, 2002 indicate that chemical remediation occurred very quickly at this site with no evidence of cumulative effects.

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