Final Project Report-

Surf to Turf Mussel Compost: Removing and Recycling Nutrients from Budd Inlet

National Estuary Program (NEP) Toxics and Nutrients

Award No. G1500057

Prepared for

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EXECUTIVE SUMMARY

Between 2012 and 2014, the Pacific Shellfish Institute (PSI) was awarded NEP funds to test nutrient bioextraction using native blue mussels as a way to address eutrophication in Budd Inlet. Nutrient bioextraction is the process of growing and harvesting shellfish or algae for the main purpose of removing nutrients from a watershed. This work demonstrated the effectiveness of cultivating, harvesting and composting Budd Inlet mussels, while simultaneously engaging the community in nutrient reduction efforts. This report expands on the 2012 "proof of concept" work by addressing the next step— establishing a connection between nutrient removal and the related impacts on water quality (i.e., pH, DO, Chl *a*, etc.) within and beneath the mussel installations.

The goal of this project was to evaluate nutrient bioextraction using mussels as a way to improve water quality in lower Budd Inlet. Project objectives were to: 1) maintain and expand the network of waterfront businesses, residents, marinas, and restaurants that implement mussel demonstration sites; 2) study how the mussel installations affect water quality via nutrient bioextraction, mussel filtration, and other processes; 3) harvest and compost the mussels growing on temporary nylon straps and permanent structures for terrestrial agricultural application; 4) provide opportunities for outreach and education via public presentations and classroom curriculum; and 5) make recommendations for innovative solutions to multi-parameter TMDLs.

During our pilot trials, over 5,000 lbs. of mussels were harvested removing 50 pounds of nitrogen, 2.75 pounds of phosphorus, and 225 pounds of carbon while generating over four cubic yards of organic compost. The mussel compost was incorporated into gardens and landscapes at The Evergreen State College's Organic Farm, Department of Corrections – Cedar Creek Facility, City of Olympia's Capitol Campus, and in backyards of Thurston County residents.

The impacts to water passing through the WB mussel installation was evaluated using two flow-through experiments. Results indicated that plankton cell counts, Chl *a*, and POC/PON decreased as the water moved through the system. This removal of phytoplankton via filter feeding resulted in slight increase in dissolved nutrients (i.e., nitrates, nitrites, silica) and decrease in pH moving through the system as fewer phytoplankton remained in the water column to assimilate nutrients and carbon dioxide. During peak growth rates, mussels also released the waste product ammonium, which was detected in concentrations above historic ambient conditions for lower Budd Inlet.

Mussel biodeposition at both sites was 3-5 time higher under the mussel installations when compared to control stations. During July and August, pH and DO were typically lower under mussels compared to the reference site, but the difference was slight and likely to have no

biological significance. By September, no difference was detected at either site due to changing weather patterns that improved water quality conditions considerably.

Nutrient bioextraction results in a net reduction of nutrients post-harvest due to the fact that shellfish growth requires no additional food supplementation. Instead, mussels obtain their nutrients directly from surrounding waters. Despite this benefit to water quality, care must be taken when siting larger mussel installations in locations characterized by poor water circulation. Circulation maps and models of Budd Inlet indicate that flow velocities and DO concentrations are historically lower at EB, approaching complete hypoxia in mid-summer. Based on this knowledge, EB would not be an ideal candidate for a larger scale nutrient bioextraction project. Nutrient bioextraction would be suitable, however, in a lower Budd Inlet location that experiences adequate water circulation. In such instances, nutrient removal would occur via physical harvest (measurable) and denitrification, although denitrification was not measured as part of this study.

Various, theoretical, scaled-up mussel farming scenarios are presented in this report – both of which would result in the harvesting of approximately 500,000 to 600,000 pounds of mussels and remove 5,000 pounds of nitrogen from Budd Inlet. Should future nutrient bioextraction projects be pursued, however, ongoing monitoring of biological communities within and beneath installations, mussel "drop-off" times, and water quality at depth is recommended.

Water quality trading (WQT) is a voluntary market-based approach that, if used in certain watersheds, might achieve water quality standards more efficiently and at lower cost than traditional approaches (EPA, 2004). WQT has been encouraged by various agencies as part of the 2014 Recommendations for Improving Water Quality Assessment and Total Maximum Daily Load Programs in Washington State (Interagency Project Team, 2014). Establishment of WQT in Budd Inlet should, however, be predicated by TMDL established load allocations. When PSI began the "Surf to Turf" research, we expected to correlate required nutrient reductions in the Deschutes Basin to confirmed nutrient reductions from our bioextraction efforts in Budd Inlet. Unfortunately, with the separation of the upper and lower Deschutes Basin TMDL process, work remains in developing load allocations for Budd Inlet.

These results demonstrate that nutrient bioextraction with shellfish can be a viable component toward improving Budd Inlet water quality. The shared ecosystem functions of nutrient remediation, water clarification, biodeposition, and habitat creation make suspension-feeding bivalves a valued provider of ecological services to the shallow-water ecosystems. In addition, nutrient bioextraction engages the public, encourages Puget Sound stewardship, and supports larger nutrient removal efforts being made by LOTT Clean Water Alliance, the TMDL advisory group, participating government agencies, non-profit organizations, and the community at large.

INTRODUCTION

Water quality, particularly dissolved oxygen (DO), has been studied extensively in Budd Inlet for decades. Between 1988 and 2004, DO levels in bottom waters of the Inlet were in a state of slow decline. At the same time, dissolved inorganic nitrogen concentrations in the Deschutes River were on the rise (Roberts et al., 2009). Fish and marine organisms require adequate DO levels for survival. During the process of eutrophication, excess nutrients stimulate algae resulting in oxygen depletion upon decomposition.

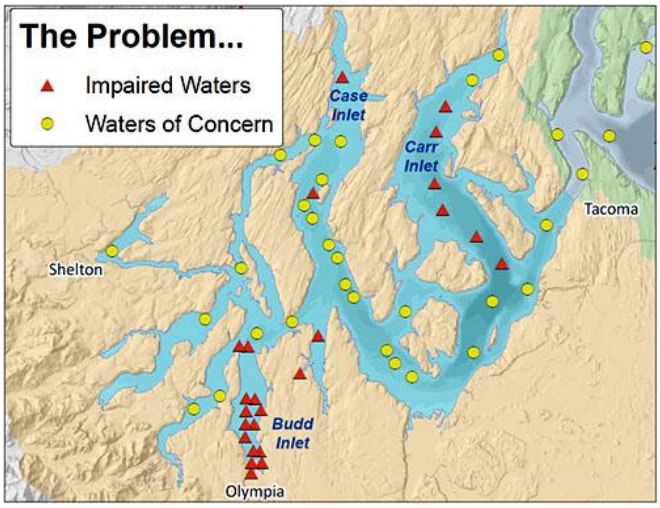

Figure 1. 2008 Water Quality Assessment for dissolved oxygen (Mohamedali et al., 2011).

In 1994, the LOTT (Lacey, Olympia, Tumwater, Thurston) Alliance implemented denitrification technology at the wastewater treatment facility. Despite this effort, DO levels continued to decline in the face of nutrient inputs from a multitude of sources – river inputs, Puget Sound, and sediments to name a few. In 2008, Washington Department of Ecology identified Budd Inlet as "impaired" for dissolved oxygen (Mohamedali et al., 2011) (Figure 1). After portions of the Deschutes River and Budd Inlet failed to meet water quality standards for DO and other parameters, they were placed on the federal Clean Water Act Section 303(d) list triggering the total maximum daily load (TMDL) process (WDOE, 2012).

Between 2012 and 2014, the Pacific Shellfish Institute (PSI) was awarded NEP funds to test nutrient bioextraction using native blue mussels as a way to address eutrophication in Budd Inlet (PSI, 2014). Nutrient bioextraction is the process of growing and harvesting shellfish or algae for the main purpose of removing nutrients from a watershed (Figure 2). This work demonstrated the effectiveness of cultivating, harvesting and composting Budd Inlet mussels, while simultaneously engaging the community in nutrient reduction efforts. To this end, over 4,000 pounds of mussels were harvested from pilot sites removing at least 40 pounds of nitrogen and 3 pounds of phosphorus. Mussels were harvested from each site and provided to The Evergreen State College's Organic Farm, Washington State University - Puyallup's Research and Extension Service, and the Washington Department of Correction's Cedar Creek facility for compost trials. Results indicated that the mussel compost was of suitable quality for agricultural and garden use, and that all metals were within compost limits set by the Ecology solid waste handling standards for composting facilities.

This report expands on the 2012 "proof of concept" work by addressing the next step—establishing a connection between nutrient removal and the related impacts on Budd Inlet DO. This research took a closer look at how water quality parameters, particularly DO, differed between the two study sites; throughout the entire water column; and within, beneath and surrounding mature mussel installations. It also evaluated changes in nutrients, carbon, and phytoplankton as water passed through the mussel installation. The quantity and composition of mussel biodeposits beneath study sites were also studied. Finally, stable isotope analysis was performed to determine the origin of nitrogen and carbon contained within mussel tissues.

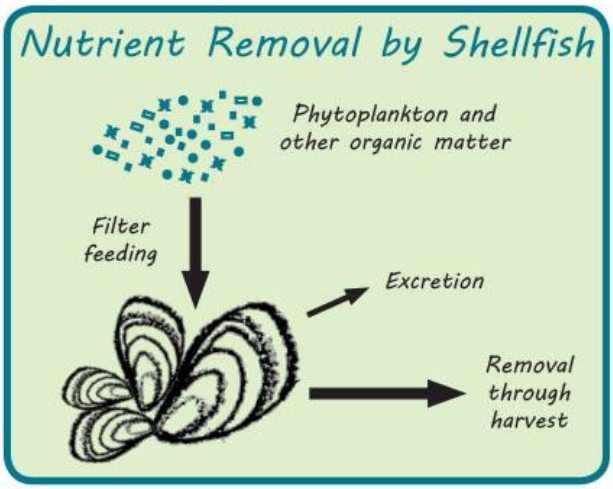

Figure 2. Simplified graphical representation of how shellfish remove nutrients through filter feeding.

Similar to the work performed in 2012, the project continued to grow mussels and harvest them for nutrient removal and the generation of Surf-to-Turf mussel compost. However, in addition to harvesting mussels from nylon straps, this study also evaluated harvesting and composting mussels found on existing anthropogenic surfaces located in lower Budd Inlet. Finally, the project continued to provide outreach and education opportunities to the general public and students, both in the field and classroom; as well as developed new partnerships with schools and local agencies.

The goal of this project was to evaluate nutrient bioextraction using mussels as a way to improve water quality in lower Budd Inlet. Project objectives were to: 1) maintain and expand the network of waterfront businesses, residents, marinas, and restaurants that implement mussel demonstration sites; 2) study how the mussel installations affect water quality via nutrient bioextraction, mussel filtration, and other processes; 3) harvest and compost the mussels growing on temporary nylon straps and permanent structures for terrestrial agricultural application; 4) provide opportunities for outreach and education via public presentations and classroom curriculum; and 5) make recommendations for innovative solutions to multi-parameter TMDLs.

The outcomes of this project enable decision-makers and regulators to answer questions surrounding both the feasibility and scientific fitness of using bivalve shellfish as a nutrient reduction strategy, both in Budd Inlet and other inlets where water resides long enough for filter feeding to impact nutrients and dissolved oxygen levels.

OBJECTIVE 1 – Implement experimental mussel systems

Figure 3. Study sites, lower Budd Inlet.

Site Selection

Mussel demonstration sites were established at two lower Budd Inlet locations (Figure 3). The first was located at West Bay Marina along the western shore of the main lower basin. The second was located at Port of Olympia's Boatworks facility in East Bay. The two sites were expected to have different water properties based on natural circulation patterns and the influence of Capitol Lake. Water from the greater Puget Sound enters Budd Inlet along the western shore and exits along the eastern shore, carrying fresh water from Capitol Lake with it, especially during low tide. While Budd Inlet experiences adequate flushing overall, very little water entering Budd Inlet reaches the southernmost portion (Aura Nova Consultants, 1998). This circulation pattern suggests that water properties might be different between West Bay and East Bay providing an opportunity for comparison.

A third site was established on the concrete pilings located along the southern edge of Port of Olympia's Port Terminal. This site was used to evaluate the suitability of harvesting mussels from existing anthropogenic structures and converting them into compost.

On May 11, 2015, 134 nylon straps were affixed to two dock fingers at West Bay Marina. The straps were either tied directly to the dock or attached to boards that were affixed beneath the dock (Figure 4). Each strap extended 5 feet beneath the water's surface, was weighted down

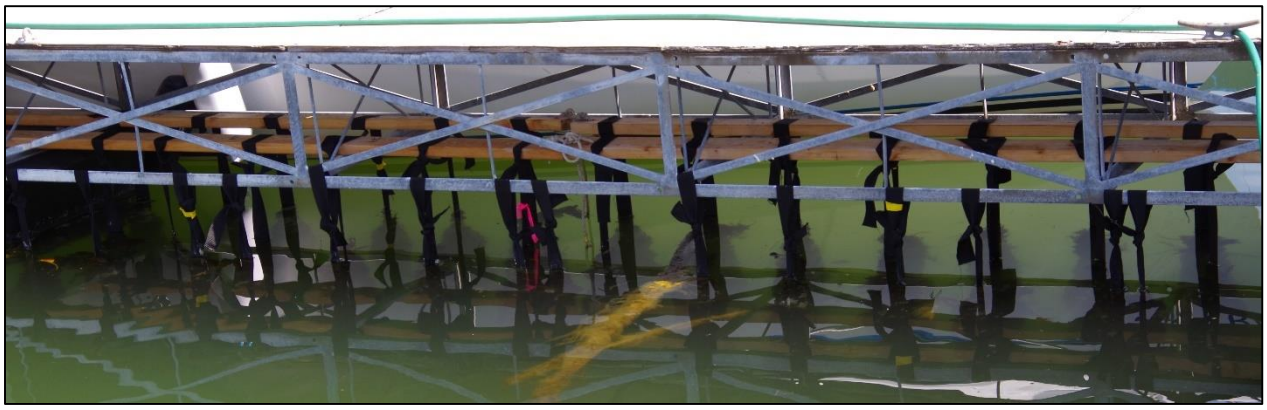

Figure 4. Straps affixed to dock at West Bay Marina, Budd Inlet.

with a small piece of rebar, and spaced at 1-foot intervals. The specific location of the demonstration site at West Bay Marina was selected based on preliminary visual flow observations. Surface currents appeared to travel parallel to the selected dock fingers during incoming and outgoing tides making it suitable for flow-through tunnel experiments.

On May 14th, 2015, 240 5-foot nylon straps were affixed to 30 boards running along the northern and southern edge of the main dock at Boatworks in East Bay. Similar to West Bay Marina, the straps were spaced every foot along the length of the dock (Figures 5 and 6). Additional straps (5-10) were placed on docks adjacent to the Oyster House and Hearthfire Restaurants for potential outreach opportunities, but only attracted adequate spat at Hearthfire.

Figure 5. Nylon straps prior to being affixed to the dock at Boatworks, East Bay.

Interpretive signage was placed at each of these locations describing the goals and objectives of the project and highlighting actions that individuals can take to prevent nutrient pollution.

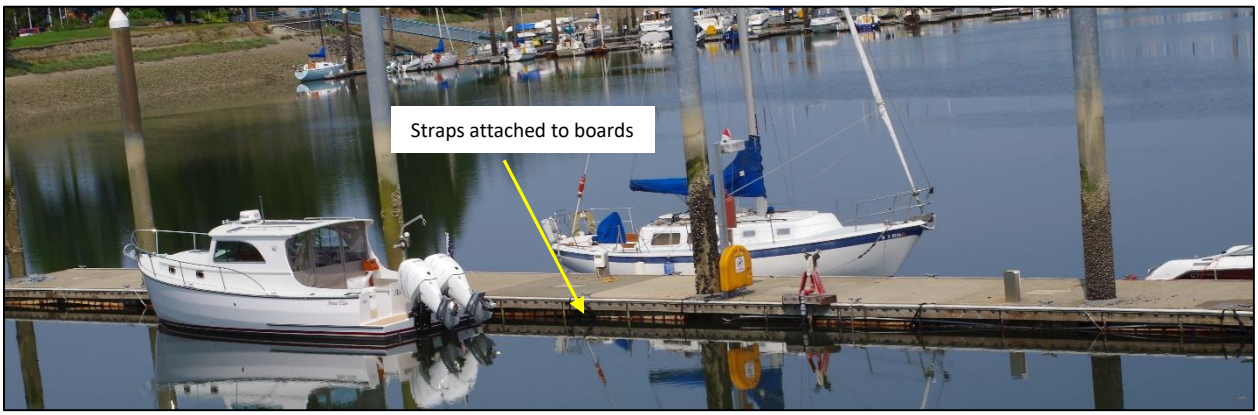

Figure 6. Mussel demonstration site: Boatworks in East Bay, Budd Inlet.

OBJECTIVE 2 – Study the impact of the mussel systems on water quality

Results from 2012-2014 demonstrated that nutrient bioextraction was indeed possible in Budd Inlet. Blue mussels proved to set and grow well on straps, remove nutrients when harvested, and generate a suitable, potentially marketable soil amendment. The amount of nutrients removed, however, was small (40 lbs. of nitrogen) in light of the size of the demonstration sites. When scaled up, the potential amount of mussels harvested and nitrogen removed could be considerable, in the 4,000-5,000 lbs. range.

Perhaps one of the first, and widely recognized, nutrient bioextraction trials using large scale mussel installations was performed by Odd Lindahl et al. in Sweden (Lindahl et al., 2005; Lindahl, 2011). While successful in many regards, this study, among others, cautioned against siting large mussel installations in locations characterized by poor water circulation (Chamberlain et al., 2001; Hargrave et al., 2008; Carlsson et al., 2012). Decomposing biodeposits beneath dense mussel assemblages can increase the biological oxygen demand resulting in depleted DO conditions. Too much organic matter under raft systems can also create a sulfidic environment inhibiting the beneficial process of denitrification which produces un-biologically available nitrogen gas (Carlsson et al., 2012). This project begins to address these concerns by taking a closer look at how the mussel installations – still quite small – impact the surrounding environment.

Under Objective 2, this work evaluates how the mussel installations impact water quality in a number of ways. This section first compares mussel cultivation (set, biofouling, growth rates) between the installations at West Bay (WB) and East Bay (EB). Then, the potential and actual amount of nutrients removed through nutrient bioextraction is calculated, along with the possibility of harvesting mussels from existing anthropogenic surfaces in lower Budd Inlet. In addition to calculating nutrients removed, the origin of nitrogen and carbon is evaluated through stable isotope analysis using mussel tissue.

Also under Objective 2, the quantity and composition of mussel biodeposition is evaluated at both sites. Water quality parameters are compared between not only WB and EB, but also between the mussel installations and nearby reference stations. Finally, changes in water quality (nutrients, carbon, plankton, etc.) are measured as water passes through the WB mussel installation using flow-through tunnel experiments.

While general methods are provided throughout this section, detailed laboratory and field procedures are explained in the Quality Assurance Project Plans (WDOE, 2013; WDOE, 2015).

Mussel Cultivation – Spat settlement and biotic community

Nylon straps were placed at both sites in mid-May to provide settling substrate for the blue mussels. Mussel seed appeared by mid-June, but while mussel set was uniform at WB, the seed at EB was sparce and patchy along the straps – sometimes difficult to detect at all. It is unclear if the window of opportunity for peak mussel set was missed at EB or if natural interannual variation was a factor. In 2013, nylon straps were placed at sites in early-May, two weeks earlier. Even in 2013, mussel set was similarly less robust and uniform at EB, but to a lesser degree. Other factors that may have inhibited seed settlement was early biofouling at EB as well as disturbance caused by a large barge moored adjacent to a sizable section of straps during several weeks in May (Figure 7).

Figure 7. Nylon straps in mid-July at EB (top) and WB (bottom).

While an array of algae, invertebrate, and fish species were observed using the mussel installations at both sites, a larger number was detected on straps at EB (Table 1)(Appendix A). The greater diversity was likely due to more available space provided by the smaller percent mussel cover. Most notable was the number of colonial ascidians and solitary tunicates that were detected on straps at EB (Figures 8 and 9). East Bay also had more macroalgae, anemones, nudibranchs, and crustaceans such as caprellids, shrimp and shore crab. Both sites contained hydroids, flatworms, annelids, amphipods, small fish and moon jellies. No sea stars were observed at either site, unlike the large quantities that were detected on straps at WB in 2013. Barnacle fouling, a significant problem in trials performed in Quartermaster Harbor in 2011, was also absent at both WB and EB.

	West Bay			East Bay		
Species	July	Aug	Sept	July	Aug	Sept
Ulvoids				X		
Porphyra				X		
Hydroids	X	X		X	X	X
Colonial ascidians	X			X	X	X
Solitary tunicates				X	X	X
Encrusting bryazoans	X					
Orange sponge						X
Anemones						X
Nudibranchs				X		
Flatworms		X		X	X	X
Annelids		X			X	
Amphipods	X	X	X	X	X	X
Caprellids						X
Shrimp					X	
Shore crabs					X	
Shiner perch	X	X	X			X
Sticklebacks	X	X	X			X
Moon jellies	X	X	X	X		X

Table 1. Biodiversity at WB and EB.

Figure 8. Solitary tunicates and orange compound ascidian at EB in August.

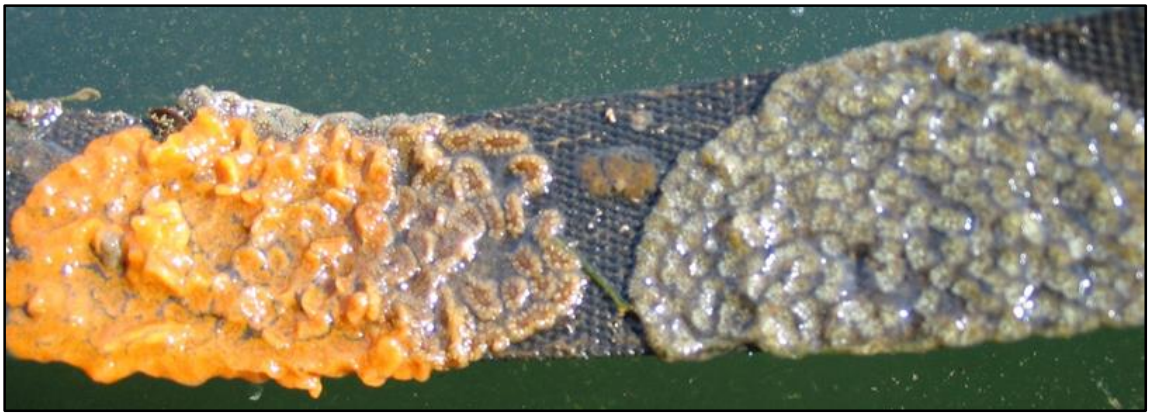

Figure 9. Compound ascidians, *Botrylloides violaceous* (left) and *B. schlosseri* (right) at EB in July.

Growth Rates, Biomass, and Harvest

Mussel growth measurements (lengths and individual weights) were collected every two weeks between July and October (Appendix B). Despite the poor mussel set at EB, mussels ultimately reached greater lengths and weights by season's end – 32 mm and 2.8 g compared with 28 mm and 1.8 g at WB (Figure 10). For comparison, 2013 growth rates were slightly slower at EB than

WB with average mussels lengths and weights reaching 28 mm and 2.0 g compared with 30 mm and 2.4 g respectively. The accelerated growth rates at EB were likely due to the benefit afforded by less competition for space and resources on the straps. This was most apparent in late summer when the smaller density of mussels took advantage of the extra space and food, reaching a maximum growth rate of 0.05 g/day (Figure 11).

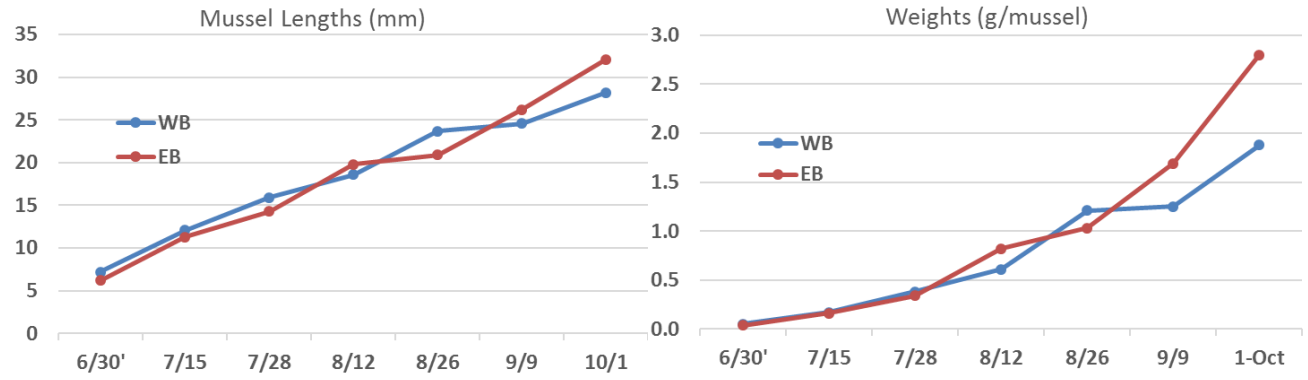

Figure 10. Average mussel lengths (mm) and weights (g/mussel) at WB and EB.

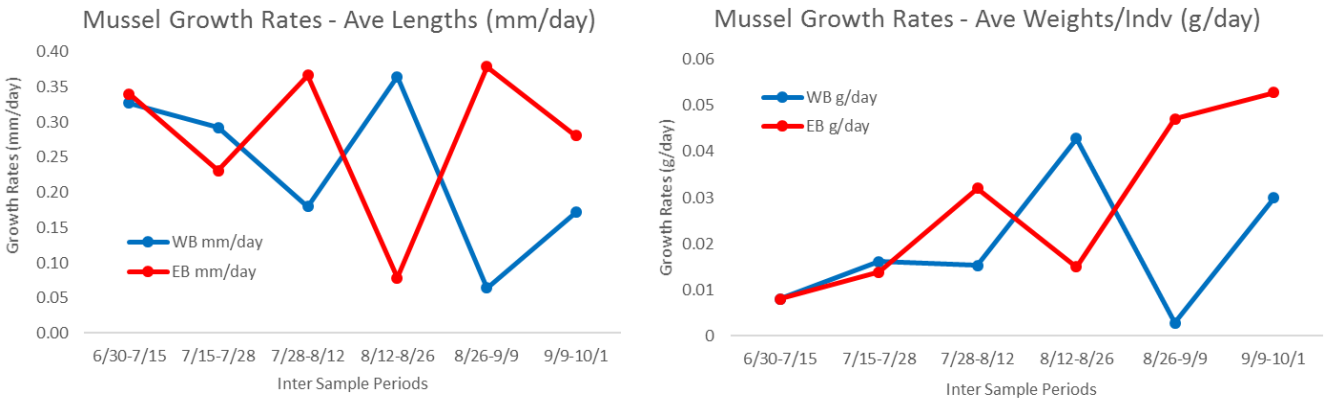

Figure 11. Growth rates (mm/day and g/day) per Inter Sample Periods at WB and EB.

While average mussel lengths and weights were higher at EB by season's end, the average biomass, or total weight of mussels found on each 5-foot strap, was much higher at WB. By October, the average weight per strap exceeded 31 lbs. at WB compared with only 18 lbs. at EB (Figures 12 and 13). In 2013, average weights per strap were 32 lbs. and 40 lbs. respectively. The significant reduction in weight per strap at EB was due to poor initial mussel set in May at this location.

Figure 12. Typical strap at WB (above) and EB (below) in late August, 2015.

Mussels were harvested on October 1st at WB and October 12th at EB. At WB, 134 straps were affixed to the dock yielding 2,210.5 lbs. of whole mussels, or 2,037 lbs. of chipped mussel slurry. At EB, 240 straps were affixed to the dock yielding 2,808 lbs. of whole mussels, or 2,570 lbs. of chipped slurry. Mussels from WB were transported to The Evergreen State College's Organic Garden for composting. EB mussels were composted at Washington Department of Corrections' Cedar Creek facility (1745 lbs.) and on a PSI staff member's private property (825 lbs.)

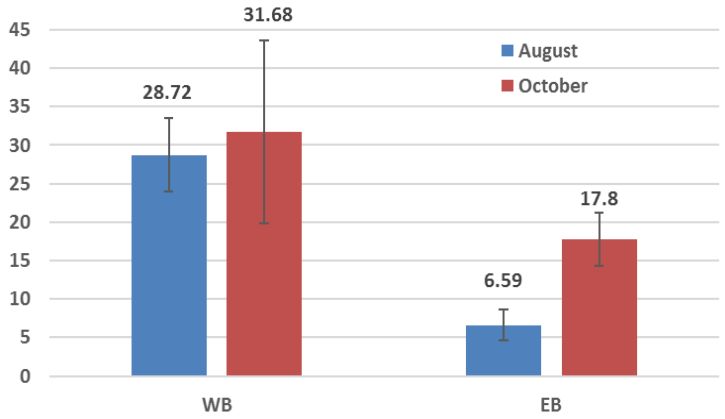

Figure 13. Average weight of mussels per strap (lbs.) at WB and EB.

Nutrient Bioextraction – Mussel Systems

One project goal was to remove a quantifiable amount of nutrients from Budd Inlet. To this end, both the *potential* and *actual* amount of nitrogen, phosphorus, and carbon were determined based on the potential pounds of biomass generated (4,245 lbs. at WB + 4,272 lbs.

at EB = 8,517 lbs.) and the actual pounds harvested (2,210 lbs. at WB + 2,808 lbs. at EB = 5,018 lbs.) at the 2 sites. The amount of nutrients removed through bioextraction was calculated by multiplying the amount of potential biomass or actual harvested mussels by the total percent of nutrients. Laboratory results indicated that total percent nitrogen (wet weight) in mussels (tissues and shell combined) was 0.94% at WB and 1.04% at EB for an average of 0.99%, or essentially 1%. Percent phosphorus was 0.05% at WB and 0.06% at EB for an average of 0.055%. 2013 values were similar at 1% and 0.08% respectively. Percent carbon was 4.47% at WB and 4.53% at EB for an average of 4.5% (Figure 14). The two sites had the *potential*, therefore, to remove 85.17 pounds of nitrogen, 4.68 pounds of phosphorus, and 383.26 pounds of carbon from the original 374 straps. The potential amount of nutrients removed *per 5-ft strap* at WB was 0.32 lbs. of nitrogen, 0.02 lbs. of phosphorus and 1.43 lbs. of carbon. This information may be used to extrapolate the number of straps required to remove any given amount of nutrients.

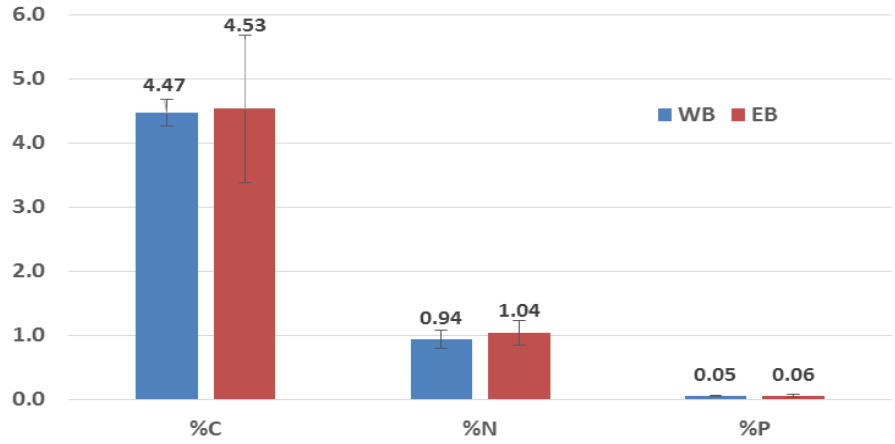

Figure 14. Average carbon, nitrogen, and phosphorus (%-wet) at WB and EB.

The actual amount of mussels harvested from the sites was 5,018 pounds. The difference between the potential and actual amount of mussels harvested was 3,500 pounds. Half of this loss, or 1,742 lbs., was attributed to mussels removed for biomass measurement collection (12 straps), education and outreach (10 straps), and sloughing off from late season weight (40 straps). These losses accounted for 1,457 lbs. at WB and 285 lbs. at EB. The *actual* amount of nutrients removed from the remaining straps (n=312) at both sites was 50 pounds of nitrogen, 2.75 pounds of phosphorus, and 225 pounds of carbon. In 2013, the 3 demonstration sites removed 43 lbs. of nitrogen and 3.4 lbs. of phosphorus.

Determining the exact amount of nitrogen removal needed to meet TMDL requirements for Budd Inlet is still underway. Once this value is known, it will reveal how important of a role nutrient bioextraction might play in improving water quality in Budd Inlet. Preliminary results

suggest that a large amount of mussels would be required to reduce a worthwhile amount of nitrogen. For example, 500,000 lbs. of mussels would be required to remove 5,000 lbs. of nitrogen, or the equivalent annual nitrogen output of 500 people based on 4.5 kg (10 lbs.) per year.

The 2013 report estimated that if straps were hung from both sides of all of the docks and boat slips at West Bay Marina (an area of 13 acres), the site could support 16,400 straps and yield approximately 492,000 pounds of mussels at 30 lbs. per strap (Figure 15). This is merely a theoretical example to illustrate the approximate size needed to remove 4,920 lbs. of nitrogen.

A second theoretical example of a system that would yield a similar amount of mussels would be a series of floating rafts. Mussels are often grown commercially from suspended lines attached to raft structures. Some, like the rafts pictured in Figure 16, support 4,320 lines each (Cheney et al., 2003). At 30 lbs. of mussels per strap, 4 rafts would generate 518,400 lbs. of mussels, or remove 5,184 lbs. of nitrogen. The rafts pictured in Figure 16 actually support 65 lbs. of mussels per line which, at these densities, could generate 561,600 lbs. from only 2 rafts. Considering that every 1,500 lbs. of mussels would yield a pick-up truck's worth of compost, both of these scenarios would generate over 350 truckloads of compost. For comparison, one of the long-line experimental mussel farms monitored in the Swedish nutrient bioextraction study was of similar size – with the potential to generate approximately 250 tons of mussels, or 500,000 lbs. every 16 months (Carlsson et al., 2012).

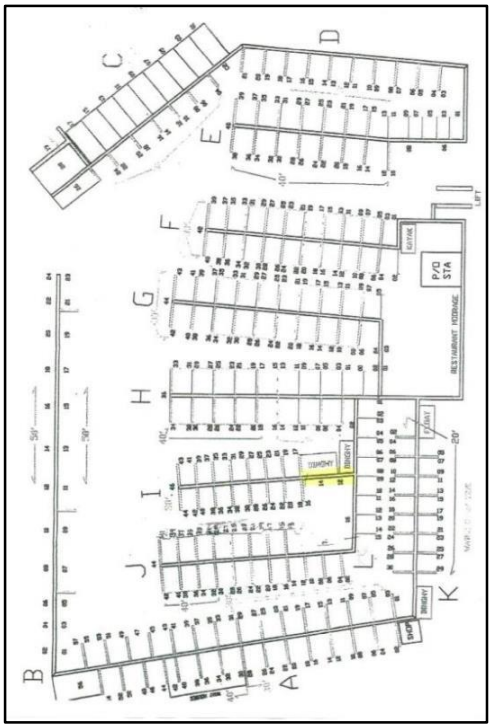

Figure 15. West Bay Marina. Highlighted section represents 120 straps.

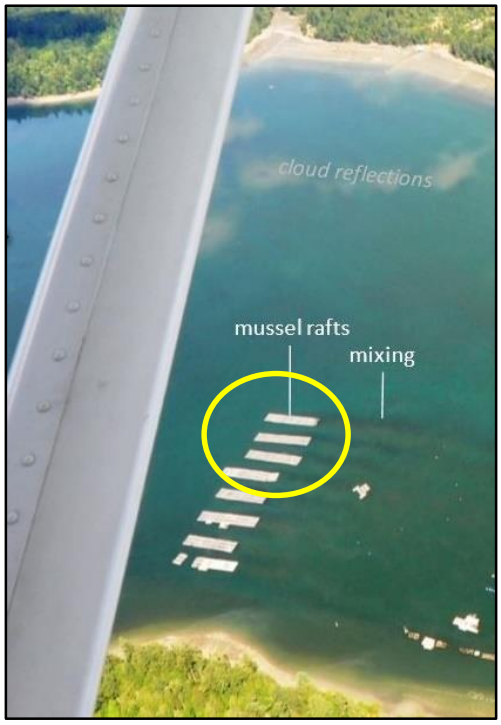

Figure 16. Commercial mussel rafts, July 2016. Photo: WDOE Eyes Over Puget Sound.

Nutrient Bioextraction – Anthropogenic Surfaces

Growing and harvesting mussels could yield a commendable amount of mussels for nutrient bioextraction. However, the amount of mussels naturally observed growing on pilings, docks, boats, and rocks in lower Budd Inlet is notably greater. These mussels serve important ecosystem services in the watershed by providing shelter and food to other organisms. As filter feeders, they improve water clarity and transfer nutrients from surface waters to benthic invertebrates via biodeposition. This experiment does not endorse large scale removal of these naturally occurring, beneficial organisms. Instead, it explores the possibility of removing a subset of mussels already growing from anthropogenic clean surfaces (as opposed to creosote pilings or treated boat hulls) and testing their suitability/safety as mussel compost. The objective is to estimate the amount of mussels (nutrients) that could be harvested, ensure future recruitment, and test for compost safety. These results would simply open the discussion for including this subset as a potential source of additional nutrient removal.

The site selected for this trial was located on a row of concrete pilings situated beneath the Port of Olympia's Marine Terminal (Figure 17). At this site, three pilings (4, 7, 10) positioned

perpendicular to the shoreline were studied in greater detail. The pilings selected were one row inward from the southern edge of the Terminal with Piling 4 closest to shore (Figure 18).

Figure 17. Port of Olympia's Marine Terminal (left) and pilings beneath the Terminal showing mussel patchiness due to natural disturbance and ongoing drop-off (right).

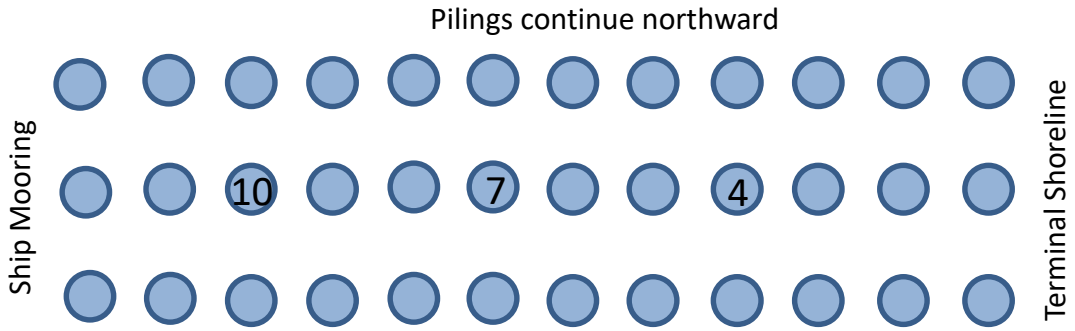

Figure 18. Piling arrangement beneath Terminal and location of pilings selected for data collection.

In May, 2016, a 0.25-meter squared quadrat was surveyed at the 0.0 MLLW line at each of the three pilings. Within each quadrat, species biodiversity was assessed, mussel lengths (n=50) and composite weights were collected, and the entire biomass removed and weighed. Mussel composites were also sent to AmTest Laboratory for analysis of nutrients, trace metals, PAHs and PCBs. In addition, 1,000 pounds of mussels were harvested from 6-8 adjacent pilings for composting. Biological recruitment was then monitored in July, August, and September.

The pilings under the Marine Terminal were dominated by mussels and, to a lesser extent, barnacles. Other invertebrate species identified included shore crabs, amphipods, isopods, green ribbon worms, nereid worms, limpets, chitin, encrusting sponge, plumose anemone and sea stars (Table 2). One Olympia oyster was found within the quadrat on Piling 4. A total of 5 Olympia oysters were found on the entire piling. Gunnels and green gunnel egg clusters were

observed within the mussel clusters and additional fish species such as stickleback and shiner perch were witnessed feeding among the pilings. Kingfishers and a river otter were also observed under the Marine Terminal.

Piling 4	Piling 7	Piling 10
shore crabs	shore crabs	shore crabs
amphipods	amphipods	isopods
barnacles (lg)	barnacles (lg and sm)	barnacles (lg and sm)
gunnel egg clusters	green ribbon worms	green ribbon worms
gunnels	isopods	nereis worm
olympia oyster		

Table 2. Pre-harvest species diversity within quadrats on the 3 pilings.

After removing the mussels, recruitment was initially slow. By mid-July, small barnacles appeared on the bare quadrats and several clusters of remnant barnacles had increased in size. By mid-August, a new set of mussels appeared, with most preferring to settle on Piling 10 (Figure 19). Percent cover of mussels within the quadrats at Piling 4, 7, and 10 was 0.1%, 2% and 25% respectively. By September, the percent cover of mussels was 5%, 5% and 85% (Figure 20). Barnacle set dominated the scraped surfaces on Pilings 4 and 7. Piling 4 had the slowest mussel

Figure 19. Typical species composition prior to harvest (left), quadrat 1-month post-harvest (middle), and new mussel recruits in August (right).

recruitment. This piling was closest to shore and also the only piling in which the entire band of mussels was removed for composting.

Figure 20. New mussel recruitment on Piling 10 in September (left), and Olympia oyster valve (right).

Large mussel clusters were frequently observed dropping off surrounding pilings during all visits due to weight. In general, the succession of species recruiting to bare surfaces was barnacles followed by mussels. New mussels also tended to prefer setting at tidal elevations below the 0.0 MLLW, particularly on the deeper water pilings. Mussel set was more robust immediately adjacent to existing mature mussels and on the barnacles themselves, which may partially explain the low recruitment on Piling 4 which was completely stripped for composting. Even in this instance, mussel recruitment was occurring, but at a much slower pace. By September, species observed within quadrats on the three pilings included barnacles, green ribbon worms, amphipods, limpets, and a chitin.

Mussel lengths and weights were recorded in May, prior to their removal (Figure 21). Average mussel lengths and weights ranged from an average of 26.9 mm and 2.5 g/mussel on Piling 4 to 38.5 mm and 6.3 g/mussel on Piling 10. For comparison, the mussels harvested from East Bay at the end of the season were 32 mm and 2.8 g per mussel. By September, the

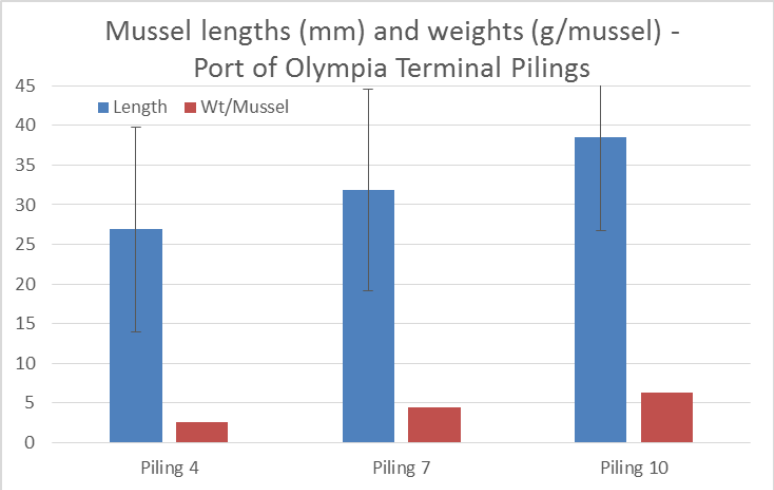

Figure 21. Mussel lengths and weights at initial harvest.

newly recruited mussels were on average 16 mm and 0.78 g/mussel.

To estimate the potential amount of nutrients that could be removed by harvesting the mussels under the Marine Terminal, the total biomass (all species combined) was multiplied by the percent nutrient content in the mussels. The total biomass was estimated in two ways. First, the mussel biomass removed from one entire piling (221 lbs.) was multiplied by the total number of pilings. The estimated number of pilings under the Terminal was 1,044 based on 87 rows and an average of 12 pilings per row. The estimated total biomass under the Marine Terminal would, therefore, be 230,724 pounds. The second method for estimating biomass involved calculating the surface area of the mussel band based on a piling circumference of 1.5 meters and average band height of 2.6 meters. Since the biomass removed from the three 0.5-meter squared quadrats averaged 13.8 pounds, the weight of the mussel biomass per piling would be 115 pounds. This weight multiplied by 1,044 pilings equals a total estimated biomass of 120,000 pounds - significantly less than the first estimate. This discrepancy may, in part, be due to the extra water weight that accompanied the mussels as they filled the totes during the scraping process. Based on these two estimates, the biomass is likely somewhere in the middle – around 170,000 pounds. Leaving 25% of the mussels behind to spawn and recolonize the pilings would yield 128,000 pounds of mussels for potential harvesting.

Laboratory results indicated that the slightly larger mussels harvested from the pilings had higher nutrient content compared with mussels from WB and EB, particularly for carbon and phosphorus. The total percent nitrogen (wet weight) in mussels was on average 1.2% compared with 1% at WB and EB. Percent phosphorus was on average 0.10% compared with .055% at WB/EB. Percent carbon was on average 10.6% compared with 4.5% at WB/EB (Figure 22). Harvesting 128,000 pounds of mussels from all 1,044 pilings has the potential to, therefore, remove 1,536 pounds of nitrogen, 128 pounds of phosphorus, and 13,568 pounds of carbon.

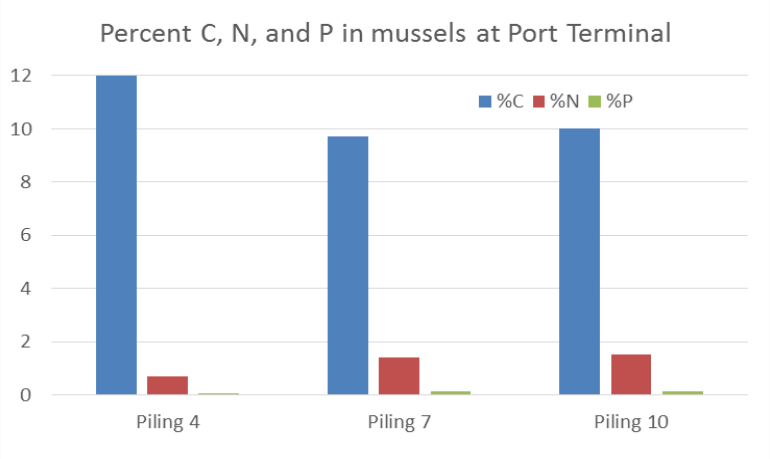

Figure 22. Percent carbon, nitrogen, and phosphorus in mussels collected at the Marine Terminal.

According to these estimates, the combination of growing a “scaled up” number of mussels (~500,000 lbs.) on nylon straps (or similar method) and harvesting mussels from pilings beneath the Marine Terminal (~100,000 lbs.) could yield approximately 600,000 lbs. of mussels removing 6,000 lbs. of nitrogen. With these larger estimates in mind, it is important to take a

closer look at how the experimental mussel installations – albeit smaller in scale – impact the surrounding environment.

Mussel Biodeposition

Filter-feeding shellfish perform the ecosystem service of transferring nutrients from surface waters to the benthic organisms below. They do so by filtering plankton from the water column and eliminating waste products in the form of feces and pseudo-feces. Pseudo-feces are ingested particles that are not used for food, but instead wrapped in mucus and expelled without passing through the digestive tract. Both of these expelled products settle to the bottom as biodeposits.

Prior research indicates that biodeposits released from the cultivation of dense assemblages of mussels can result in localized oxygen depletion when improperly sited in areas with low flushing rates (Chamberlain et al., 2001; Hargrave et al., 2008; Carlsson et al., 2012). Biodeposits are similar to phytoplankton in that they settle to the bottom and are either ingested by detrital feeders or decomposed by bacteria in a process that utilizes dissolved oxygen. This project evaluates the relative amount of biodeposits generated by mussels at each of the sites and later compares these results to DO concentrations at depth.

During August and September, sediment traps were placed beneath each of the mussel systems to measure the deposition rate, amount, and composition of biodeposits (Figure 23). Sediment traps were comprised of a 4" diameter PVC pipe 10" in length with a capped bottom end. Baffles (1cm^2 in diameter) were placed near the open top end to reduce effects of currents

Figure 23. Biodeposit collection unit (left) and transferred deposits (right).

from tides and boats that could wash sediments from the traps. The traps were weighted and lowered to a depth approximately 1-foot beneath the base of the mussel straps.

Three replicate traps were spaced evenly beneath the mussel lines and an additional three traps were placed at reference stations suspended from docks at least 50 feet away. Traps were monitored every few days to assess accumulation and retrieved after approximately 10 days. To retrieve traps, chambers were raised slowly as to not disturb surrounding mussel lines. If seawater in the traps was clear with a defined layer of deposition at the bottom, a quarter of the water was slowly poured off for transport back to the laboratory. Salinity was measured at a 6-foot depth to calculate the weight of the salt in dried biodeposit samples. Biodeposit samples (n=12) remained upright in a cooler with ice to allow suspended materials to settle overnight. The supernatant was carefully removed with a syringe to a level 2-3 cm above the deposits. The base of the chamber (containing the sediments) was separated from the rest of the unit and the deposits were transferred to a pre-weighed polypropylene container using a spoon. A small portion of the supernatant was used to rinse remaining deposits from the base. Samples were again placed in the fridge to settle overnight. Once settled, a small pipette was used to remove additional supernatant without disturbing the settled deposits. Samples were held on ice and delivered to UW Marine Chemistry Lab.

At the UW Marine Chemistry Lab, samples, including shell fragments, were dried and weighed using a Mettler-Toledo balance (Model #PR8002). The approximate weight of salt crystals was subtracted from final weights. From the dried deposit samples, 2-5 mg were subsampled (avoiding shell fragments) to analyze for total particulate carbon and nitrogen using a Leeman Labs Model CEC440 Elemental Analyzer.

The biodeposition traps remained at both sites for 11.9 days in September and 8.8 days in August. While mussel lines were absent at the reference stations, they still captured deposits from naturally occurring mussels on the dock. Because of fecal drift in the water column at both sites, this data cannot be used to quantify the exact amount of deposition per strap. Instead, these measurements can only be used to

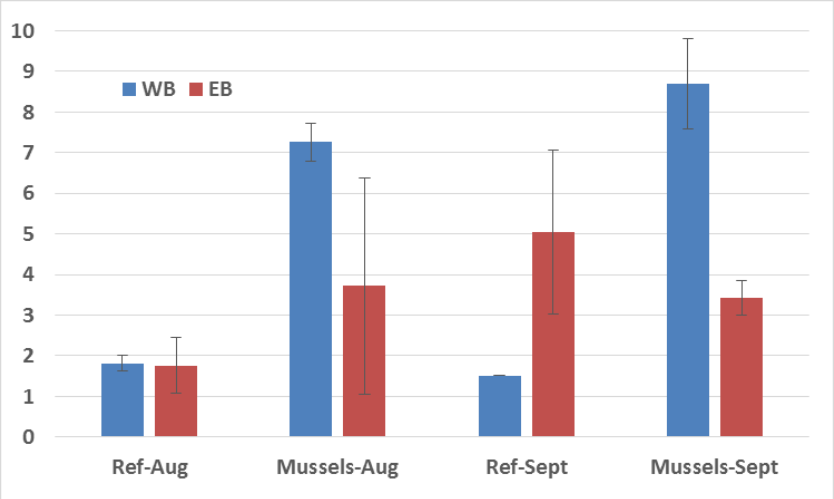

Figure 24. Average fecal deposition (g) at the reference station (Ref) and beneath mussels (Mussels) at WB and EB during Aug and Sept.

compare one site against the other or compare the mussel installation against the reference station.

Results indicate that the average amount of biodeposits collected at the reference stations remained low (< 2g) with the exception of EB in September (Figure 24). The elevated biodeposits at EB's reference station may be the result of naturally occurring mussels growing on all portions of the dock at concentrations much greater than WB. Collection tubes at WB collected more deposits than EB (>7g) and the amount increased in September as the mussels grew larger. Traps at EB collected considerably less and the amount remained consistent over time. For WB, average biodeposition rates per trap were 0.61 g/day in August and 0.98 g/day in September. For EB, rates were 0.31 g/day and 0.39 g/day respectively. The difference is likely due to the arrangement of the mussel rows at each site and the mussel biomass per strap. At EB, the mussel lines had fewer mussels and were arranged in two long rows separated by 6 feet of space. At WB, mussel lines were more densely arranged in 3 rows separated by 2 feet of space.

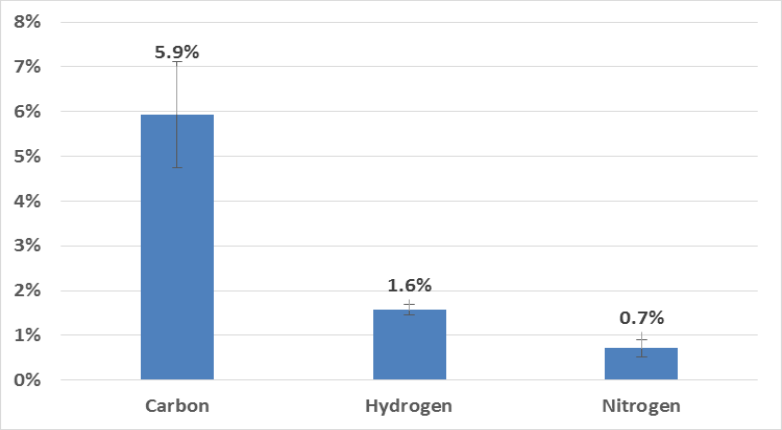

Figure 25. Percent carbon, hydrogen and nitrogen measured in mussel biodeposits – both sites and months combined.

Biodeposit composition consisted on average of 5.9% carbon, 1.6% hydrogen and 0.7% nitrogen (Figure 25). No significant difference in fecal composition was detected between the two sites or between August and September.

Water Quality

The microbial decomposition of mussel biodeposits, similar to any type of organic detrital matter, increases biological oxygen demand and also releases sequestered carbon. The Inlet is not a closed system, however, and many additional factors influence DO and pH levels including phytoplankton concentrations and water circulation. For this reason, a number of water quality parameters were measured in an effort to better understand the impact of the mussel installations on surrounding water quality.

Water quality parameters (DO, pH, water clarity, plankton, salinity, temperature) were collected at WB and EB every two weeks between July and October 2015. This data was collected in three ways using a handheld YSI Professional Plus unit as well as two YSI 6600 units. First, temperature, salinity, pH and DO were measured with the handheld YSI *Professional Plus* unit capturing one reading per visit, at 3 depths – surface, 2.5 feet (halfway down the strap) and 1 foot off-bottom. Second, once per month, the two YSI 6600 units collected side-by-side continuous data (every few seconds) as they were lowered slowly from surface to depth and back to the surface over the course of 10-15 minutes (Figure 26). Third, a YSI 6600 unit was programmed to collect continuous data within the mussel installation at a 2.5-foot depth every 15 minutes over a two week period alternating between WB and EB. For each of these three sampling scenarios, identical sampling was performed at a reference station located on a dock 50-feet away from each mussel system.

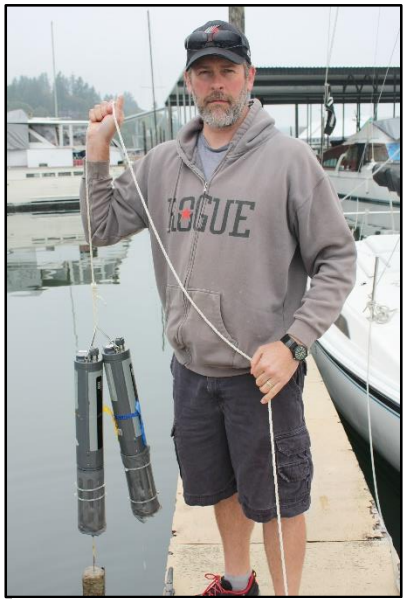

Figure 26. YSI6600 instruments

Every two weeks, water clarity was also measured at the two mussel systems and reference stations using a secchi disk. A vertical net tow was performed using a 20-micron plankton net pulled from a depth of 3-meters. Samples were collected in 125-ml glass jars and preserved with Lugol's solution until further processing. Species diversity and relative density were determined using an Olympus microscope and 0.1-ml Palmer-Maloney counting chamber.

Dissolved oxygen and pH

One concern about growing dense mussel installations is the potential to impact water quality, particularly dissolved oxygen and pH concentrations at depth. Biodeposits beneath installations can undergo several processes: ingestion by benthic organisms, decomposition by bacteria, or burial via sedimentation. Decomposition increases the biological oxygen demand resulting in decreased DO levels at depth. This process also releases, or mineralizes, particulate organic carbon into the surrounding waters in the form of carbon dioxide which can lower pH. Studies indicate that between 24-40% of the POC contained in mussel biodeposits is mineralized into carbon dioxide (Carlsson et al., 2010; Giles and Pilditch, 2006).

When considering larger scale mussel installations, the impact of mussel biodeposition on DO and pH at depth must be considered. The DO and pH data generated from this project addresses two key questions. First, do differences in DO and pH below the mussel installations at WB and EB exist when compared to their reference stations? And second, do differences exist between WB and EB overall?

In general, YSI profile data indicates that while differences did exist between DO and pH beneath rafts compared to reference stations, the differences were small, inconsistent, and likely to have no biological significance. For example, DO averages at WB were slightly lower beneath mussels in July and August, but the same in September (Table 3). At EB, DO levels beneath mussels were significantly lower in July, the same in August, but higher in September. The significant difference in DO at EB in July is difficult to explain given the small size of the mussels at this time. Instead, this calls into question the strength of the reference station itself. Boatworks at EB is a dynamic environment, and the control site, while located as far away from the mussels as possible (while still remaining on the dock), was positioned adjacent to an active boat launch/hoist. The possibility of the launch creating a more mixed environment during short, temporary periods of time, is possible. The handheld YSI data yielded similar results for August and September with small and inconsistent differences (± < 0.5 mg/l) between the mussel installations and control sites (Figure 27).

	WB		EB	
	Mussels	Ref	Mussels	Ref
Jul.	7.2*	7.4*	4.6*	6.3*
Aug.	4.9*	5.1*	1.4	1.4
Sept.	5.8	5.8	5.5*	5.3*

Table 3. DO (mg/l) at depth between mussel installations and reference stations at WB and EB. * Significant difference (p<0.05).

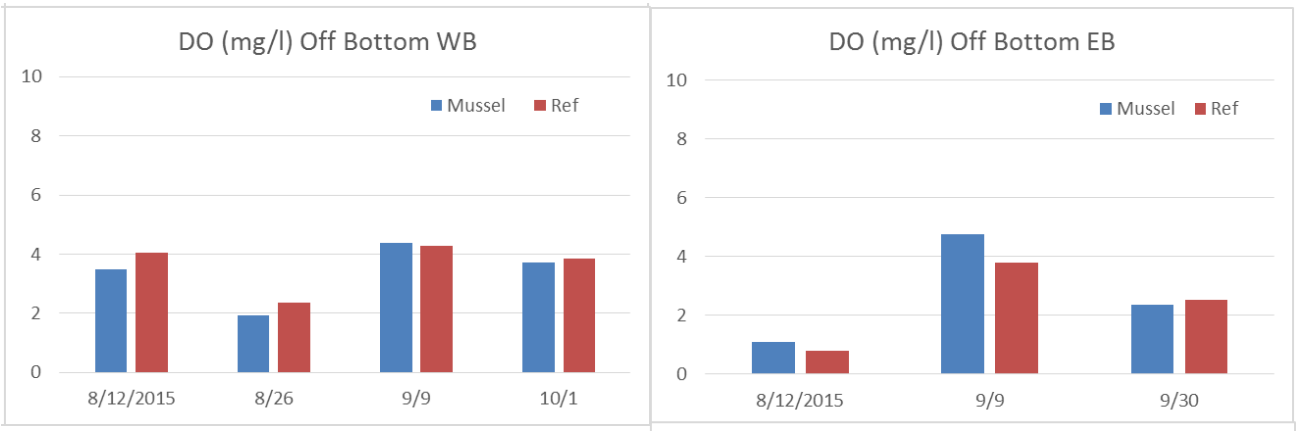

Figure 27. DO (mg/l) between mussels and reference stations at depth at WB & EB. (Source: Handheld YSI.)

YSI profile data indicates that pH differences between mussel installations at depth and the reference station were also subtle and likely to have no biological significance. In fact, mean pH values were identical at WB in July, August, and September.

At EB, pH was slightly lower under the mussels in July, similar in August, and higher in September (Table 4). While the YSI handheld data detected consistently lower pH values under the mussels, the differences were less than .1 pH units. Normal error values for this instrument are ± 0.2 as reported by the manufacturer (Figure 28). In summary, while slight differences did exist below the mussel installations for DO and, to a lesser extent pH, these differences were small and likely resulted in no biological significance.

	WB		EB	
	Mussels	Ref	Mussels	Ref
Jul.	8.0	8.0	7.7*	7.9*
Aug.	7.9*	7.9*	7.5*	7.5*
Sept.	7.8	7.8	7.8*	7.7*

Table 4. pH at depth between mussel installations and reference stations at WB and EB. * Significant difference (p<0.05).

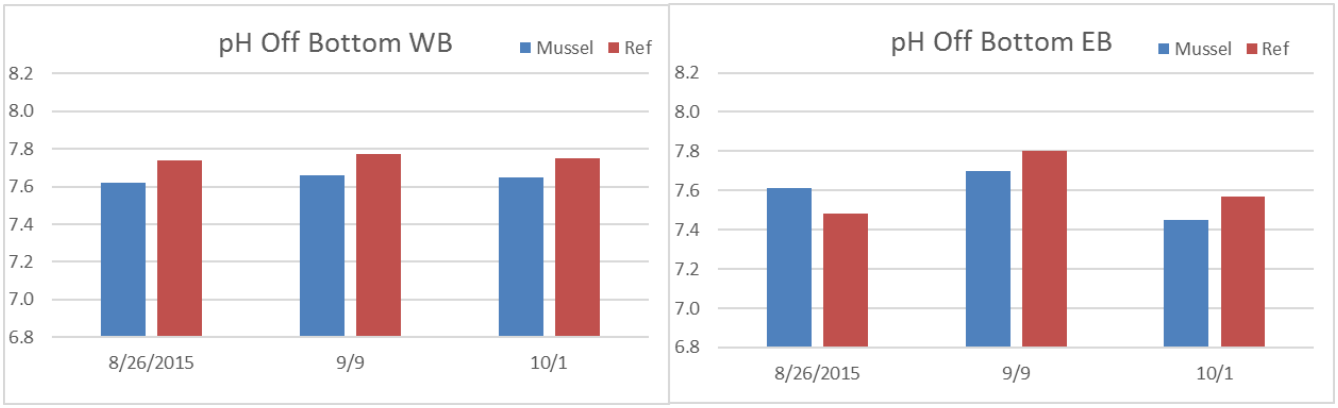

Figure 28. Difference in pH between mussels and reference sites at 2 depths at WB & EB. (Source: Handheld YSI.)

The second key question asks if differences in DO and pH exist (at depth) between WB and EB overall. Results strongly demonstrate that WB was consistently higher in DO and pH across all months and stations (mussel and reference data averaged) (Table 5, Figure 29). This does not hold true for surface waters, however, where EB remained quite rich in oxygen throughout August and September, despite oxygen depletion at depth. During this same time, pH also increased in surface waters at EB – both increases likely the result of a surface bloom of phytoplankton.

	DO		pH	
	WB	EB	WB	EB
Jul.	7.3*	4.6*	8.0*	7.8*
Aug.	4.9*	1.4*	7.9*	7.5*
Sept.	5.8*	5.5*	7.8*	7.75*

Table 5. DO (mg/l) and pH levels at depth (average between mussel installation and reference station data) at WB and EB. * Significant difference (p<0.05).

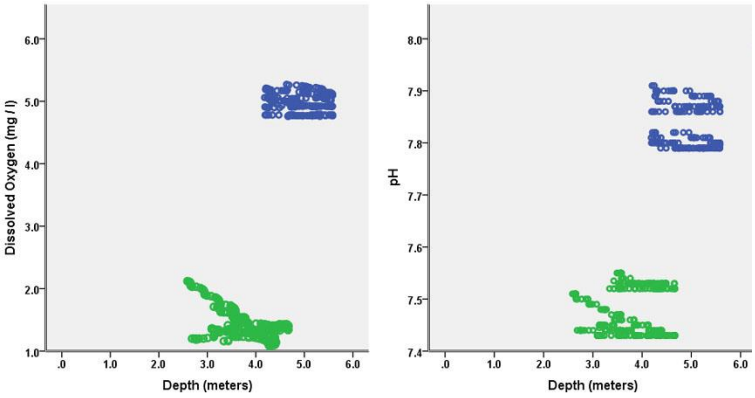

Figure 29. DO and pH at WB and EB during August. EB=Green, WB=Blue

Both the handheld YSI data and YSI6600 profile data indicate that DO in surface waters was over 100% saturated in July and decreased throughout the growing season with the lowest levels occurring in August. During this time, DO levels at depth were measured at 4.9 mg/l and 1.4 mg/l at WB and EB respectively. Handheld YSI data detected levels as low as 0.28 mg/l (4.2%) at EB during this time (Figure 30). Conditions improved considerably in September as weather patterns changed.

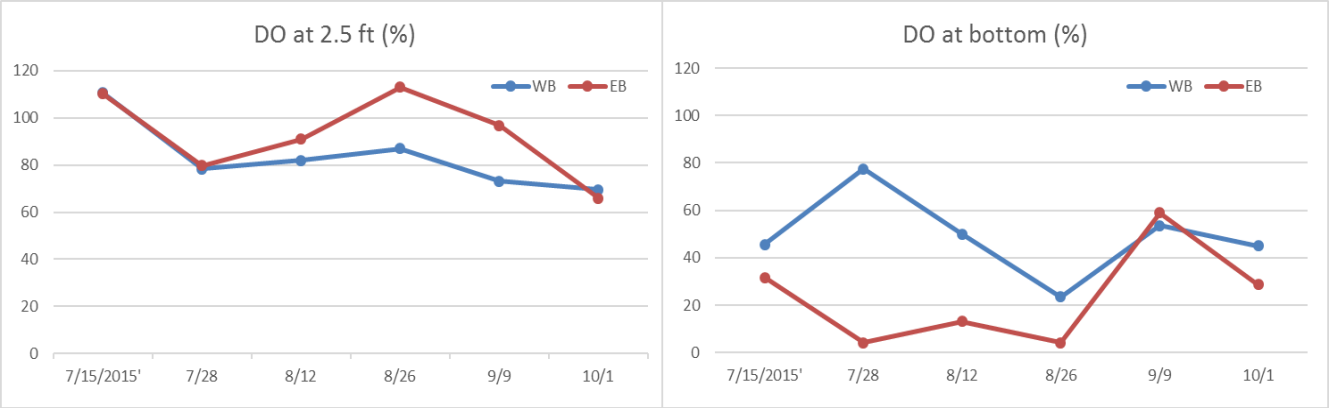

Figure 30. DO (%) at WB & EB in upper meter and off-bottom. (Source: Handheld YSI.)

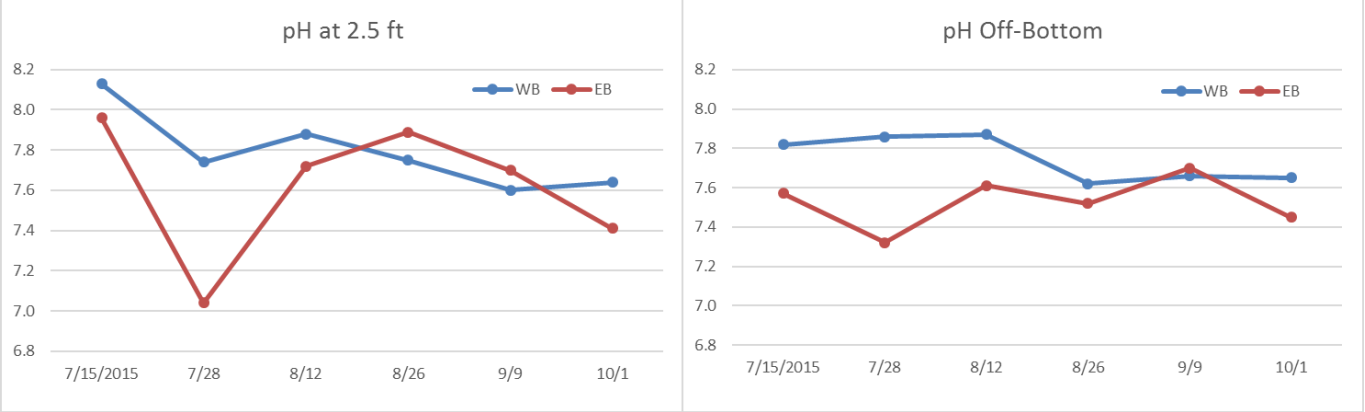

Figure 31. Seasonal pH at WB and EB. (Source: Handheld YSI.)

At WB, pH levels were also consistently higher at depth throughout most of the growing season, but the difference disappeared in September (Figure 31). Between August 28th and September 3rd, a storm system moved through the region bringing consistent rain and decreasing air temperatures from 87°F to 65°F (Weather Underground, searchable data). This cooling and mixing event improved both oxygen and pH conditions at both locations.

A significant drop in pH was detected in surface waters during late July using the handheld YSI unit. This drop coincided with a sharp decline in surface salinity (18 ppt at surface, 29 ppt at depth) on that same day. Fresh surface water pulses are common along the eastern shore of

lower Budd Inlet, particularly during peak low tides and Capitol Lake dam releases. A surface lens of fresher water could be responsible for the decline in pH given fresh water's lower pH than salt water. This would not be the case, however, at depth where salinity remained high. Overall, pH at depth was more elevated at WB throughout most of the growing season.

Results indicate that biodeposits may have slightly impacted DO and, to a lesser extent, pH early in the season as this organic matter decomposed beneath the mussel installations. These differences were very small in August and all but disappeared by September. However, the significant difference in DO and pH at depth between WB and EB suggests that other factors, such as phytoplankton and circulation, were influential on a larger scale.

Water Clarity, Chorophyll, and Phytoplankton

Lower Budd Inlet frequently experiences dense phytoplankton blooms between spring and fall as observed by WDOE's Eyes Over Puget Sound, archived WDOE marine flight data, and on-the-ground plankton sampling. Phytoplankton are important drivers of mussel growth, DO, pH, and nutrients. For this reason, biological productivity was monitored in an effort to better explain potential differences between the two sites. As such, PSI performed 3-meter vertical net tows, in addition to collecting YSI6600 Chl *a* measurements and water clarity data using a secchi disk. While suspended sediments flowing out of the Deschutes River into lower Budd Inlet have been observed after rainfall events on occasion, water clarity predominantly reflects phytoplankton concentrations.

Water clarity measurements, coupled with phytoplankton net tow observations, indicate that phytoplankton concentrations were typically more concentrated at WB, with the exception of late July, with visibility falling to less than 1-meter in mid-August during a thick *Ceratium fusus* bloom (Figure 32). This generally held true during the 2013 season as well, with secchi readings declining to 1.8 meters during that same time period.

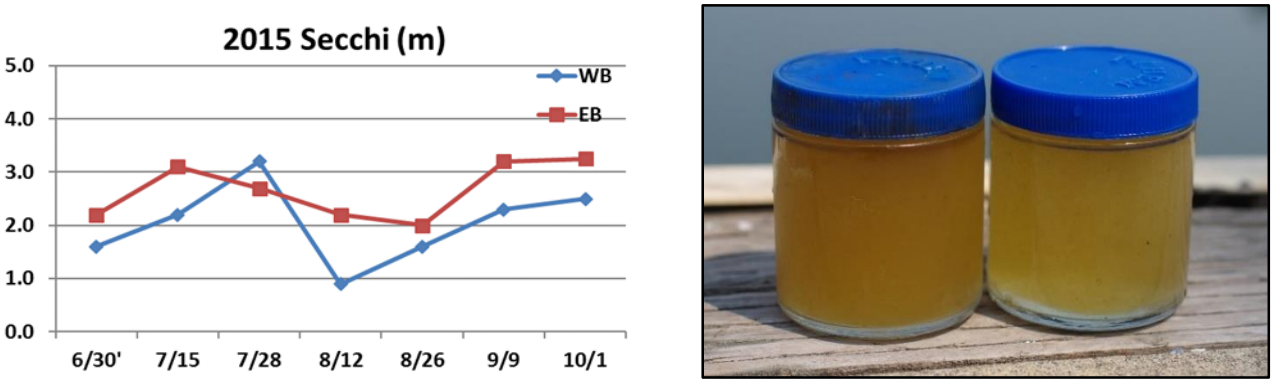

Figure 32. Water clarity (m) at WB and EB (left). Net tow samples collected on 8/12/15 at WB (left) and EB (right).

Species diversity was also higher at WB than EB during August, but similar during September (Figure 33). In August, the water at WB was particularly thick with a bloom of *C. fusus* (Figure 34). By the end of the month, the dominating species at both sites shifted to *Akashiwo sanguinea*. During August, EB also experienced blooms of *Noctiluca, Scrippsiella* and *Euglenoids*. In September, EB maintained quite a few dinoflagellates (*Scrippsiella* and *Heterocapsa*), while both sites experienced blooms of centric diatoms such as *Chaetoceros, Thalassiosira, Coscinodiscus* and *Ditylum*. In early October, both sites experienced a bloom of *Pseudo-nitzschia,* the species associated with amnesic shellfish poisoning. The EB sample included a large number of solitary cells that looked similar to *Alexandrium,* the genus associated with paralytic shellfish poisoning (*A. tamarense*?) as well as blooms of centric diatoms (*Skeletonema, Thalassiosira, Cerataulina*).

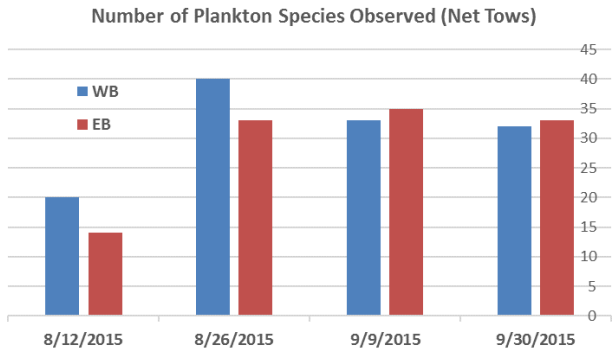

Figure 33. Species diversity (# species) at WB and EB.

Figure 34. WB mussel lines during the 8/12/15 bloom of *C. fusus* (left). *C. fusus* bloom in Budd Inlet observed by WDOE's Eyes Over Puget Sound on 7/21/16 (center), *C. fusus* and *Akashiwo sanguinea* - common Budd Inlet species (right).

Chl *a* readings from YSI6600 profile data similarly demonstrated that WB experienced more phytoplankton than EB during August with mean levels at 11.4 mg/l and 3.0 mg/l respectively and 11.8 mg/l and 6.3 mg/l in September. In late July, however, this was reversed with Chl *a* reaching 4.2 mg/L at WB and 19.5 mg/l at EB. This early bloom of phytoplankton at EB helps to explain the off-bottom decline in DO and pH by month's end.

After comparing plankton concentrations, growth rates, mussel biodeposition rates, and mussel biomass per strap between the two sites, one would expect WB to exhibit lower DO at pH at depth, particularly in August. For example, during August, WB had greater phytoplankton concentrations (higher Chl *a* values and lower water clarity), faster shellfish growth rates (0.35 mm/day vs. 0.05 mm/day), higher biodeposition rates (0.61 g/day vs. 0.31 g/day), and greater biomass per strap (28.7 lbs. vs. 6.6 lbs). The decomposition of phytoplankton and biodeposits should have resulted in greater oxygen depletion and lower pH at WB. And yet, while DO and pH levels dropped at both sites in August, the levels were significantly lower at EB. These findings suggest that circulation patterns have a strong influence on off-bottom DO and pH.

Circulation patterns have been studied extensively during the Lacey Olympia Thurston Tumwater (LOTT) wastewater treatment plant study (Aura Nova et al., 1998), TMDL process (Roberts et al., 2012), and more recently by Ahmed et al. 2017 using the Generalized Environmental Modeling System for Surface Waters (GEMSS) (Figure 35). This model uses virtual tracers (dye) that account for riverine and subtidal flows that are unique to each sub-basin. Aura Nova's 1998 results indicate that water flow is stronger near West Bay Marina (143 m3/s) as compared to the northern tip of downtown's peninsula, near Swantown Marina (0.5 m3/s). Recent findings illustrate that the concentration of dye remaining at the end of the flushing time is greater at EB than WB and that the flushing time for the remote cells is 19 days compared with 13 days across the entire Inlet (Table 6). For all 5 South Puget Sound inlets, the highest concentration of remaining dye was found at the head of the inlets, farthest away from the main basin tidal exchange. In most cases, flushing times improved with greater freshwater inflow as seen when comparing Oakland Bay with Eld Inlet (Table 6). Flushing times are also known to vary seasonally as freshwater inputs wax and wane.

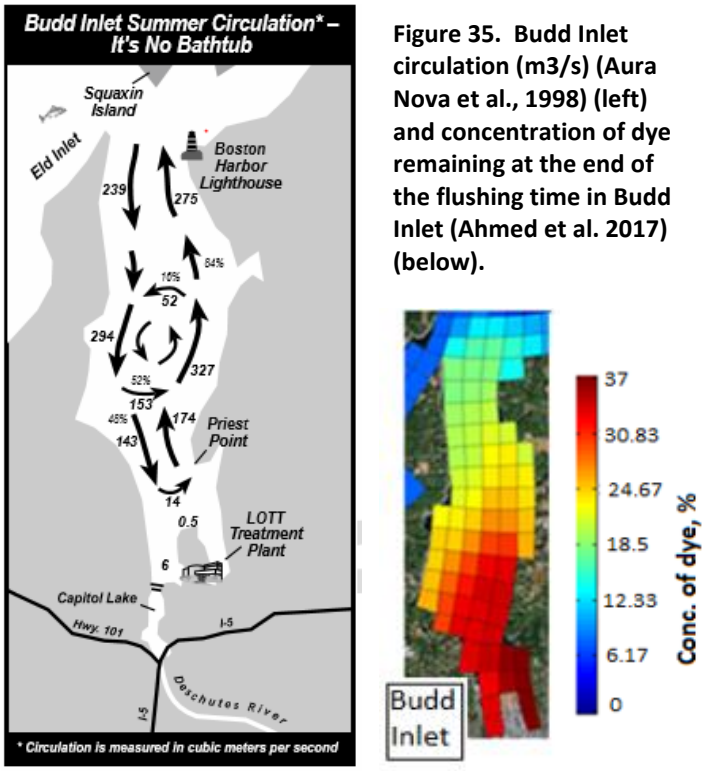

Figure 35. Budd Inlet circulation (m3/s) (Aura Nova et al., 1998) (left) and concentration of dye remaining at the end of the flushing time in Budd Inlet (Ahmed et al. 2017) (below).

Table 6. Flushing times (days) for South Puget Sound Inlets and freshwater inflows (m3/s) (Ahmed et al. 2017).

	Flushing Time (days)		
Finger Inlets	**Whole Inlet**	**Remote Cell**	**Freshwater Inflows (m3/s)**
Eld	15	36	0.31
Budd	13	19	1.87
Totten	11	16	0.82
Oakland	8	10	2.44
Henderson	3	9	0.34

Salinity and Temperature

Surface salinities were slightly saltier at WB compared with EB indicating an inflow of water from the greater Puget Sound along the western shore of Budd Inlet. East Bay is more susceptible to periodic drops in salinity, especially after rainfall events, Capitol Lake dam releases, and the combined effect of Deschutes River/Capitol Lake outflow during low tides. Moxlie Creek, while notably smaller, empties into EB as well. The greatest difference in salinity between the two sites was detected in July with surface salinities measuring 27.6 ppt at WB and 18.4 ppt at EB. Similar drops in salinity were observed in 2013 as well. At depth, salinity values were consistently between 29-30 ppt throughout the sampling season at both sites (Figures 36). While off-bottom pH dropped sharply in late-July, the decline was not attributed to low salinity. Although fresh water has a lower pH than salt water, the fresh water lens was contained within the upper meter (Figure 37). No significant difference in salinity was detected between the control sites and mussel installations at either WB or EB.

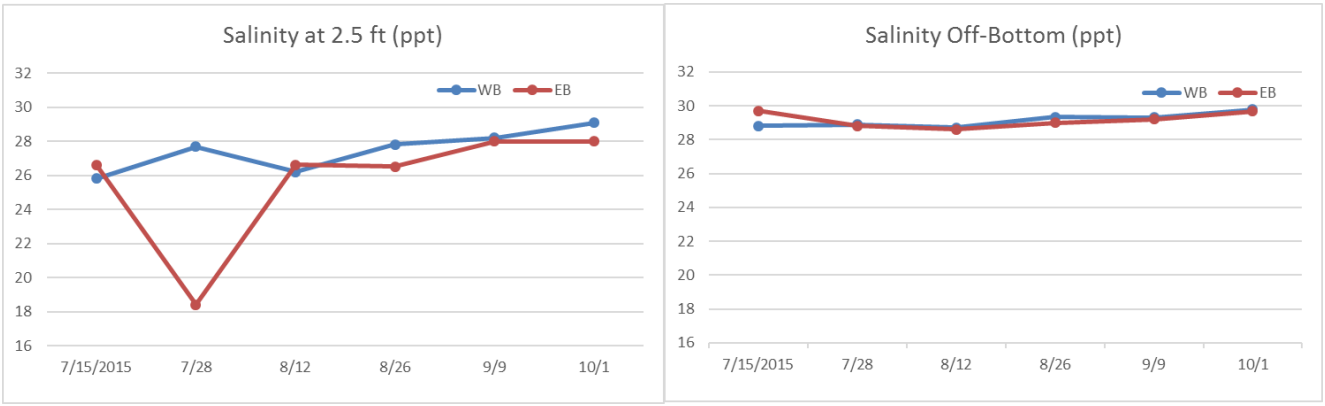

Figure 36. Seasonal salinity (ppt) at WB and EB. (Source: Handheld YSI.)

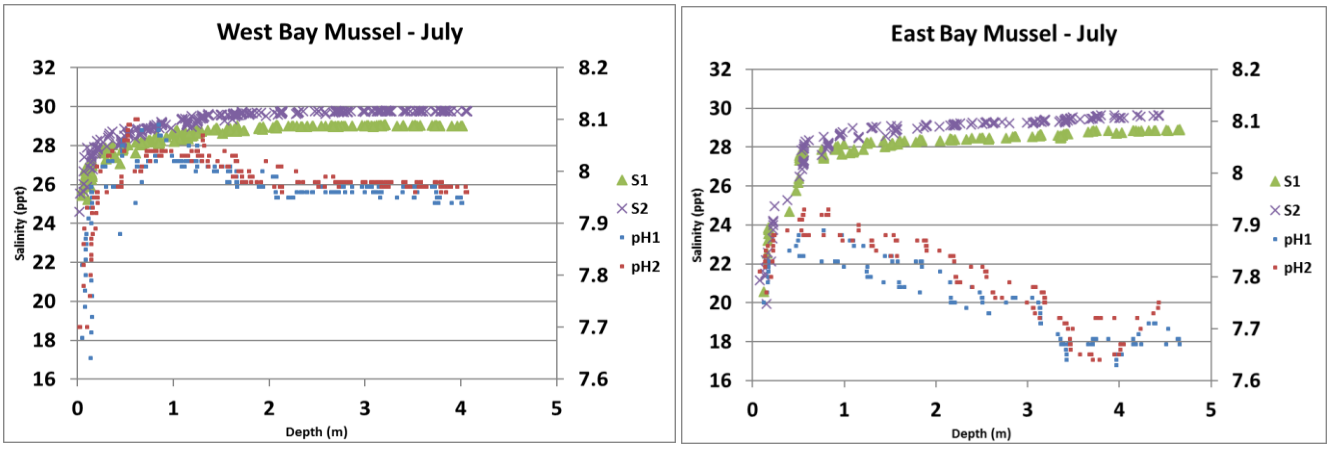

Figure 37. Salinity (S1,S2) (ppt) and pH at WB and EB using the YSI 6600, July 31, 2015.

Surface temperatures were slightly cooler at WB than EB during the latter part of the growing season. This was consistent with data collected during 2013. According to combined sources of temperature data, the warmest surface temperatures were reached during the end of July and early August exceeding 20 °C at both locations (Figures 38 and 39). The coolest surface temperatures were measured in October reaching 16-17 °C with the handheld YSI and 15°C using the YSI 1600. At depth, temperatures were consistently between 15-17°C throughout the sampling season at both sites. No significant difference in temperature was detected between the control sites and mussel installations at either WB or EB.

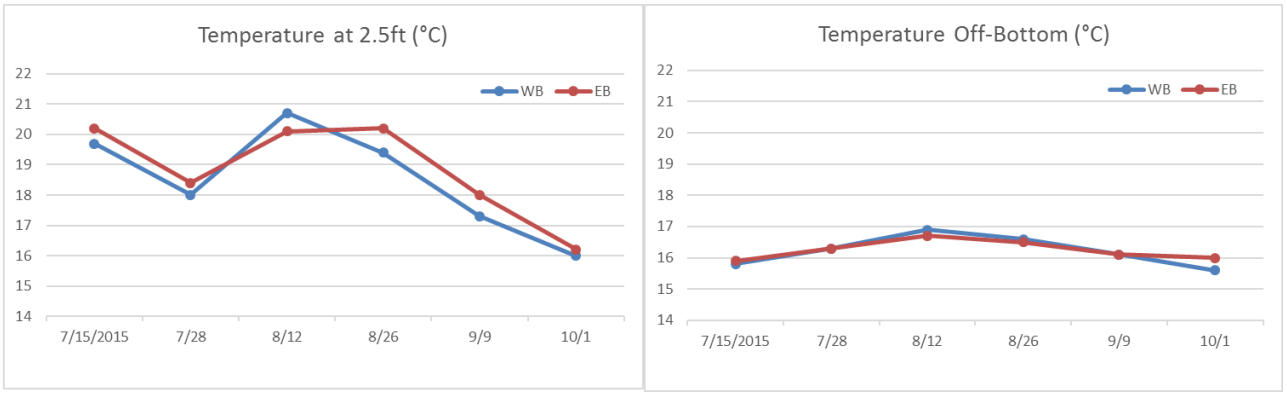

Figure 38. Seasonal water temperature (°C) at WB and EB. (Source: Handheld YSI.)

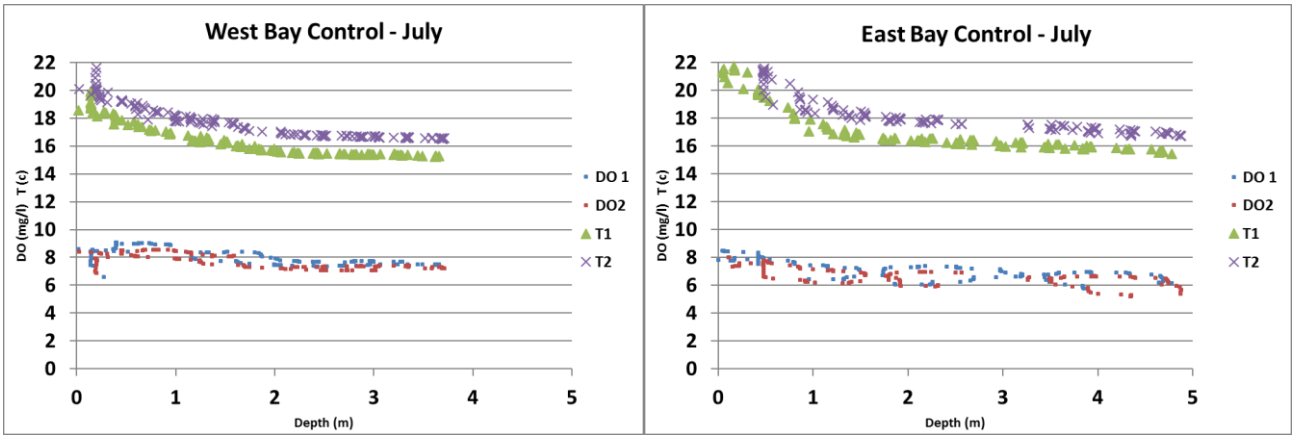

Figure 39. Water temperature and DO at WB and EB using the YSI 6600, July 31, 2015.

In summary, water temperatures were high (~20°C) and water clarity was low in July (secchi=1.5-2m) at both sites indicating that phytoplankton concentrations were rich throughout both locations. As the algae photosynthesized, surface DO levels were high– at times supersaturated – while pH was also elevated due to the removal of carbon dioxide from the water column. As early as late-July, decomposition of organic matter on the seabed floor, particularly at EB, severely robbed the water of oxygen only improving after surface temperatures dropped and water column mixing occurred in September. The lower DO and pH levels were most pronounced at EB given its poor circulation. Additionally, while slight

differences did exist below the mussel installations for DO and, to a lesser extent pH, the differences were small and likely resulted in no biological significance.

Flow-through Experiments

These results provide insight into water quality differences between WB and EB and also the impact of the mussel installations on DO and pH at depth. In order to evaluate the impact on water quality as water passes *through* a mature mussel installation, flow-through experiments were conducted. These experiments were performed in August and September at the WB location only.

For each trial, a suite of instruments were deployed at each end of the mussel installation at a 2.5-foot depth to record continuous data over a 4-5 day period. Data collected included current direction and velocity (Sontek Argonaut Acoustic Doppler Velocimeter), temperature, salinity, pH, DO (YSI 6600), and fluorescence (Turner Fluorometer and Seabird 19 unit). For the first 2 days, or 2 complete diel tidal cycles, weighted tarps were placed alongside the installation to create an isolated flow-through tunnel. The tarps extended from the surface of the water to a depth of at least 6 feet and ran along the length of the dock for a minimum of 30 feet. The tarps did not connect beneath the mussel lines. While the tarps were in place, triplicate whole water samples were collected at the downcurrent edge (N), center (C), and upcurrent edge (S) of the mussel installation using a pumped intake system from a depth of 2.5 feet (Figure 40). The sample collection time was selected based on current speed – constant but not too swift – with the current direction most closely aligned with the direction of the mussel installation. Based on this information, sampling occurred mid-way through the outgoing daytime tide, starting at the downcurrent edge of the system. Triplicate samples were also collected at a reference station (R) positioned approximately 50-feet east of the mussel installation.

Whole water samples were evaluated for phytoplankton concentration (cells/L) and speciation, dissolved nutrients (NH4, SiO4, PO4, NO3, NO2), dissolved organic carbon (DOC), particulate organic carbon (POC) and particulate nitrogen (PN). Samples, including equipment blanks, were collected using supplied autoclaved bottles, syringes, and filters and adhering to standard procedures described under Laboratory Sampling Procedures for DOC, POC, PN and nutrients (www.ocean.washington.edu/file/Sampling+Procedures). Chemical analyses were performed at the University of Washington Marine Chemistry Laboratory. Phytoplankton samples were preserved with Lugol's solution until further processing at PSI.

Figure 40. Flow through experiment at WB. S-south, C-center, N-north, R-reference. Gold stars indicate sampling locations and red arrow indicates direction of tidal flow during water sampling.

At the UW laboratory, carbon and nitrogen samples were run on a Leeman Labs Model CEC440 Elemental Analyzer with appropriate QA/QC. Refer to the Quality Assurance Project Plan for detailed methods, detection limits and quality control. Plankton samples were settled overnight at PSI's laboratory, concentrated 10-fold and quantified using an Olympus microscope and 0.1-ml Palmer-Maloney counting chamber. Cell counts for over 40 species were tallied individually and then grouped as centric diatoms, pennate diatoms, dinoflagellates, zooplankton, and other (Appendix C).

Results indicate that the tarps increased the current speed from approximately 5 cm/sec to 30 cm/sec, with a maximum recorded velocity of 60 cm/sec (in August) and 90 cm/sec (in September). The tarps also directed water flow in a more aligned path with the mussel installation (190°/340°) (Figure 41). Whole water samples were collected at the south edge, center, and north edge of the installation during current velocities of approximately 4-6 cm/sec. Current direction and speed plots were similar during both the August and September flow through experiments.

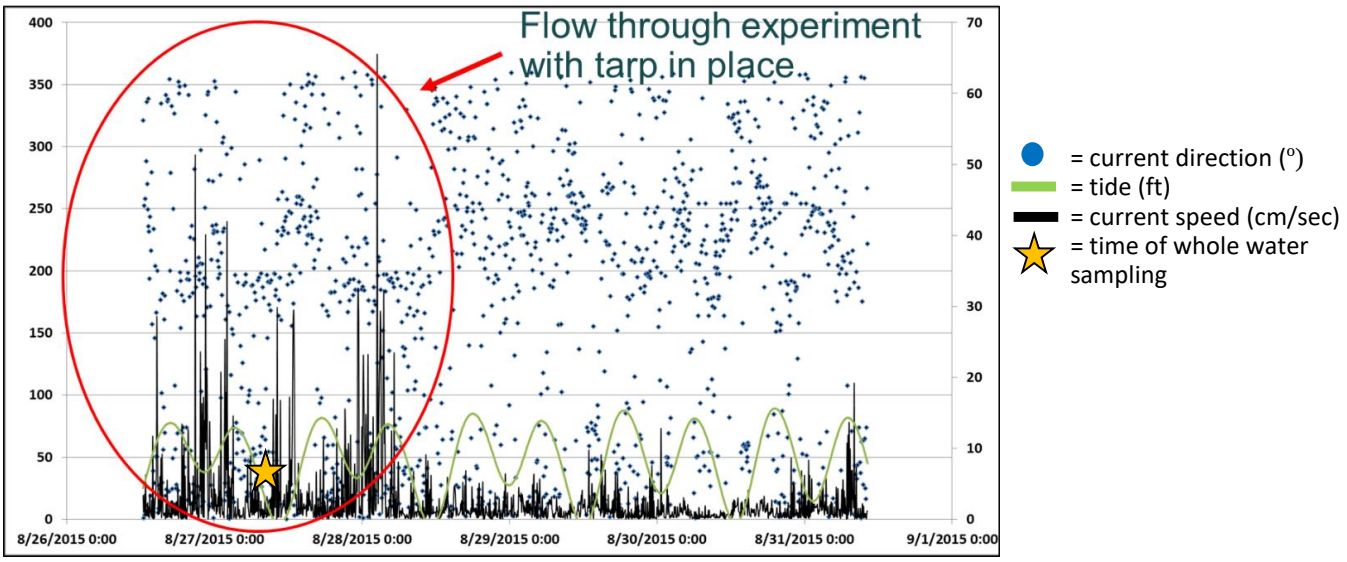

Figure 41. Current direction (degrees, left axis) and speed (cm/second, right axis) during August flow-through experiment.

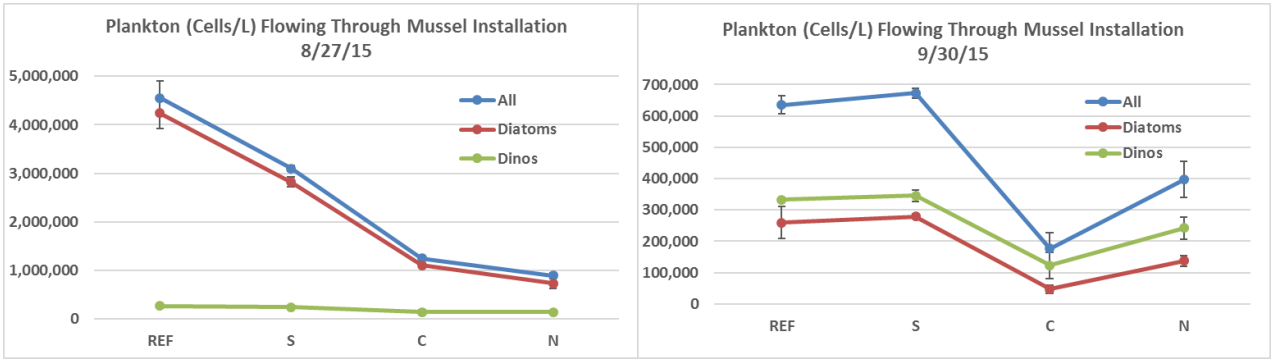

Figure 42. Plankton (cells/L) passing through the mussel installation at WB.

As water passed through the mussel installation from south to north, phytoplankton cell counts significantly declined. In late August, cell counts decreased by 71% between the south edge and north edge of the mussel system (Figure 42). Maximum cell concentrations were high – over 4 billion cells/L – and dominated by diatoms such as *Leptocylindrus minimus*, *Chaetoceros spp.*, and *Pseudo-nitzschia*; dinoflagellates such as *Akashiwo sanguinea* and *Prorocentrum*; and

the chlorophyte *Euglena*. In late September, cell counts decreased by 41% between the south and north position, and 74% between south and center. The increase in cell counts along the north edge of the installation likely indicates outside water leaking into the tunnel system.

In late September, maximum cell concentrations were moderate – over 600,000 cells/L – and dominated by larger dinoflagellates such as *Akashiwo sanguinea*, *Ceratium fusus*, *Heterocapsa* and *Protoperidinium spp*. and, to a lesser extent, diatoms such as *Thalassiosira spp*. The species composition of phytoplankton shifted between late August and late September with blooms of chain forming diatoms (93% diatoms) being replaced by predominantly dinoflagellates (41% diatoms). This composition shift is typical for this time of year and location. Diatoms tend to thrive under moderate light and high nutrient conditions encountered during spring and fall. Dinoflagellates, on the other hand, are better adapted to high light, low nutrient and stratified conditions found during mid to late summer. The shift from diatoms to dinoflagellates has been associated with various factors including a decrease in available silica, limited nutrient availability in surface waters, temperature, and grazing by dinoflagellates. Silica is an essential nutrient for diatom growth. Diatoms require silica to build their frustules, whereas dinoflagellates require cellulose to build their plates. Additionally, dinoflagellates, with some mobility, maintain an advantage over diatoms in that they can migrate vertically to access nutrients found at depth.

The ratio of N:P and Si:N have also been used to evaluate the cause of community shifts from diatoms to dinoflagellates (PSMW, 2012). Our data reports a low, but rising N:P ratio (0.09 µM to 0.87 µM) and declining Si:N ratio (153 to 19) perhaps suggesting that silica may have been a driver in the community shift (Table 7).

	Aug	Sept
% diatoms	93%	41%
N:P	0.089	0.874
Si:N	153	19

Table 7. Shift in plankton composition related to nutrient ratios (µM). P=PO4, N=NO3+NO2

Our results indicate that silica concentrations were higher when sampled in August than September with an average of 70.1 ± 0.31 µM and 61.6 ± 0.12 µM

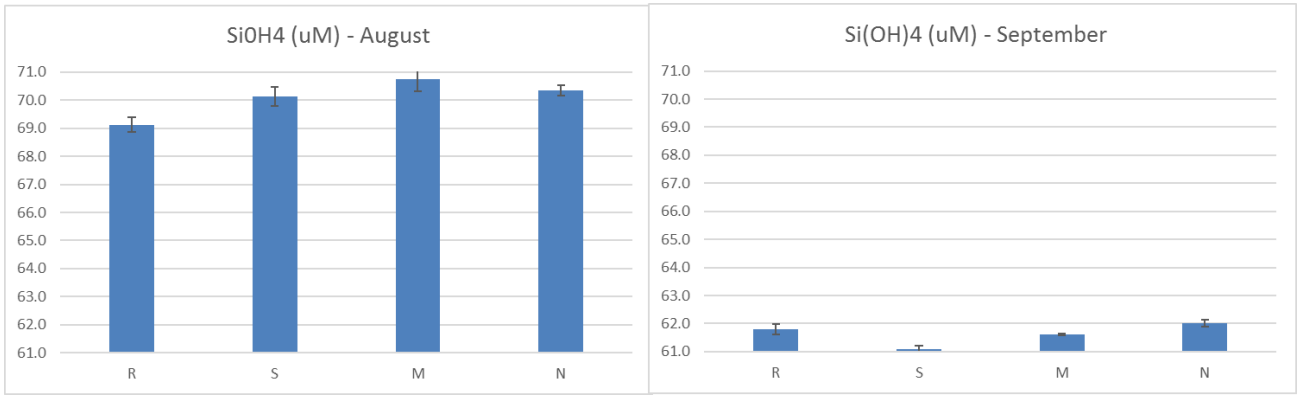

Figure 43. Silica concentrations (µM) passing through the installation.

respectively (Figure 43). Silica concentration data collected from lower Budd Inlet during historic WDOE's marine flights ranged from 6 – 184 µM with the highest values occurring during the winter and lowest values in spring (WDOE, searchable database, BUDD002). WDOE readings between 60-70 µM have been detected during August and September indicating that our data was within the normal range. A slight increase in silica as water passes through the mussels may suggest that as phytoplankton were filtered out of the water column (from south to north), more available silica remained.

Fluorescence sensors were used to measure chlorophyll *a* (Chl *a*), one of the main pigments used by phytoplankton during photosynthesis. August readings ranged from 1 - 28 µg/l with an average of 13.8 µg/l over a 5-day period. September values ranged from 1-22 µg/l with an average of 5.3 µg/l – levels waxing and waning with the tides (Figure 44). The strongest fluorescence readings were detected mid-way through the outgoing tide at the southern edge of the installation. The notable decrease between the southern and northern edge indicates removal of phytoplankton via mussel filtration. The lowest readings were measured during slack low tides. Once mussel lines were harvested on October 1st, fluorescence values were practically identical on each side of the dock during incoming and outgoing tides.

Chl *a* values typically range between 0-20 µg/l throughout Puget Sound with Budd Inlet capable of exceeding this range four-fold during strong spring and summer blooms (PNNL, 2012). Historic WDOE marine water quality data for lower Budd Inlet reported Chl *a* readings between

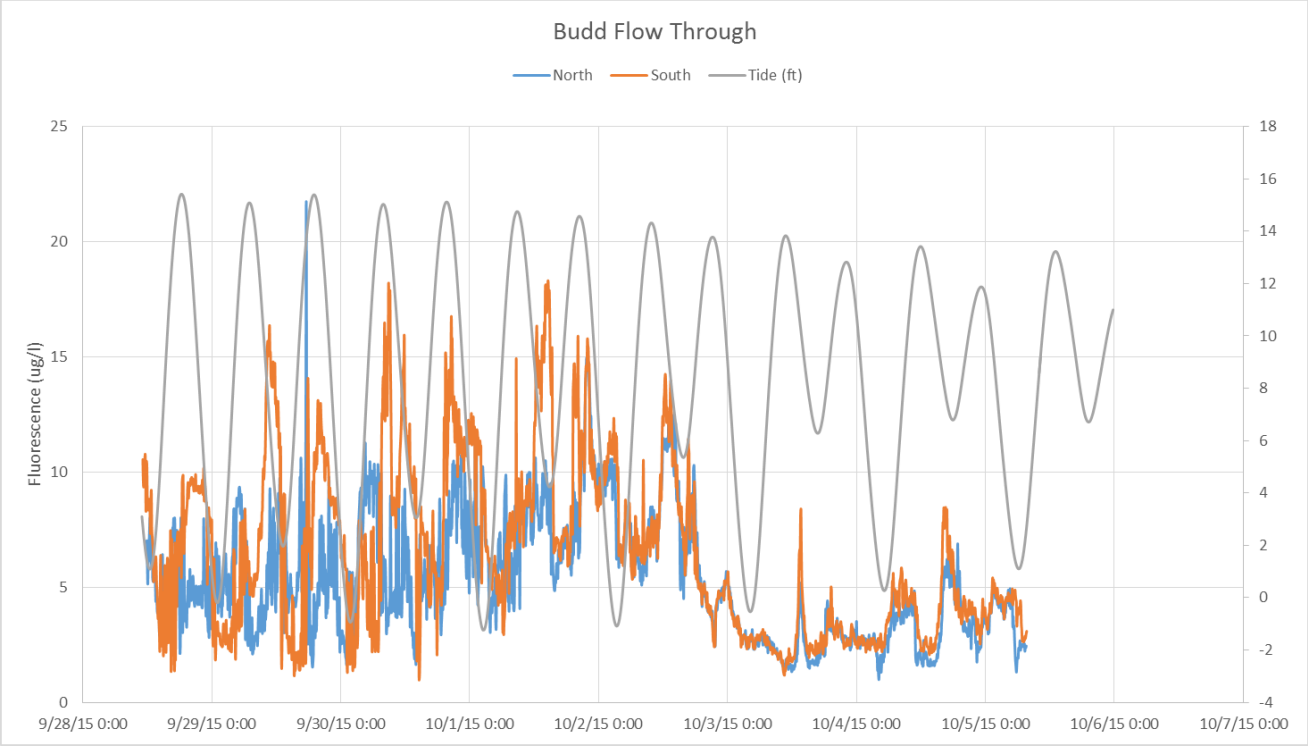

Figure 44. Fluorescence values (µg/l) at north and south edge of the mussel installation at WB.

0.5 and 88 µg/l throughout the year with elevated levels in spring and fall and lower readings in early summer. More specifically, Chl *a* data was detected between 2-7 µg/l in August and 15 µg/l in September (small sample size). These values are consistent with our data for this time of year.

In summary, the fluorescence measurements were positively associated with phytoplankton cell count data. As water flowed through the mussel installation, the mussels removed phytoplankton resulting in lower fluorescence readings. Over the course of several days, fluorescence values remained slightly higher at the north edge of the installation during incoming tides and significantly higher on the south edge during outgoing tides.

As water passed through the mussel installation, particulate organic carbon (POC) and particulate nitrogen (PN) concentrations also declined (Figure 45). POC is comprised of heterotrophic bacteria, phytoplankton, zooplankton and detritus. As mussels remove plankton from the water column, POC and PN levels would be expected to decline as well. In August and September, POC and PN decreased on average by 58% and 60% respectively as water passed from the south edge to center position of the installation. This decline was consistent with decreases in phytoplankton cell count data and fluorescence.

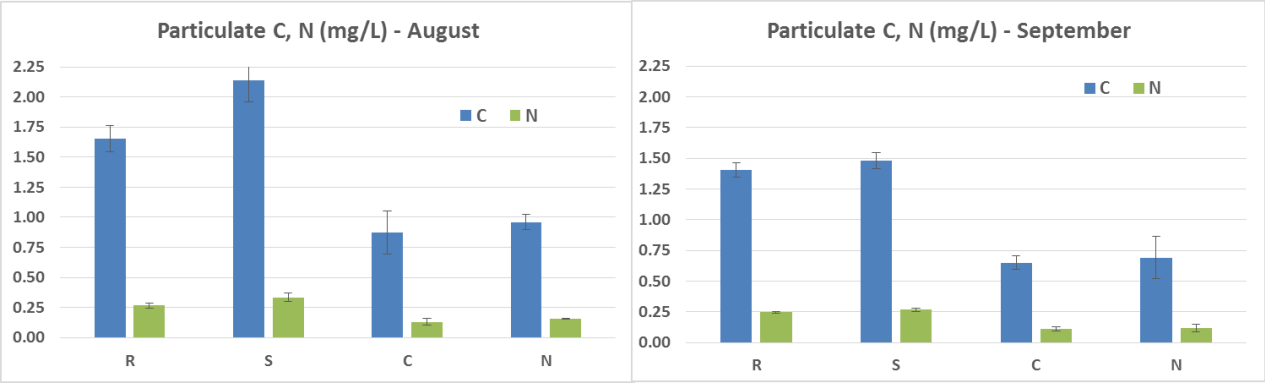

Figure 45. Particulate organic carbon and particulate nitrogen (mg/L) in water passing through the mussels.

Dissolved nitrate and nitrite concentrations were approximately 6 times greater during late September than late August (Figure 46). In late August, both of these nutrient concentrations were higher within the mussel installation when compared to the reference station. Similar to silica, this increase immediately surrounding the mussels might be due to a reduction in nutrient sequestering diatoms. Nutrients increased slightly as the water passed through the mussels, although the difference was not statistically significant. In late September, no difference was observed in nitrates and nitrites between the reference station and mussels or as water flowed through the system.

The surface concentration of nitrates and nitrites *combined* is typically between 25-30 µM in most parts of Puget Sound throughout the year (PNNL, 2012). This concentration decreases during the summer particularly in shallow fjordal sub-basins like Budd Inlet where levels below 2 µM are not uncommon due to phytoplankton sequestration. WDOE marine flight data for lower Budd Inlet measured nitrate concentrations ranging from 0.06-30.0 µM throughout the year with the highest concentrations found during winter months and lowest concentrations during the summer (WDOE, searchable database, BUDD002). For example, August and September nitrate levels averaged 1 µM and 3.5 µM respectively. Marine flight data for nitrites ranged from 0.01-1.3 µM in lower Budd Inlet with an average of 0.04 µM in August and 0.30 µM in September. The nitrate and nitrite levels measured during our sampling events, therefore, were normal for August and September and the mussels appeared to slightly increase nutrient levels within the installation during August, but not September.

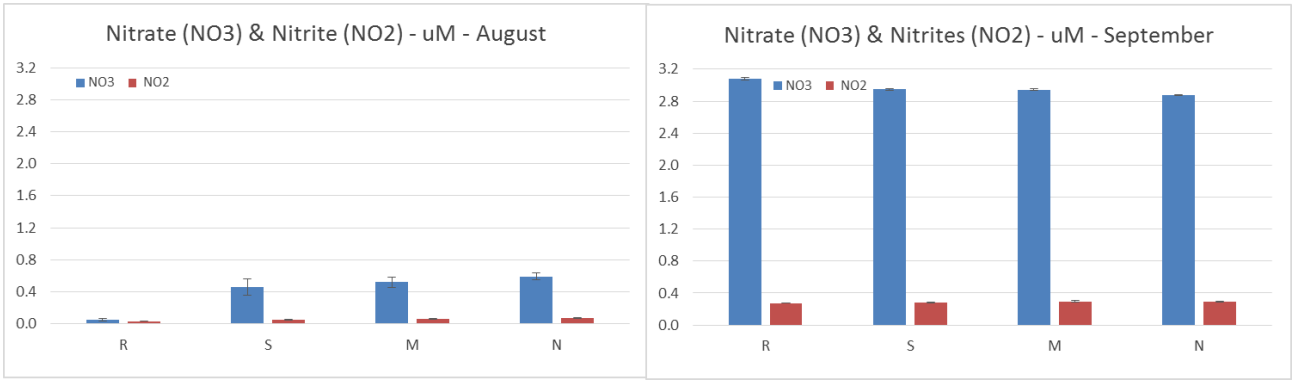

Figure 46. Dissolved nitrates and nitrites (µM) in water passing through the mussel installation.

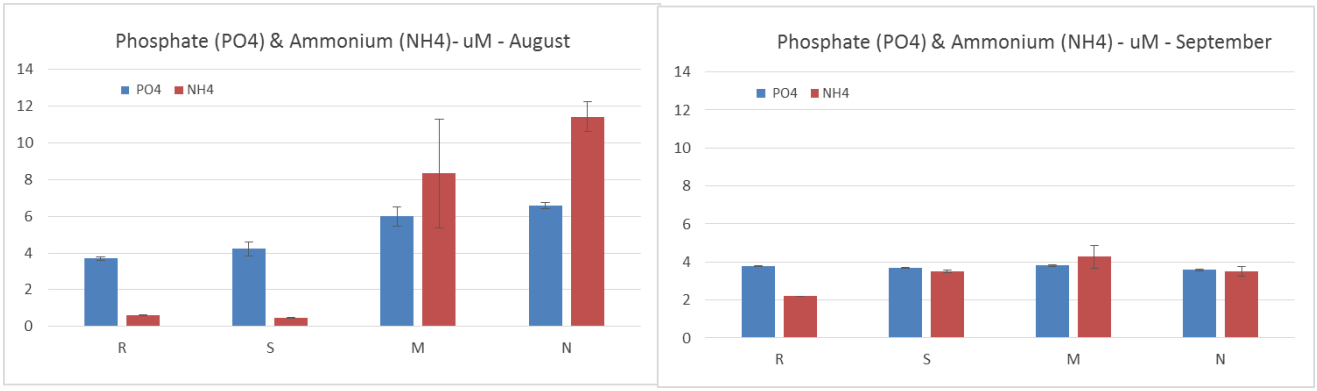

Figure 47. Dissolved phosphates and ammonium (µM) in water passing through the mussel installation.

Dissolved phosphates and ammonium concentrations were elevated within the mussel system in August (Figure 47). Ammonium, in particular, increased as water passed through the mussels indicating the release of this nitrogenous waste products. In September, no increase in phosphates and a slight increase in ammonium was observed as water passed through the mussel system. The elevated levels of ammonium within the mussel installation appear to be

associated with peak recorded growth rates for that time period. From mid to late August, mussels grew 0.36 mm/day in length and 0.04 g/day in weight compared with 0.17 mm/day and 0.03 g/day between mid to late September. As mussel growth declined, so did the release of ammonium into the surrounding water column.

Phosphate levels measured throughout Puget Sound surface waters are typically between 2-3 µM dropping as low as 0.8-2.5 µM in Budd Inlet during the summer (PNNL, 2012). Marine flight data for lower Budd Inlet measured phosphate concentrations ranging from 0.9 µM to 3.3 µM throughout the year with the lowest concentrations found during spring and the highest during late summer. Specifically, phosphate levels averaged 2.4 µM in August and 2.8 µM in September. Similar to the seasonal pattern of phosphates, historic ammonium concentrations in lower Budd Inlet ranged from 0.7 µM to 10.7 µM with the lowest concentrations in spring and highest concentrations in late summer. Average ammonium levels were 3.4 µM in August and 8.7 µM in September. Based on this historic data, phosphates and ammonia were slightly elevated within the mussel installation in August, but phosphates were normal and ammonia was below normal in September.

The amount of dissolved organic carbon (DOC) in seawater was slightly higher in August than September, but not significantly different between the reference site and mussel installation (Figure 48). DOC is a result of the decomposition of dead organic matter such as fresh or marine plants and animals. It serves as an important food supplement for microorganisms in the marine environment. The mussel installation does not appear to be removing or adding DOC to the water column. Typical values in undisturbed watersheds range from 1-20 mg/l with the Everglades at the upper range and the open ocean at the lower range. Additional approximations range from Puget Sound river tributaries at 4 mg/l to wastewater as high as 70 mg/l (PNNL, 2012).

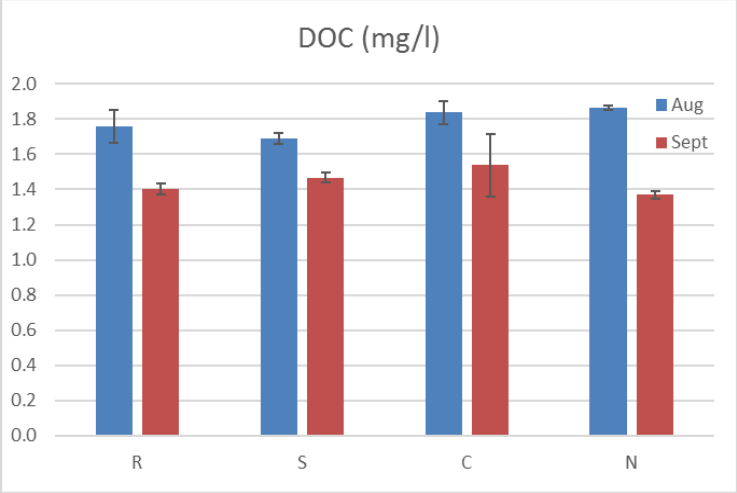

Figure 48. Dissolved organic carbon (mg/l) in water passing through the mussel installation.

The impact of the mussel installations on Chl *a*, DO and pH within the upper meter of the water column was also evaluated. Results indicate that all three parameters moved in sync with one another – waxing and waning with the tides (Figure 49). In general, incoming tides tend to

carry more phytoplankton which, through photosynthesis, produce more DO and reduce carbon dioxide levels thus increasing pH. The water in the center of the mussel installations would be expected, therefore, to be lower in Chl *a*, lower in DO and have a lower pH.

Chl *a* concentrations were, in fact, significantly lower within the mussel installations when compared to the control station in July and September. DO levels were slightly lower adjacent to mussels during most months, although the difference was only significant in August (Table 8). On the other hand, pH was lower within the mussel installations at both sites during all four months.

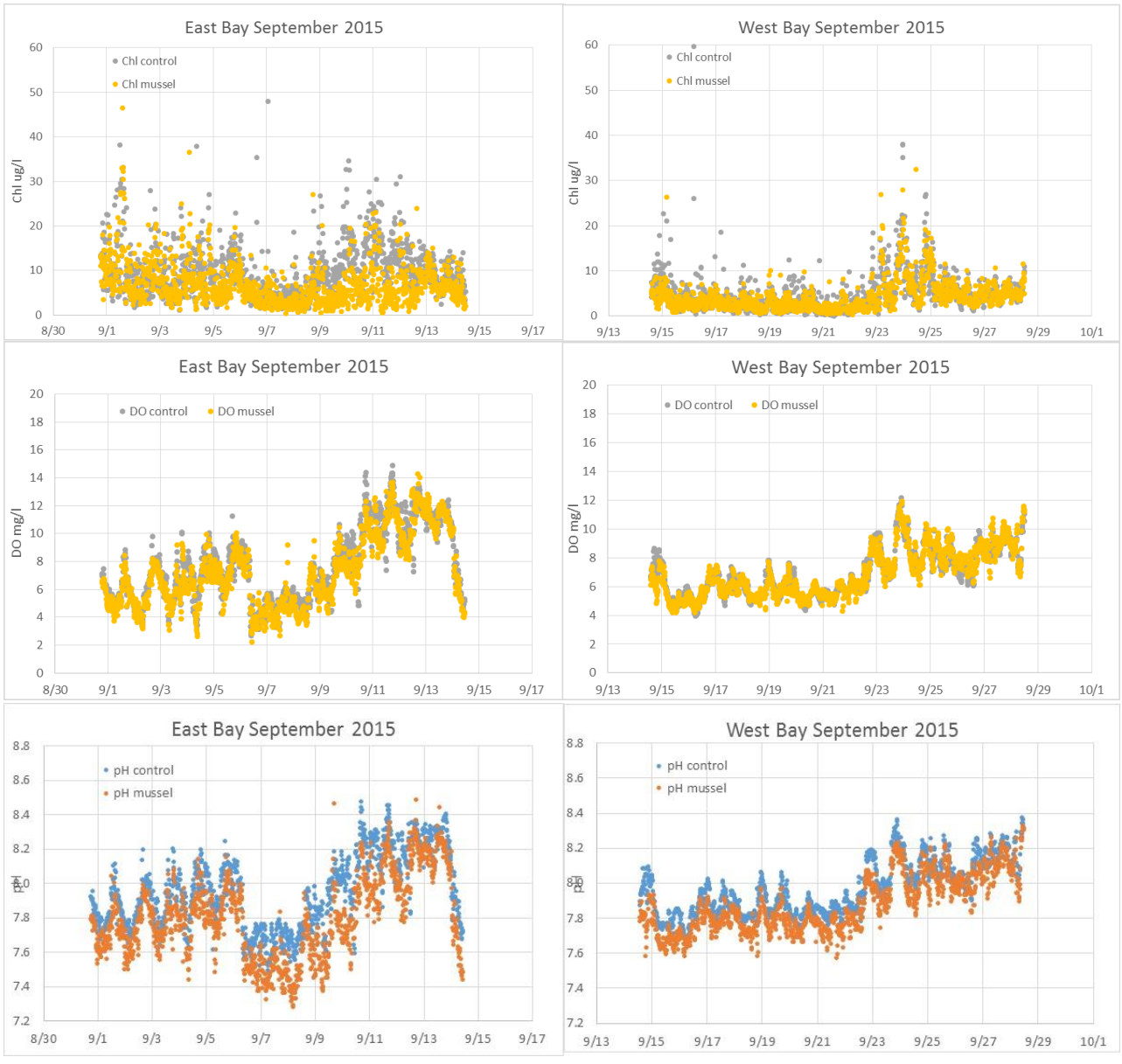

Figure 49. Chl *a* (top), DO (middle), and pH (bottom) in upper meter of mussel installation and reference station over 2-week intervals. (Source: YSI6600.)

DO	EB		WB	
	Mussels	Ref	Mussels	Ref
Jul.			7.2	7.6
Aug.	5.8*	6.2*	6.2*	6.7*
Sept.	7.2	7.6	6.9	6.9
Oct.			7.2*	6.8*

pH	EB		WB	
	Mussels	Ref	Mussels	Ref
Jul.			7.8*	7.9*
Aug.	7.8*	8*	7.8*	8.1*
Sept.	7.8*	7.9*	7.9*	8*
Oct.			7.8*	7.9*

Table 8. DO and pH averages between mussels and reference stations in the upper meter at WB and EB.
*** Significant difference ($p<0.05$).**

In summary, as water passed through the mussels, plankton cell counts, Chl *a*, and POC/PON concentrations declined. As phytoplankton (and photosynthesis) decreased, the amount of nutrients such as silica, nitrates, nitrites and phosphates remained slightly elevated. The pH, and to a lesser extent DO, also tended to remain lower. The waste product ammonia was significantly elevated near mussels particularly during times of rapid growth. With the exception of ammonia in August, all parameters were within normal ranges for lower Budd Inlet.

OBJECTIVE 3 – Compost mussels for agricultural application.

Over 6,000 pounds of whole mussels were harvested and turned into Surf-to-Turf mussel compost. On 10/1/15, 2,210 lbs. were harvested from WB and delivered to The Evergreen State College's Organic Farm. On 10/12/15, 2,800 lbs. were harvested from EB and delivered to the Washington Department of Corrections' Cedar Creek facility and to PSI employee, Dr. Steven Booth's, home residence. On 5/9/16, 1,000 lbs. were harvested from pilings at the Port of Olympia's Port Terminal and also delivered to Dr. Booth's residence. At each of these destinations, PSI staff, in combination with composting partners, weighed the whole mussels, chipped them using a wood chipper, and re-weighed the final material. The Port of Olympia provided space at Boatworks for chipping, pressure washing, and strap disposal for mussels harvested from the EB location.

At TESC's Organic Farm, mussels were combined with a mixture of green waste from the garden, compost, and wood chips. Feedstocks were placed into a manure spreader and mixed directly into a "negative aeration compost reactor" lined with a 6-inch layer of wood chips (Figure 50). The compost remained in the reactor for one month during which time pile temperatures were monitored. The finished compost was transferred to a covered storage bay to cure (Figure 51). No odor problems were encountered after the feedstocks were mixed. After the curing phase, triplicate samples were collected from various points in the pile and sent to Soiltest Farm Consultants for analysis.

Figure 50. Chipping mussels (left) and loading mussels and green waste into the "reactor" (right) at TESC.

Figure 51. Finished compost at TESC Organic Garden.

At the WDOC location, mussels were composted by inmates under the guidance of Environmental Planner, Eric Heinitz. The composting facility hosts an Enviro-Drum, an in-vessel composting system (8-yard operational capacity) made by DT-Environmental (Lynden, Washington) originally designed to compost dairy waste (Figure 52). The system is equipped with a biofilter for odor control. Chipped mussels and additional feedstocks (recycled and chipped bed mattress frames, unscreened compost, kitchen food waste, shredded paper) were loaded into a mixer at a ratio of 1:3 (mussels : other) where they were mechanically shredded, blended and conveyed into the rotating drum via a feed auger. The compost was discharged from the drum after 20 days and transferred to a covered curing bay.

Figure 52. The Enviro-Drum at WDOC's Cedar Creek Facility.

Two batches of mussel compost were generated at Dr. Booth's residence in fall and spring. The first batch contained chipped mussels and Douglas fir hog fuel purchased at Great Western Supply in a 1:3 ratio. Feedstocks were combined manually using pitchforks and shovels. For the second batch, mussels were mixed in the same ratio but with a blend of sawdust and fir shavings. The combined chipped mussels were then loaded into an aerated 2.5-cubic yard capacity micro-bin compost system constructed by Dr. Steven Booth (PSI) modeled after the O2Compost Bin used at WSU's Composting facility in Puyallup, WA (Figure 53).

Figure 53. Micro-bin composting system.

The unit was covered with a loose lid to divert rainfall but still allow airflow. The bin was equipped with an aeration system consisting of a blower connected to two perforated 4-inch pipes placed on the bottom of the composting chamber. The blower was operated on a cycle to maintain temperatures between 55° C and 70˚ C (131° F - 158° F) during active composting. To meet this temperature range, the blower typically ran 30-60 seconds on and 30-60 minutes off over a period of several months. Specific cycling times were adjusted based on temperatures and moisture trends in the pile. Temperatures were monitored regularly and both batches exceeded 150° F within the first week of composting. After curing, triplicate compost samples were collected and sent to SoilTest for analysis (Figure 54).

Figure 54. First batch of finished compost.

The Surf-to-Turf mussel compost was used on-site at TESC's Organic Farm and WDOC's Correctional facility. Several totes were also delivered to Marshall Middle School's Citizen Science Institute (CSI) Program where students used the compost to perform vegetative growth trials. Approximately 1.5 cubic yards of compost were distributed to the general public during the City of Olympia's Great Yards

Figure 55. Compost giveaway at the Great Yards Get Together event, Capitol Lake.

Get Together event (Figure 55). The event was covered in a full-feature article in The Olympian on September 11, 2016 titled, *Great Yards Get Together – Event guides gardeners to eco-friendly approach*. An additional 0.5 cubic yards were disseminated to the public at community events, presentations, and meetings including the Turning of the Tides Festival, Point Defiance Zoo and Aquarium presentation, Return to Evergreen event, Department of Enterprise Services staff meeting, and a Deschutes Advisory Committee meeting. In May, 2017, 2 cubic yards of mussel compost will be applied to landscapes on Capitol Campus managed and maintained by staff at Washington State Department of Enterprise Services. Interpretive signage will be placed on site.

Mussel Analysis – Trace Metals, PAHs, PCBs

Immediately prior to harvest, mussel samples were collected at all 3 sites for laboratory analysis. Three composites of 30 mussels each were collected for nutrients (total nitrogen, total organic carbon, phosphorus) and metals (copper, nickel, arsenic, cadmium, lead, mercury). Samples harvested from Port Pilings were also tested for PAHs and PCBs. Because PAHs and PCBs were analyzed at WB and EB in 2014, coupled with the high cost of testing, these elements were only tested at the Port Pilings in 2016. In the field, mussels were randomly collected from three equally dispersed straps at a depth of 2.5 feet, and from quadrats placed on three pilings at the Port Pilings. Lengths and composite weights were recorded prior to placing the mussels into sealable plastic bags and holding on ice. Mussels were frozen and delivered to AmTest Laboratories for processing.

Once delivered to AmTest Laboratories, mussel composites were homogenized (tissue and shell combined) and analyzed following Standard Operating Procedures found at: www.amtestlab.com/aboutus/QC_Manual.pdf. Results were reported in wet and dry weights. QA/QC standards were upheld including digestion blanks, duplicates, spiked test portions, appropriate standard reference material (SRM) and recovery calculations. Refer to the Quality Assurance Project Plan for details.

Results indicate that trace metal levels in whole mussels (tissue + shell) were low (Table 9, Figure 56). In fact, the concentrations were slightly lower than those measured in 2014. Unlike 2014, copper levels were not elevated contradicting the previous hypothesis that the mussels were naturally high due to the shell's affinity to bind onto this metal. This theory was based on research demonstrating that green lipped mussels were shown to have a significant capacity for removing zinc and copper from solution due to an exchange mechanism with calcium carbonate (Craggs et al., 2010). Instead, it seems more likely that the mussels in 2014 may have actually been exposed to elevated levels of copper in the ambient water.

	WB	EB	PP
Arsenic	1.00	0.82	2.37
Cadmium	*0.38	*0.38	0.76
Lead	*0.41	*0.26	2.29
Copper	*0.41	*0.26	9.29
Nickel	*2.05	*1.29	*0.19
Mercury	0.013	0.019	0.034

Table 9. 2016 metal concentrations (ppm) in mussels at WB, EB, and PP (Port Pilings).
*** Indicates value lies below this detection limit.**

Lead and copper concentrations were elevated in mussels collected from the Port Pilings only. While their concentrations were lower than the national mean, they were higher than historic averages collected from Budd Inlet (Table 10).

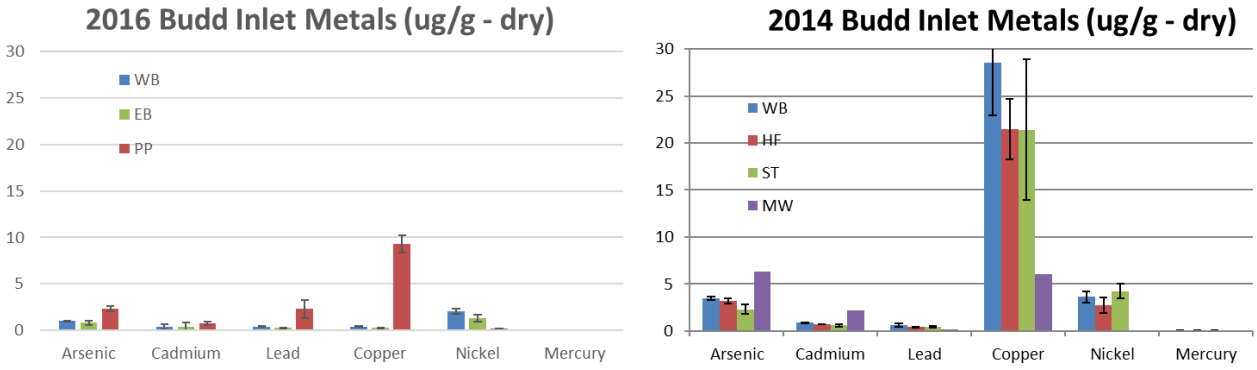

Figure 56. Trace metal concentrations (µg/g-dry) in mussels (tissue + shell). WB (West Bay), EB (East Bay), HF (Hearthfire Restaurant), ST (Swantown = East Bay), MW (average Mussel Watch data for Budd Inlet).

	Budd*	Budd**	National Mean**	National Range**	Compost Limit***
Arsenic	6.62±0.96	7.4	10.5	4.8-23.7	<20
Cadmium	2.11±0.50	2.5	2.68	0.4-10.4	<10
Copper	6.20±1.19	5.2	11.9	5.2-22.0	<750
Lead	0.92±0.80	0.57	2.62	0.02-11.6	<150
Mercury	.012±0.03	0.15	0.18	0.04-0.70	<8
Nickel	1.00±0.41	1.2	3.1	0.59-11.3	<210
*Mussel tissue (ppm-dry), 1986-2010 (NCCOS-Mussel Watch)					
**Mussel tissue (ppm-dry), 1997-1998, National Mean and Range Data (Mearns, 2001)					
***Source: WAC173-350-220 Composting facilities - Metal Limits (ppm-dry)					

Table 10. Average trace metal concentrations (µg/g-dry) in mussels nationally and locally.

Overall, results indicate that metal concentrations for arsenic, cadmium, copper, lead, mercury, and nickel in mussels harvested from Budd Inlet were all below the national mean and well within compost limits set by the Ecology solid waste handling standards for composting facilities (WAC 173-350-220).

2-methylnaphthalene, at a concentration of 50 ppb, was the only PAH detected in mussels collected from the Port Pilings (Figure 57). All other PAH and PCB results for 2014 and 2016 were below the detection limit. According to the Agency for Toxic Substances and Disease Registry, 2-methylnaphthalene is a solid that is used to make chemicals such as dyes and resins. The compound is present in cigarette smoke, wood smoke, tar, asphalt, and some hazardous waste sites. Naphthalene does not accumulate in the flesh of animals and fish that are consumed, but naphthalene and the methylnaphthalenes have been found in very small amounts in some samples of fish and shellfish from polluted waters (ATSDR, 2005).

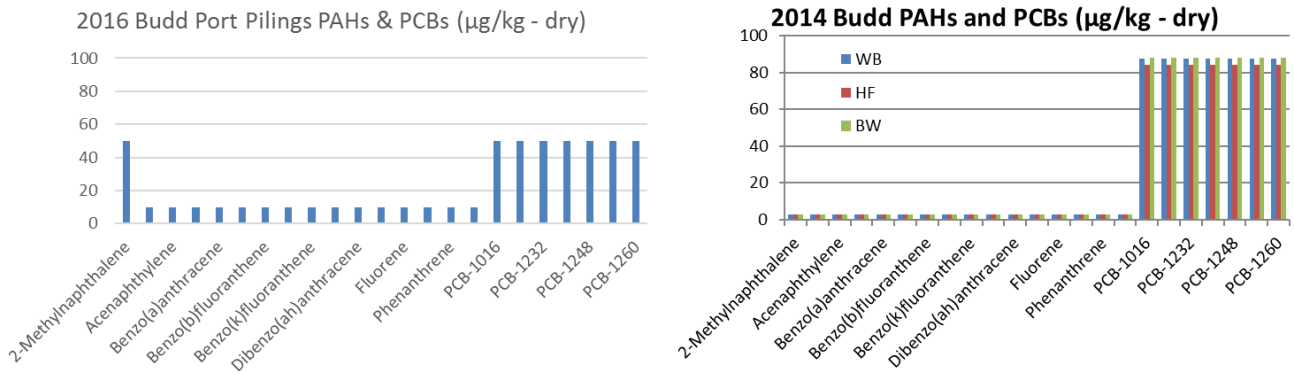

Figure 57. PAH and PCB concentrations (µg/kg = ppb) in Budd Inlet mussels from 2014 and 2016.

The EPA recommends that children not drink water with over 0.5 ppm (500 ppb = µg/kg) naphthalene for more than 10 days or over 0.4 ppm for any longer than 7 years. Most naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene that enters the body is expected to leave quickly within 1–3 days. In soil, some microorganisms break down naphthalene. Microorganisms present in the soil will break down most naphthalene in 1–3 months (ATSDR, 2005). Based on this information, the amount of 2-methylnaphthalene detected in the mussels from the Port of Olympia is very low and any trace amounts potentially retained in the Surf-to-Turf compost would be broken down by microorganisms in the soil.

Compost Analysis – Nutrients and Trace Metals

Triplicate samples were collected from cured mussel compost piles at TESC's Organic Farm and at PSI's backyard location. Each sample consisted of placing 10 small handfuls of compost collected from various locations throughout the pile into a 1-gallon sealable plastic bag. The

samples were sent to Soiltest Farm Consultants, a Seal of Testing Assurance (STA) Certified compost testing laboratory located in Moses Lake, Washington.

SoilTest protocols for laboratory sample preparation and analysis for compost samples are described in TMECC 02.02 Laboratory Sample Preparation for Analysis (USDA, 2001). The laboratory follows a strict QA/QC program to ensure accurate results. A minimum of 12% of the 52 samples analyzed include quality control samples comprised of blanks, references and duplicates. All procedures are documented under Standard Operating Procedures.

Results indicate that both batches of mussel compost were of suitable quality for agricultural and garden use and received a "PASS" rating (Table 11) (Appendix D). Both batches had a moisture content around 45%. Wet compost can lead to odor problems, whereas dry compost can be dusty and irritating to work with. Compost with a moisture content between 40 and 50 is ideal. The percent nitrogen was 1.2% (dry weight) in TESC's compost and 1.3% in PSI's. Most compost contains approximately 1% total nitrogen indicating that an application of 1,000 lbs. per acre would add 10 lbs. of nitrogen, or in this case, 12 lbs. at 1.2% nitrogen.

The C/N ratio was 24.7 for TESC and 20.3 which was comparable to similar trials at TESC in 2014 (22), but higher than compost generated from WSU in previous years (14). Typical compost recipes may start as high as 30, but decline steadily as the composting process proceeds and microbes utilize the carbon. A ratio of less than 25 likely indicates a finished product in which nitrogen will be readily available in the form of nitrate and ammonium (soilplantlab.missouri.edu). A C:N ratio of 14 is low enough to expect net mineralization of nitrogen in the soil during the first season and continued slow release of N in subsequent years. A C:N ratio in the 20s is ideal for crops (i.e. strawberries) that benefit from less nitrogen which promotes fruiting and flowering as opposed to extensive leaf development.

Of the macronutrients (e.g., phosphorous, potassium, calcium, and magnesium) only calcium has ever exceeded the typical range, a unique signature reflecting the calcium carbonate contained within the mussel shells. In the past three trials, however, a strong calcium signature was not observed. The discrepancy may depend on how much shell was included in each particular grab sample collected for analysis. Soils west of the Cascade Mountains are often depleted in calcium making mussel compost an attractive soil amendment.

While sodium was in a safe range, electrical conductivity (EC) was high in both TESC and PSI batches. EC measures the soluble salt content and can be harmful to germinating seeds and plants if too high. This can be mitigated by using the compost as soil amendment and not using the product straight. Rinsing compost with fresh water or exposing the compost to rain may help decrease the EC. Micronutrients (e.g., boron, zinc, copper and iron) were within or below

the typical range for compost and all heavy metals were well below Washington State compost standards (WAC 173-350-220, Table 220-B "Testing Parameters").

	WSU	TESC	WSU	TESC	PSI		
	2013 QMH	2014 WB	2014 HF	2016 WB	2016 PP	Units	Typical Range
Moisture	0.00	58.00	20.63	46.00	41.5	%	15 to 40
Solids	100.00	42.00	79.37	54.00	58.5	%	60 to 85
Total N	1.52	1.37	1.45	1.18	1.3	%	1 to 5
Organic C	22.30	30.37	21.27	28.77	26.8	%	18 to 45
Phosphorus	0.28	0.26	0.19	0.24	0.2	%	
Potassium	0.48	0.52	0.39	0.74	0.6	%	
Calcium	12.40	13.27	4.70	4.60	7.5	%	0.5 to 10
Magnesium	0.35	0.30	0.26	0.30	0.3	%	0.05 to 0.7
Sodium	0.48	0.48	0.36	0.30	0.4	%	0.05 to 0.7
Sulfur	0.32	0.28	0.29	0.20	0.3	%	0.1 to 1.0
Boron	24.50	19.33	16.33	11.67	15.8	mg/kg	25 to 150
Zinc	138.00	66.33	147.67	50.00	88.0	mg/kg	100 to 600
Manganese	251.00	295.00	228.00	353.33	292.1	mg/kg	250 to 750
Copper	61.00	29.00	17.67	25.67	24.1	mg/kg	100 to 500
Iron	5581.00	6185.00	4439.67	9959.67	6861.4	mg/kg	1000 to 25000
C/N ratio	14.70	22.00	14.33	24.67	20.3	ratio	18 to 24
pH	NA	NA	7.00	6.73	7.2	SU	5.5-8.5
EC	NA	NA	4.70	6.64	8.6	mmhos/cm	<5
							WAC Limit
Arsenic	2.83	0.40	2.77	3.93	2.4	mg/kg	20
Cadmium	0.57	0.33	0.37	0.10	0.3	mg/kg	10
Chromium	10.73	11.13	10.27	17.37	12.9	mg/kg	
Cobalt	2.40	2.30	2.03	5.03	3.1	mg/kg	
Coper	61.00	29.00	17.67	25.67	24.1	mg/kg	750
Lead	21.50	1.03	3.57	5.80	3.5	mg/kg	150
Mercury	0.05	0.01	0.04	0.04	0.0	mg/kg	8
Molybdenum	1.70	0.40	2.23	2.00	1.5	mg/kg	9
Nickel	8.90	8.67	7.50	13.37	9.8	mg/kg	210
Selenium	0.80	0.80	0.50	0.17	<.53	mg/kg	18
Zinc	138.00	66.33	147.67	50.00	88.0	mg/kg	1400
	PASS	PASS	PASS	PASS	PASS		

Table 11. SoilTest compost analysis from all nutrient bioextraction projects to date. Moisture and Solids reported "as received" whereas all other data reported as dry weights.
QMH =Quartermaster Harbor, Vashon Island, HF = Hearthfire Restaurant, PP = Port Pilings

Mussel Analysis – Stable Isotopes

Bivalves, as sessile filter feeding organisms, have been used to monitor variations in trace metals, organic contaminants, and nutrients in marine waters (Piola et al., 2006; Martinetto, 2006). Sources of nitrogen and carbon such as particulate organic matter from the terrestrial environment, phytoplankton, macroalgae and wastewater all have unique isotopic signatures. Stable isotope analysis has, therefore, been used to better understand the origins of nitrogen and carbon in the shellfish diet. These isotopic signatures vary, however, between shellfish species, seasonally and across geographic regions (Fry, 1999; Ruesink, 2014). For this reason, this analysis is best used when comparing nearby sites or evaluating changes over time.

For this study, three mussel composites (20 mussels/each) were collected from each site prior to harvest for analysis of their stable isotopic (d15N and d13C) signatures. Mussels of average, to above average, size were collected from three straps along the length of the dock at a depth of 2.5 feet. Mussels were placed into mesh seed bags, submerged in buckets of local seawater, and purged for 24-28 hours to remove stomach contents. Once purged, lengths and composite weights were recorded prior to freezing the mussels in plastic sealable bags for processing at the University of Washington's Isolab.

At the laboratory, 5 individual mussels were randomly selected from each composite. For each group of 5 mussels, the individuals were hand thawed, shucked, and the tissues lyophilized for several days. The dried tissue masses were combined and crushed into a powder. One subsample was immersed in HCL to test if a secondary de-carbonation step was required, but it was not. Stable isotope analysis on the 6 dried tissue samples was performed according to procedures described in Sample Preparation and Analysis for solid d13C and d15N (isolab.ess.washington.edu/isolab/sample-prep-analysis/solid-cn). The samples were analyzed on a ThermoFinnigan MAT 253 / Costech EA instrument.

Mussels were slightly larger at EB than WB with average lengths and weights of 36.0 ± 3.2 mm and 4.0 ± 0.2 g/mussel compared with 34.3 ± 2.8 mm and 3.2 ± 0.3 g/mussel respectively (Figure 58).

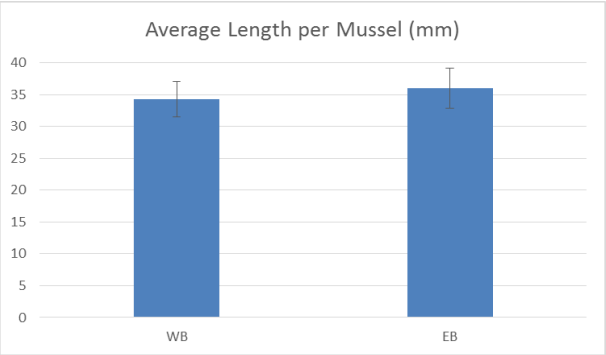

Figure 58. Average length per mussel (mm) for samples collected for stable isotope analysis.

Results demonstrated that the mussels collected at the two sites had slightly different d15N and d13C isotopic signatures with less variation among the WB samples (Figure 59). For d15N, mussels at WB ranged from 9.75 to 9.84‰ with an average of 9.8‰ ± 0.05. EB mussels ranged from 9.32 to 9.67‰ with an

average of 9.5‰ ± 0.18. For d13C, mussels at WB ranged from -19.66 to -19.54‰ with an average of -19.6‰ ± 0.06. EB mussels ranged between -20.63 and -20.33‰ with an average of -20.5‰ ± 0.15.

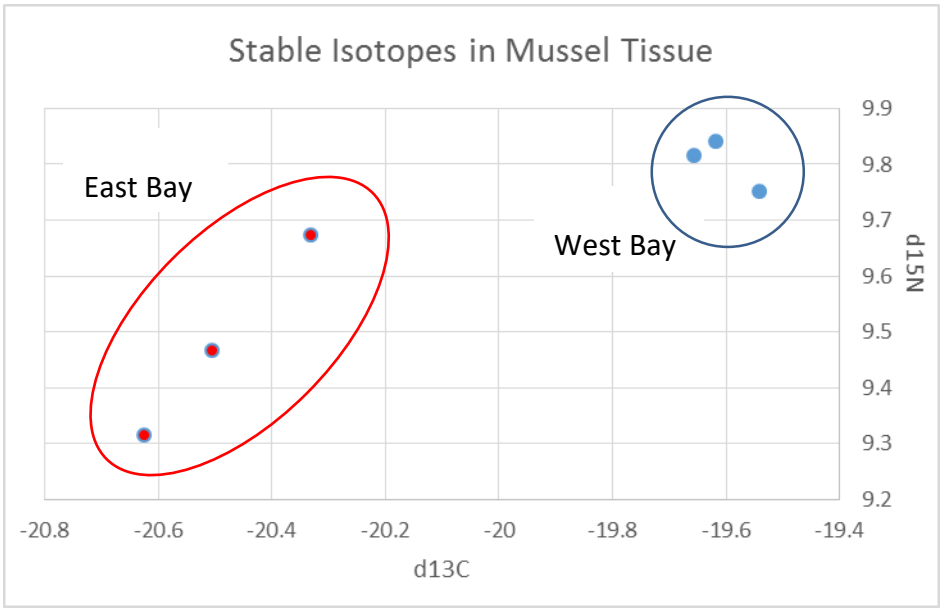

Figure 59. Stable isotope signatures in mussel tissue collected from EB and WB.

Previous works have shown that d13C decreases with decreasing salinity due to more terrestrially derived POM from riverine inputs (Wissel et al., 2005; Richard, 1997). Findings also demonstrate that d15N is higher in estuaries characterized by high primary productivity. Studies performed in Totten Inlet and San Francisco Bay support these trends, while also demonstrating differences between shellfish species and geographic locations.

In a 2014 study by Ruesink et al., oyster growth and stable isotopes (d13C and d15N) were evaluated along the length of Totten Inlet in South Puget Sound. This work determined that isotopic signatures did indeed change along the Totten Inlet gradient. For example, salinity was lower in lower Totten Inlet indicating fresh water inputs from Kennedy Creek and resulting in more depleted d13C (-19‰), enriched N15 (11‰) and faster shellfish growth rates. At the entrance to the Inlet, salinity was higher, d13C enriched (-18‰), N15 lower (10‰) and oyster growth rates slower. Pacific oysters tested from Browns Point (near Tacoma) and Carkeek (near Seattle) had d13C signatures between -18‰ and -17 ‰ (less POM influence); and d15N signatures as low as 8‰ (less productivity). In a 1999 study by B. Fry, this trend was also observed in clams tested along the length of the San Francisco Bay. In this case, d13C ranged

from -27‰ near the Sacramento River to -21‰ closer to the Pacific Ocean; while d15N ranged from 11.1‰ to 10.7‰ respectively.

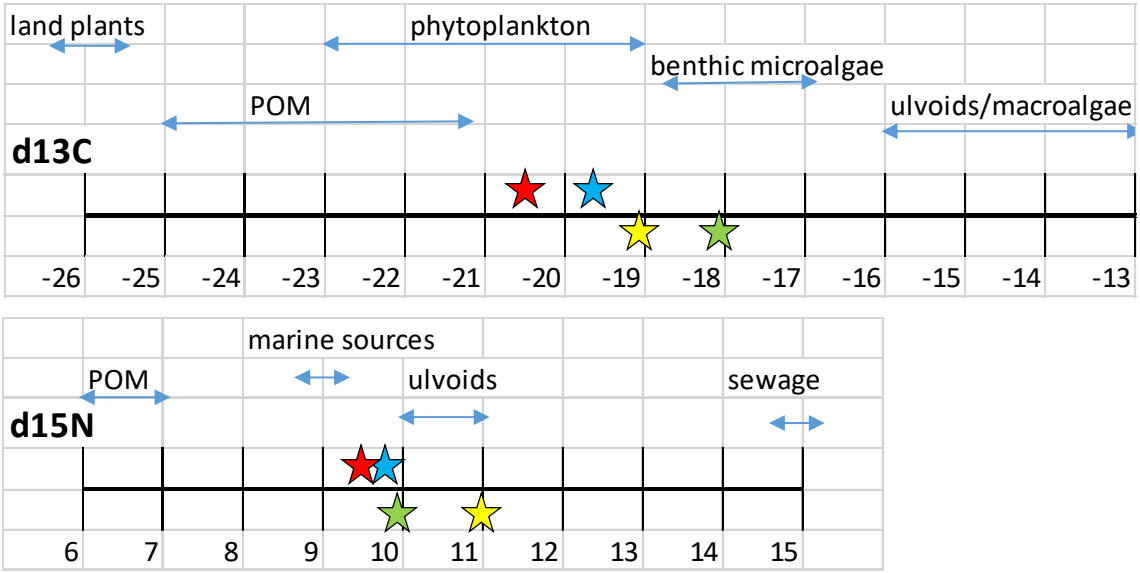

Figure 60. Typical isotopic signature ranges for various food sources (Ruesink, 2013; Ruesink et al., 2014). Stars indicate mussel tissue from EB (red) and WB (blue) and oyster tissue from Ruesink's work in lower Totten (yellow) and upper Totten (green).

According to scientific literature, typical isotopic signature ranges for various food sources are listed in Figure 60 (Ruesink, 2013; Ruesink, 2014). Based on these ranges, the Budd Inlet mussels derived their carbon predominantly from phytoplankton, with POM perhaps having a slight influence at EB. Literature indicates that nutrients derived from marine sources typically have a d15N signature around 9‰ suggesting that nitrogen in mussels was not strongly influenced by stormwater runoff or sewage.

OBJECTIVE 4 – Provide opportunities for outreach and education.

This project provided the community with locally based opportunities to connect with Puget Sound, learn about nutrient pollution, and participate in ways to improve water quality through engineering solutions and personal behavior change. These opportunities were provided to the general public, students, and relevant government employees (Appendix E).

Classroom and field presentations were offered to K-12 students within the North Thurston, Olympia, and Tacoma School Districts. During 2015 and 2016, PSI educators conducted a total of 35 classroom presentations to 914 students from 10 schools. Five additional presentations were conducted at the Port of Olympia's nutrient bioextraction site at Swantown Boatworks reaching 134 students. For the field presentations, PSI partnered with LOTT's WET Science Center. Students engaged in a 50-minute presentation about nutrients and LOTT's waste water treatment facility prior to walking along the waterfront to Boatworks for a 50-minute hands-on presentation about water quality and the nutrient bioextraction project. Thurston Conservation District's South Sound GREEN program provided life preservers for the field trips.

The Shellfish at Work - Reducing Nutrients in Budd Inlet curriculum offered hands-on activities that included: viewing live plankton under a microscope, performing a mussel filtration demonstration, collecting mussel growth measurements, handling water quality monitoring equipment, and learning about local efforts to address nutrient pollution (Figure 61) (Appendix F). The curriculum is available on PSI's web-site and advertised on Thurston County's ECO Network web page available to the general public and environmental educators (thurstoneconetwork.org/). PSI has secured additional funds to continue offering this curriculum beyond the NEP grant cycle.

Figure 61. 5th grade students getting a close look at mussels and the many small invertebrates hidden among byssal threads.

Students also participated in various aspects of the project including initial mussel site preparation and compost trials. In April 2015, students from Avanti High School learned about nutrient bioextraction and prepared mussel lines for installation by cutting rebar and straps to size. During fall of 2015, a Western Washington University graduate student assisted with data collection and mussel composting. In spring 2016, students at Marshall Middle School conducted vegetative growth experiments using mussel compost

generated from the project (Appendix G). In 2017, Marshall Middle School students disassembled the final mussel compost bin, relocated its contents, and prepared samples for laboratory testing during a Martin Luther King's Day of Service event (Figure 62).

Figure 62. Marshall Middle School students assist with composting activities for MLK Day of Service.

During the summers of 2015 and 2016, 29 citizen monitoring events were offered to the general public under PSI's contract with the City of Olympia under the "What's Blooming in Budd?" program (Figure 63). The events resulted in 564 contacts. During these events, community members met at the Port Plaza dock to collect weekly data on weather conditions, water temperature, salinity, and water clarity. Volunteers also performed a net tow and viewed live plankton under field microscopes. Samples were transported to LOTT's WET Science Center where live plankton was projected onto a large screen for public viewing. The samples were screened for harmful algal bloom species and data was shared with other programs such as NOAA's SoundToxins, Washington Dept. of Ecology's Eyes Over Puget Sound, and Washington Department of Health's Biotoxin Program.

PSI also provided opportunities for outreach and education at an array of meetings, community events, and professional conferences. For example, PSI presented information about the nutrient bioextraction project at Thurston County South Sound GREEN's Annual Teachers' Training on Biomimicry (50 participants), Department of Ecology's Sustainability Team lunchtime lecture series (25 participants), a Deschutes Advisory Group meeting (25 participants), Washington Department of Enterprise Services staff meeting (15 participants), Return to Evergreen alumni event (15 participants), and the Point Defiance and Zoo staff/volunteer lecture series (20 participants). Staff presented information at community events hosted at the Hands on Children's Museum (3 events - 400 contacts), South Sound

Estuary Association's Turning of the Tides festival (57 contacts), Shellfest at Twanoh State Park (200 contacts), and the City of Olympia's Great Yards Get Together (100 contacts). At The Great Yards Get Together, attendees learned about nutrient enrichment in lower Budd Inlet, ways to reduce their own nutrient inputs into Puget Sound, and the benefits of using organic compost. PSI distributed 10 totes of Surf-to-Turf mussel compost samples to the public at the event. PSI also delivered presentations at two professional conferences including the Pacific Coast Shellfish Growers Association/National Shellfisheries Association's (PCSGA/NSA) Annual Shellfish Conference in Hood River, OR (2015) and the PCSGA/NSA Annual Shellfish Conference in Lake Chelan, WA (2016).

Figure 63. Citizen volunteers at Port Plaza, September, 2016.

One full-feature article was published in StreamTeam's Spring 2016 newsletter titled, "What's Blooming in Budd?" The article highlighted Budd Inlet water quality, PSI and Stream Team's citizen plankton monitoring program, ways to keep excess nutrients out of Puget Sound, and links to PSI's nutrient bioextraction study (Appendix H). The nutrient bioextraction project and "Surf-to-Turf" mussel compost was also featured in an Olympian newspaper article on September 11, 2016, titled, "Great Yards Get Together – Event guides gardeners to eco-friendly approach" (Appendix I).

OBJECTIVE 5 – Make recommendations for innovative solutions to multi-parameter total maximum daily loads (TMDLs)

The Deschutes Basin TMDL

Since inception of the "Surf to Turf" project detailed in this final report, the Deschutes Basin total maximum daily load (TMDL) process advanced significantly. However, the portion of direct relevance to this project—Budd Inlet—was separated from the original TMDL process. The Clean Water Act requires that states develop a TMDL for each of the water bodies on the state's 303(d) list. The Department of Ecology, Washington's water quality program manager, began monitoring, modeling and analysis for the Deschutes Basin in 2003. Ecology, with the help of an advisory group of affected stakeholders, special interest groups, and interested citizens, subsequently worked to develop a plan to solve the basin's pollution problems. The end result of that process is the Deschutes River, Capitol Lake, and Budd Inlet Temperature, Fecal Coliform Bacteria, Dissolved Oxygen, pH, and Fine Sediment Total Maximum Daily Load Water Quality Improvement Report and Implementation Plan (Ecology, 2015), which was finalized by Ecology staff in December 2015, and submitted it to the U.S. Environmental Protection Agency (EPA).

As described earlier in this report, the Deschutes River flows into Capitol Lake, which empties into Budd Inlet. Unfortunately, both Capitol Lake and Budd Inlet are not included in the 2015 implementation plan. Ecology and the Deschutes Advisory Group (DAG; the stakeholder advisory group for the TMDL process) continue to work toward a plan to solve pollution problem in these remaining portions of the Deschutes Basin. Budd Inlet exceeds water quality standards for dissolved oxygen, while Capitol Lake is 303(d) listed for total phosphorus. Ecology and DAG identified more computer modeling needed in Budd Inlet, which Ecology began in 2016. At the time of this report's preparation, DAG had not met during 2017, but planning for a late spring meeting is underway, and Ecology continues to conduct identified information gaps in the Budd Inlet modeling.

The remaining portions of the Deschutes TMDL process were briefly termed the "Deschutes Phase 2 TMDL". However, this title was revised in early 2016 to avoid confusion with a separate Washington Department of Enterprise Services (DES) Capitol Lake/Lower Deschutes Watershed management plan. The DES process is a phased approach for a long-term Capitol Lake management plan, largely focused on sediment management. DES maintains Capitol Lake as part of the Capitol Campus, and the Washington State Legislature directed DES to "make tangible progress on reaching broad agreement on a long-term plan" for the Capitol Lake/Lower Deschutes Watershed. Through that process, representatives from local and tribal governments, state agencies and the community completed the first of three phases of an overall Capitol Lake management plan in 2016. In recognition of the DES process, and the

uncertainty surrounding future Capitol Lake sediment management, the remaining Deschutes Basin TMDL has focused on the marine waters of Budd Inlet.

Water Quality Trading

When our team began this "Surf to Turf" research, we expected to correlate required nutrient reductions in the Deschutes Basin to empirically confirmed nutrient reductions from mussel cultivation in Budd Inlet. We envisioned that the Deschutes TMDL implementation plan would establish wasteload allocations (WLA) for individual and general permittees, and load allocations (LA) for nonpoint sources, which could in-turn be used in a nutrient trading system (Figure 64). Unfortunately, with the separation of the upper and lower Deschutes Basin TMDL process, substantial work remains to identify and develop both WLAs and LAs for Budd Inlet. TMDL implementation plans set LAs for nonpoint sources and can provide details relevant to trading, including a schedule and phased milestones for achieving the TMDL cap, and direction regarding the actions expected of nonpoint sources (Willamette Partnership et al., 2015). The 2003 U.S. EPA Trading Policy and 2007 U.S. EPA Toolkit for Permit Writers both state that the nonpoint source trading baseline should be established or derived from LAs as the baseline for nonpoint source trading in the context of a TMDL.

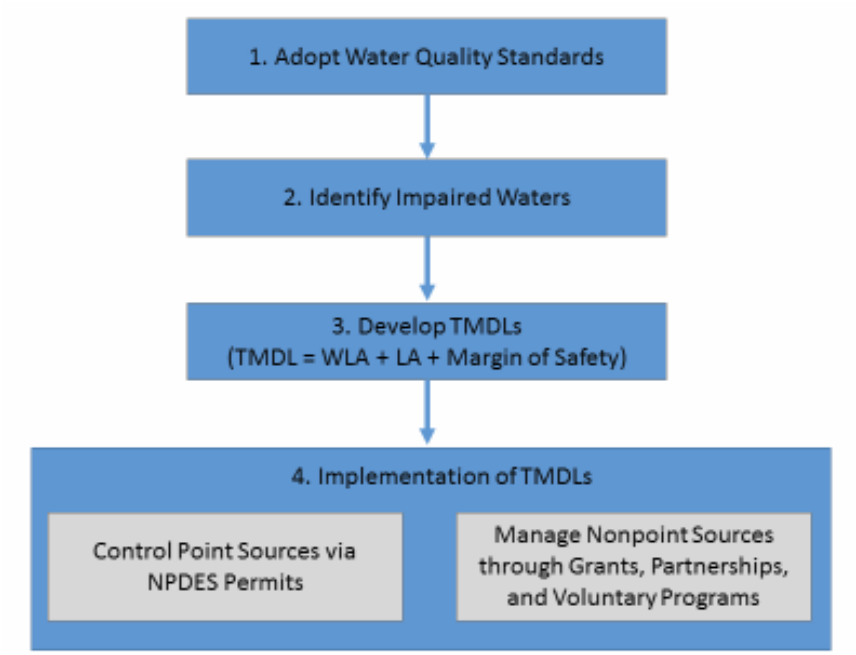

Figure 64. Water Quality-Based Approach of the Clean Water Act. (Image source: EPRI, 2015.)

Water quality trading (WQT) is a voluntary market-based approach that, if used in certain watersheds, might achieve water quality standards more efficiently and at lower cost than traditional approaches (EPA, 2004). As implied by the TMDL discussion above, water quality trading proposed here is intended to achieve compliance with an existing permitted discharge. In other words, trading to achieve regulated permit requirements allows the option to purchase credits in lieu of potentially expensive technology upgrades or installation (Willamette Partnership et al., 2015).

Trades must ensure that regulated discharges do not cause or contribute to an exceedance of applicable water quality criteria. To ensure that trades do not result in temporary exceedances above water quality standards, a trading program may require buyers to purchase credits only from upstream sources (Willamette Partnership et al., 2015). In the Deschutes Basin, delineating the trading area to allow inclusion of credit generation in the marine waters of Budd Inlet will be important. If WQT required the trading area to be upstream of the point of discharge, although National Network on Water Quality Trading participants commented that this approach may be overly limiting (Willamette Partnership et al., 2015), nutrient bioextraction with shellfish would not be an allowed credit source.

Building Successful WQT Programs

In his forward to Building a Water Quality Trading Program: Options and Considerations (Willamette Partnership et al., 2015) David Taylor, Chair of the National Association of Clean Water Agencies WQT Workgroup, affirms that:

> "Successful water quality trading programs involving point source discharges have demonstrated that trading can provide much-needed flexibility, while generating more cost-effective environmental benefits than traditional regulatory approaches. Faced with an ever-growing crisis on nutrient pollution and an environmental statute in need of updating to allow for more holistic, watershed-based approaches, the nation must look to further broaden the use of water quality trading and similar management approaches to find more opportunities for collaboration between point and nonpoint sources, including agriculture."

Since PSI's 2015 NEP report (PSI 2014) nutrient trading in the United States has not advanced substantially, but significant effort has been made toward developing reputable trading frameworks and providing guidance to jurisdictions considering WQT. Most notable is the National Network on WQT. The network is a dialogue among 18 organizations representing agriculture, wastewater and stormwater utilities, environmental groups, regulatory agencies, and the practitioners delivering WQT programs. Its purpose is to establish a national dialogue on how water quality trading can best contribute to achieving clean water goals. This includes

providing options and recommendations to improve consistency, innovation, and integrity in water quality trading.

The National Network on WQT identified 11 elements to consider when designing and implementing WQT programs:

1. Identifying and establishing regulatory instruments to support trading;
2. Defining who is eligible to trade, where trading can occur, and what is being traded;
3. Determining eligibility for participants in the trading program;
4. Quantifying water quality benefits;
5. Managing risk and uncertainty in the trading program;
6. Defining credit characteristics;
7. Establishing project implementation and assurance guidelines;
8. Establishing procedures for project review, certification, and tracking;
9. Ensuring compliance and enforcement;
10. Establishing adaptive management guidelines for ongoing program improvement and performance tracking; and
11. Defining roles, responsibilities, transaction models, and stakeholder engagement processes.

WQT programs continue to emerge across the country. As of 2015, fifteen states have established policies to support water quality trading, mostly focused on reducing nutrients (e.g., nitrogen and phosphorus), but also addressing temperature, sediment, and salinity (Table 12). Twenty-two states and territories were expected to have at least some waters with nitrogen and/or phosphorus criteria by the end of 2016 (EPRI, 2015).

Recommendations for Budd Inlet

This project's results demonstrate that nutrient bioextraction with shellfish can be a viable component toward improving Budd Inlet water quality. The shared ecosystem functions of nutrient remediation, water clarification, biodeposition, and habitat creation make suspension-feeding bivalves a valued provider of ecological services to the shallow-water ecosystems (Carmichael et al., 2012; Peterson et al., 2010; Rose et al., 2010; Newell, 2005). A summary of shellfish as a component of nutrient trading scenarios, and examples of buyers and sellers of nutrient credits involving shellfish were provided in previous NEP funded research (PSI, 2014).

IMPLEMENTING STATE AGENCY (ACRONYM USED)	FORM OF STATEWIDE TRADING AUTHORITY (INCLUDING CHESAPEAKE, AND MULTIPLE WATERSHED RULES FOR NORTH CAROLINA)				PERMITS ISSUED WITH TRADING
	STATUTE	RULE	POLICY	GUIDANCE	
Arkansas Department of Environmental Quality (ADEQ)	X				
Colorado Department of Public Health and the Environment (CDPHE)			X		X
Florida Department of Environmental Protection (FL DEP)	X	X			X
Idaho Department of Environmental Quality (ID DEQ)		X		X	X
Maryland Department of Agriculture (MDA) and Maryland Department of the Environment (MDE)	X		X	X	
Minnesota Pollution Control Agency (MPCA)	X				X
Montana Department of Environmental Quality (MT DEQ)		X	X		
North Carolina Department of Environment and Natural Resources (NC DENR)		X			
Ohio Environmental Protection Agency (OH EPA)		X			X
Oregon Department of Environmental Quality (OR DEQ)	X			X	X
Pennsylvania Department of Environmental Protection (PA DEP)		X		X	X
Utah Department of Environmental Quality (UT DEQ)		X			
Virginia Department of Environmental Quality (VA DEQ)	X	X		X	X
Washington Department of Ecology (WA DOE)				X	
Wisconsin Department of Natural Resources (WI DNR)	X			X	

Table 12. States with active trading authority. (Table from Willamette Partnership et al., 2015.)

In their forward to Building a Water Quality Trading Program: Options and Considerations (Willamette Partnership et al., 2015) USDA Environmental Markets Council Co-Chairs Robert Bonnie and Robert Johansson assert that:

> "Water quality trading programs provide a catalyst for developing innovative, practical solutions for improving water quality, while generating environmental benefits at lower cost and providing a new source of revenue for farmers, ranchers and forest landowners."

Through the results of this nutrient bioextraction project, we assert that WQT can extend to marine waters, providing a new source of revenue for *aquatic* farmers and *aquatic* landowners.

WQT has been encouraged by various state and local agencies as part of the 2014 Recommendations for Improving Water Quality Assessment and Total Maximum Daily Load Programs in Washington State (Interagency Project Team, 2014). Establishment of WQT in Budd Inlet should be predicated by TMDL established load allocations. Although the upper Deschutes TMDL is a multi-parameter TMDL, implementation plans for correcting pollution problems in Capitol Lake and Budd Inlet may be single parameter TMDLs. As noted previously, Capitol Lake exceeds water quality standards for phosphorus, while Budd Inlet pollution stems from low dissolved oxygen.

This project's result validates dissolved oxygen improvements stemming from mussel cultivation, provides robust empirical evidence of nutrient removal, and clear methods to quantify that nutrient removal. Furthermore, nutrient bioextraction with shellfish adheres to seasonal needs in Budd Inlet, due to the seasonal nature of eutrophic condition and dissolved oxygen levels, and the resulting National Pollutant Discharge Elimination System (NPDES) permit requirements. This is significant because EPA's 2003 Trading Policy states, "Credits should be generated before or during the same period they are used to comply with a monthly, seasonal or annual limitation or requirement specified in an NPDES permit." Additionally, this project's research—conducted by independent scientists not associated with a regulatory body or private enterprise—also adheres to recommendations made by senior scientist and policymakers for the Chesapeake Bay nutrient trading subcommittee that "independent, rigorous verification is essential" for nutrient trading (Dennison et al., 2012).

As summarized in PSI's 2014 NEP report, potential buyers of nutrient credits include NPDES permitted entities in the Deschutes River, Percival Creek, Budd Inlet Tributaries, Capital Lake, and Budd Inlet directly. However, in Budd Inlet the only permitted sources are waste water treatment plants, and "they are among the smallest contributors to the DO [dissolved oxygen] problem. If they are required to further modify their operations it will be expensive, with little benefit to improving water quality." (DAG November 17, 2016 meeting notes; available at: http://www.ecy.wa.gov/programs/wq/tmdl/Deschutes/advisorycomm/111716DAGmtgNotesFinal.pdf) While this observation mirrors the rationale for WQT—finding the least expensive option to water quality improvements—it also recognizes that solutions in Budd Inlet cannot rely on NPDES permitted entities alone. While point source contributors are often identified as the primary buyers in WQT scenarios, achieving satisfactory dissolved oxygen levels in Budd Inlet will likely need to include non-point credit buyers. (This can be expected to emulate implementation plan requirements for the future Budd Inlet TMDL, e.g. pollution abatement by non-point contributors will be necessary.) However, as technologies continue to become more effective and lower cost, the need for WQT as a cost-effective compliance option decreases (EPRI, 2015). The 2015 EPRI report cited developments in the Ohio River Basin Trading Project, where the regulatory limits for phosphorus (P) are lower (now 1mg/L total P in Indiana), the

technology cost for reaching this lower limit has also become affordable, reducing demand for WQT. This is positive from the large view of the watershed, but also a consideration for the role of WQT if regulatory drivers are the only potential buyer pool (EPRI, 2015). WQT can reduce nutrient discharges more quickly by allowing municipal stormwater utilities to purchase credits as a gap-closing strategy for meeting timeline goals for reductions if they are unable to complete all of the necessary stormwater projects in time, or if they want to spread capital costs over a longer period (WRI, 2017).

In Washington State, "…any chance of water quality trading occurring will depend on action by the legislature. State lawmakers will have to implement such a program in order for it to get off the ground. Ecology has explored this issue in the past, but the Legislature found a lack of interest to be a barrier to large-scale implementation." (Sykes, 2015). When the Washington State Conservation Commission, in partnership with Ecology, provides their December 2017 report to satisfy the directive under House Bill 2454 of the 2013-2014 legislative session, a statewide approach for WQT should be more evident. Per HB 2454, the report will explore whether there are a sufficient number of potential buyers and sellers for a WQT program to be successful in watersheds where TMDLs have been established. The summary will undoubtedly focus on terrestrial systems, per previous U.S. examples (see Table 12), but we offer the recommendations, above, for inclusion of marine TMDL solutions.

CONCLUSIONS

Over the past few decades, nutrient levels in Budd Inlet have risen steadily while dissolved oxygen levels have declined. Nutrient bioextraction, or harvesting shellfish for the purpose of removing nutrients was evaluated in 2013 and 2015 as a way to recycle excess nutrients in watersheds and return them to the upland environment in the form of organic compost. This work came at the heels of similar research both nationally and globally that yielded promising results.

The Budd Inlet nutrient bioextraction trials were successful in many ways. The experimental sites demonstrated that growing blue mussels via natural propagation was typically reliable in terms of mussel set and harvest timing, growth rates, and laboratory analysis. At the same time, similar to terrestrial agriculture, predators (e.g., overwintering diving birds, starfish), disturbance (e.g., boats), and unusual weather patterns (e.g., changes in mussel spawn timing) proved variable. Overall, the straps worked well to support blue mussels, the mussels yielded suitable compost for terrestrial application, and the end-of-season harvest removed a quantifiable amount of nitrogen, phosphorus, and carbon.

While harvesting mussels removes nutrients, the pounds required to extract a significant amount of nutrients is substantial. For example, to remove the amount of nutrients equivalent to the inputs of 500 individuals, approximately 500,000 pounds of mussels would need to be harvested. This amount is similar in size to the theoretical “scaled up” scenarios presented in this report. The size of a mussel installation required to remove 5,000 pounds of nitrogen is reasonable but raises issues about impacts to the surrounding environment.

The impacts to water passing through the WB mussel installation at a 2.5-foot depth was evaluated using two flow-through experiments. Results indicated that plankton cell counts, Chl *a*, and POC/PON decreased as the water moved through the system. This removal of phytoplankton via filter feeding resulted in slight increase in dissolved nutrients (nitrates, nitrites, silica) and decrease in pH moving through the system as fewer phytoplankton remained in the water column to assimilate nutrients and carbon dioxide. During peak growth rates, mussels also released the waste product ammonium, which was detected in concentrations above historic ambient conditions for lower Budd Inlet.

The aforementioned water quality changes were detected in waters flowing through a mussel installation that supported approximately 2,000 lbs. of mussels. The impacts to a much larger installation must also be considered, but was not within the scope of this project. Between 2001 and 2003, PSI was involved in a National Marine Aquaculture Initiative funded project that evaluated the ecological impacts of larger mussel rafts on the surrounding environment using

similar flow-through experiments (Cheney et al., 2003). Each raft unit, pictured in Figure 16, supported 4,320 lines yielding 280,800 pounds of mussels. Water samples were collected along the length of the raft and also 70-meters downstream. Results were similar to the smaller Budd Inlet trials indicating that plankton, Chl *a*, and pH decreased as the water passed through the raft (data not shown). At the same time, dissolved nutrients (nitrite, nitrate, phosphates, and silica) increased slightly. Ammonium, similar to our findings, was also notably higher within the raft system (Figure 65). All parameters (except nitrates) approached pre-raft concentrations 2-m downcurrent and differences were undetectable 70-m away. Phytoplankton cell counts (cells/L) were 70% lower in the center of the raft, 25% lower 2-m downcurrent, and showed no statistical difference 70-m away (data not shown). These results suggest that the Budd Inlet flow-through experiments, while small, reflect how larger scale systems might influence surrounding conditions.

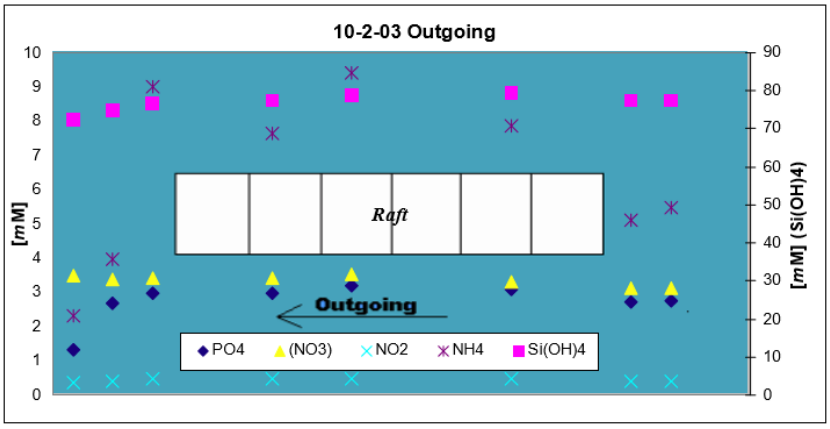

Figure 65. Totten Inlet nutrient data during outgoing tide at commercial raft. X axis from left to right: reference, north buoy (70m), north boom (2m), raft north, raft center, raft south, south boom (2m) and south buoy (70m).

The impact of large shellfish installations on sediments beneath mussel installations has also raised concerns, especially when grown in sheltered inlets with poor circulation (Carlsson et al., 2012; Stadmark and Conley, 2011). Nutrient bioextraction using mussels was tested at 3 long-line farms located off the Swedish west coast. Results demonstrated that sedimentation rates beneath the low circulation site were 3-5 times greater, and biological oxygen demand and the amount of ammonium and phosphates released from sediments were also greater (Carlsson et al., 2012). In fact, when comparing the sheltered site against one with adequate circulation, the amount of ammonium released from sediments was higher (20% vs. 10%) and the amount of nitrogen gas released from sediments was lower (1.1% vs. 13%).

Nutrient removal can occur through physical extraction of shellfish during harvest and microbial conversion of biodeposits into biologically unavailable nitrogen gas. Microbes living at the aerobic/anaerobic interface convert nitrates to nitrites and ultimately nitrogen gas in the following process: $NO3^{-} \rightarrow NO2^{-} \rightarrow NO + N2O \rightarrow N2$ (gas) (Newell et al., 2005). An anaerobic environment, however, creates a sulfidic environment that changes the benthic communities and eliminates nitrifying bacteria. Without these bacteria, the production of nitrogen gas cannot take place and is instead replaced by biologically available ammonium and phosphate.

Budd Inlet results indicate that biodeposition at both of our sites was 3-5 times higher under the mussel installations. During July and August, pH and DO were typically lower under mussels compared to the reference site, but the difference was slight and likely to have no biological significance. By September, no difference was detected at either site due to changing weather patterns that improved water quality conditions considerably.

Nutrient bioextraction results in a net reduction of nutrients post-harvest due to the fact that shellfish growth requires no additional food supplementation. Instead, mussels obtain their nutrients directly from ambient waters. During our pilot trials, over 5,000 lbs. of mussels were harvested removing 50 pounds of nitrogen, 2.75 pounds of phosphorus, and 225 pounds of carbon while generating over four cubic yards of organic compost. Despite this benefit to water quality, care must be taken when siting mussel installations in locations characterized by poor water circulation. Circulation maps and models of Budd Inlet indicate that flow velocities and DO concentrations have historically been lower at EB, approaching complete hypoxia in mid-summer. Based on this knowledge, EB would not be an ideal candidate for a larger scale nutrient bioextraction project. Nutrient bioextraction would be suitable, however, in a lower Budd Inlet location that experiences adequate water circulation. In such instances, nutrient removal would occur via physical harvest (measurable) and denitrification, although denitrification was not measured as part of this study.

Should future nutrient bioextraction projects be pursued, ongoing monitoring of biological communities within and beneath installations, mussel "drop-off" times, and water quality at depth are recommended. Nutrient bioextraction has demonstrated itself to be an effective way to remove nutrients and generate a useful end product, encourage community participation and Puget Sound stewardship, and support the larger nutrient removal efforts being made by LOTT Clean Water Alliance, the TMDL advisory group, participating government agencies, non-profit organizations, and the community at large.

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Appendix A

BUDD INLET MUSSEL BIOEXTRACTION DATA SHEET *(Monthly Sampling)*

Questions? Contact PSI staff at 360-754-2741

Site (BHM, STM, POH/HF, WBM): BW **Sample ID:** (e.g. 13BHM-0615-1)

Date: 7/28/15 **Arrival Time:** 11:48 **Leave Time:** 12:45 **Tide:** low

Mussel Collectors: SB & AC **Depth:** no gun

Data Recorder: AC **Secchi:** 2.7 m

SITE DESCRIPTION

Water Temp. (celcius): (S) 18.6 (2.5') 18.4 (B) 16.3 **DO:** (S) 80% (2.5) 79.8 (B) 4.2 **Salinity:** (S) 17.38 (2.5) 18.4 (B) 28.8

B: 6" off bottom **pH:** (S) 6.9 (2.5) 7.04 (B) 7.32 **ORP:**

Site Description: rained a little 2 days ago, Sunny + nice ~80° F

LINE DESCRIPTION

Line #1

Location: (*See Site Map Attached for Reference*) **Position #** (*e.g. 9B*): 1

Top	Mussel set (heavy, medium, light, none)	Fouling Community Description
1 ft	5-10% base.	lots of slimy brown
2 ft	10% bad top	algae, some porphyra
3 ft	10%	lots of tunicates + orange/gray colonial ascidians
4 ft	30%	Strap is overall slimy
5 ft	30% better at bottom	

Bottom

Notes:

don't see crabs
jellies, water clearer
more fuzzy brown algae at surface depth

Appendix B

Sample Data Sheet

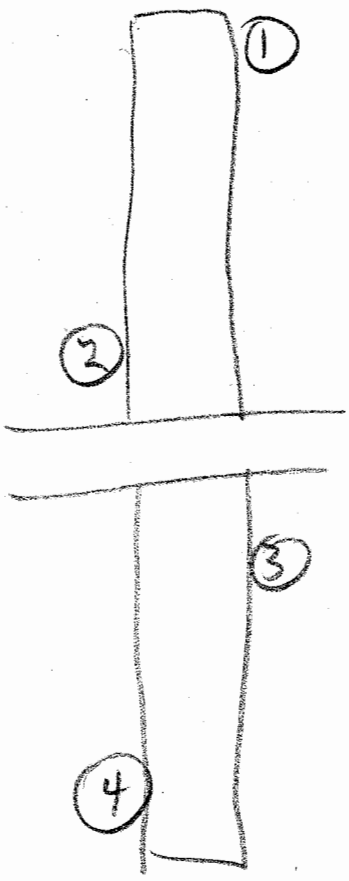
BUDD INLET MUSSEL BIOEXTRACTION DATA SHEET *(Monthly Sampling)*

Site (BHM, STM, POH, WBM): WBM **Sample ID**: (e.g. 13BHM-0615-1)

Date: 7/28/15 **Arrival Time:** 12:48 **Leave Time:** 1:23

Mussel Collectors: SB + AC

Data Recorder: AC



	Line # 1 Length (mm)	Line # 2 Length (mm)	Line # 3 Length (mm)	4
1	19	24	12	19
2	17	15	22	16
3	15	18	7	22
4	17	21	17	21
5	16	13	12	15
6	14	14	20	20
7	18	10	12	12
8	18	17	21	18
9	17	18	11	18
10	18	17	9	16
11	17	15	13	18
12	15	11	10	25
13	8	17	16	20
14	21	9	12	17
15	9	15	20	18
16	12	18	12	19
17	16	21	12	17
18	13	15	13	17
19	18	12	16	20
20	18	14	20	18
21	21	8	11	15
22	14	19	19	18
23	21	16	12	19
24	14	20	8	15
25	18	12	8	22
26	14	17	11	24
27	18	9	17	22
28	23	13	14	16
29	16	20	16	7
30	15	17	21	11

Jellies + Shallebacks

Composite wt + fouling (g):				
Fouling wt (g):				
Composite wt - fouling (g):				
BIOMASS OF ENTIRE LINE (30)	11.5	10.8	8.5	15.3

Appendix C

9 - Flow through Exp. Ref

IX

2015 NEP Phytoplankton

	Ref	N	C	S	S
Centric Diatoms					
Actinoptychus senarius					
Asteromphalus heptactis					
Aulacodiscus kittoni				2	
Cerataulina pelagica	12 90	38	12	4 32	6 28
Chaetocreros spp.		10	4	2	
Corethron criophilum					
Coscinodiscus spp.					
Dactyliosolen					
Detonula pumila					
Ditylum brightwellii	6	8	2	12	14
Eucampia					
Grammatophora					
Guinardia					
Hemiaulus					
Lauderia					
Leptocylindrus	88	48	8	98✓	96
Melosira					
Odontella					
Plagiogramopsis					
Proboscia alata					
Rhizosolenia					
Skeletonema	36	20		30	44
Stephanopyxis spp.					
Thalassiosira spp.	36	16	8	68	38
Unidentified centric					
Pennate Diatoms					
Achnanthes					
Asterionellopsis					
Cylindrotheca	6	4	2	14	2
Fragilaria					
Fragillariopsis					
Licmophora					
Navicula spp.	8 4	2			
Nitzschia acic + other					2
Petrodictyon					
Pleurosigma					
Pseudo-nitzschia	1	4	2	6	2 2
Striatella					
Thalassionema	6				
Tropidoneis					
Unidentified pennate	2			6	4
Dinoflagellates					
Akashiwo sanguinea	68	28	12	66	22
Alexandrium spp.	6*	28*	2*	9*	6
Amphidinium	26	36	14	62	4
Amylax triacantha					
Ceratium spp.	76	56	16	74	76
Dinophysis spp. ϟ	7	8		4	11
Gonyaulax spp.					
Gymnodinium					
Gyrodinium spp.	14				
Heterocapsa triquetra	34 1	6 40	16	2 40	60
Kofoidinium velleloides					
Minuscula bipes					
Nematodinium					
Noctiluca scintillans				1	2
Oxyphysis oxytoxoides					
Polykrikos					
Prorocentrum spp.	66	40	16	46 1	86
Protoceratium					
Protoperidinium spp.				6	4
Pyrophacus					
Scrippsiella		2			4
Unidentified Dinos ∞	30	30	18	34	52
Dictyoca spp.					
Heterosigma akashiwo					
Zooplankton					
Tintinnids ✓✓	30	16	8	48	10
copepoda					
barnacle nauplii					
crustacean nauplii					
rotifers					
tiarina	2	2	1	1	
Urochordata/Oikopleura					
Gastropod/bivalve larve					
Polychaete larvae (trochophore)					
other (No. different species)		1		1	

Entered 1/8/15

☺ one C. divaricatum

✓ minimus + L. danicus

A - like
* either A. Tamarense or Protoceratium

ϟ even mix fortii + acuminata

∞ most small dinos

✓✓ some ciliates, mesodinium, tintinnids

P. gracile

Some D. fortii, but mostly D. acuminata

Euglenoid Eggs

↑ maybe larvacean tail side

3 2

Appendix D

US Composting Council ®
STA Certified Lab

soiltest
farm consultants, inc.
2925 Driggs Dr., Moses Lake, Wa 98837 - www.soiltestlab.com
Office: (509)765-1622 - Fax:(509)765-0314 - (800)764-1622

Client: **Pacific Shellfish intitute**	Product: **WB-Mussel 1**	Date Reported:	01/08/16
Attn: Aimee Christy	Date Sampled:	12/16/15	Laboratory # C15-744
120 State Ave NE 1056	Date Received:	12/17/15	Reveiwed by Brent Thyssen, CPSSc
360-754-2741			Amount: $ 120.00

Nutrients

	Method	As Rcvd.	Dry Wt.	Units	Low	Normal	High	Typical Range
Moisture	70 C	**47.3**		%	**********			15 to 40
Solids	70 C	**52.7**		%	**********			60 to 85
pH	1:5	**6.7**	**NA**	SU	**********			5.5 to 8.5
E.C	1:5	**3.26**	**6.18**	mmhos/cm	**********			below 5.0
Total N	TMECC 04.02D	**0.63**	**1.20**	%	**********			1 to 5
Organic C	TMECC 04.01A	**17.0**	**32.2**	%	**********			18 to 45
Phosphorus	TMECC 04.12B/04.14A	**0.12**	**0.22**	%				
P_2O_5		**0.27**	**0.51**	%	**********			1 to 8
Potassium	TMECC 04.12B/04.14A	**0.39**	**0.73**	%				
K_2O		**0.46**	**0.88**	%	**********			3 to 12
Calcium	TMECC 04.12B/04.14A	**2.14**	**4.1**	%	**********			0.5 to 10
Magnesium	TMECC 04.12B/04.14A	**0.14**	**0.27**	%	**********			0.05 to 0.7
Sodium	TMECC 04.12B/04.14A	**0.16**	**0.30**	%	**********			0.05 to 0.7
Sulfur	TMECC 04.12B/04.14A	**0.11**	**0.20**	%	**********			0.1 to 1.0
Boron	TMECC 04.12B/04.14A	**6**	**12**	mg/kg	**********			25 to 150
Zinc	TMECC 04.12B/04.14A	**25**	**47**	mg/kg	**********			100 to 600
Manganese	TMECC 04.12B/04.14A	**161**	**306**	mg/kg	**********			250 to 750
Copper	TMECC 04.12B/04.14A	**12**	**23**	mg/kg	**********			100 to 500
Iron	TMECC 04.12B/04.14A	**4469**	**8479**	mg/kg	**********			1000 to 25000
C/N ratio			**27**	ratio	**********			18 to 24

WAC 173-350-220

	Method	Dry Wt.	Units	Low	Normal	High	WAC Limit
Arsenic	TMECC 04.12B/04.14A	**3.5**	mg/kg	**********			20
Cadmium	TMECC 04.12B/04.14A	**0.1**	mg/kg	**********			10
Chromium	TMECC 04.12B/04.14A	**15.6**	mg/kg				-
Cobalt	TMECC 04.12B/04.14A	**4.4**	mg/kg				-
Copper	TMECC 04.12B/04.14A	**23**	mg/kg	**********			750
Lead	TMECC 04.12B/04.14A	**5.3**	mg/kg	**********			150
Mercury	TMECC 04.12B/04.14A	**0.04**	mg/kg	**********			8
Molybdenum	TMECC 04.12B/04.14A	**2.2**	mg/kg	**********			9
Nickel	TMECC 04.12B/04.14A	**11.8**	mg/kg	**********			210
Selenium	TMECC 04.12B/04.14A	**0.2**	mg/kg	**********			18
Zinc	TMECC 04.12B/04.14A	**47**	mg/kg	**********			1400
		Pass					

Sample was received, handled and tested in accordance with TMECC procedures

US Composting Council ®
STA Certified Lab

soiltest
farm consultants, inc.
2925 Driggs Dr., Moses Lake, Wa 98837 - www.soiltestlab.com
Office: (509)765-1622 - Fax:(509)765-0314 - (800)764-1622

Client: **Pacific Shellfish intitute**	Product: **WB-Mussel 2**	Date Reported:	01/08/16
Attn: Aimee Christy	Date Sampled:	12/16/15	Laboratory # C15-745
120 State Ave NE 1056	Date Received:	12/17/15	Reveiwed by Brent Thyssen, CPSSc
360-754-2741			Amount: $ 120.00

Nutrients

	Method	As Rcvd.	Dry Wt.	Units	Low	Normal	High	Typical Range
Moisture	70 C	**47.3**		%	**********			15 to 40
Solids	70 C	**52.7**		%	**********			60 to 85
pH	1:5	**6.8**	**NA**	SU	**********			5.5 to 8.5
E.C	1:5	**3.39**	**6.44**	mmhos/cm	**********			below 5.0
Total N	TMECC 04.02D	**0.61**	**1.15**	%	**********			1 to 5
Organic C	TMECC 04.01A	**14.3**	**27.1**	%	**********			18 to 45
Phosphorus	TMECC 04.12B/04.14A	**0.14**	**0.26**	%				
P_2O_5		**0.31**	**0.59**	%	**********			1 to 8
Potassium	TMECC 04.12B/04.14A	**0.39**	**0.74**	%				
K_2O		**0.47**	**0.88**	%	**********			3 to 12
Calcium	TMECC 04.12B/04.14A	**2.48**	**4.7**	%	**********			0.5 to 10
Magnesium	TMECC 04.12B/04.14A	**0.17**	**0.31**	%	**********			0.05 to 0.7
Sodium	TMECC 04.12B/04.14A	**0.15**	**0.29**	%	**********			0.05 to 0.7
Sulfur	TMECC 04.12B/04.14A	**0.11**	**0.20**	%	**********			0.1 to 1.0
Boron	TMECC 04.12B/04.14A	**6**	**11**	mg/kg	**********			25 to 150
Zinc	TMECC 04.12B/04.14A	**27**	**51**	mg/kg	**********			100 to 600
Manganese	TMECC 04.12B/04.14A	**199**	**378**	mg/kg	**********			250 to 750
Copper	TMECC 04.12B/04.14A	**14**	**27**	mg/kg	**********			100 to 500
Iron	TMECC 04.12B/04.14A	**5577**	**10580**	mg/kg	**********			1000 to 25000
C/N ratio			**24**	ratio	**********			18 to 24

WAC 173-350-220

	Method	Dry Wt.	Units	Low	Normal	High	WAC Limit
Arsenic	TMECC 04.12B/04.14A	**4.0**	mg/kg	**********			20
Cadmium	TMECC 04.12B/04.14A	**0.1**	mg/kg	**********			10
Chromium	TMECC 04.12B/04.14A	**17.4**	mg/kg				-
Cobalt	TMECC 04.12B/04.14A	**5.2**	mg/kg				-
Copper	TMECC 04.12B/04.14A	**27**	mg/kg	**********			750
Lead	TMECC 04.12B/04.14A	**6.0**	mg/kg	**********			150
Mercury	TMECC 04.12B/04.14A	**0.04**	mg/kg	**********			8
Molybdenum	TMECC 04.12B/04.14A	**1.7**	mg/kg	**********			9
Nickel	TMECC 04.12B/04.14A	**13.8**	mg/kg	**********			210
Selenium	TMECC 04.12B/04.14A	**0.2**	mg/kg	**********			18
Zinc	TMECC 04.12B/04.14A	**51**	mg/kg	**********			1400
		Pass					

Sample was received, handled and tested in accordance with TMECC procedures

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Office: (509)765-1622 - Fax:(509)765-0314 - (800)764-1622

Client: **Pacific Shellfish intitute**	Product: **WB-Mussel 3**	Date Reported:	01/08/16
Attn: Aimee Christy	Date Sampled:	12/16/15	Laboratory # C15-746
120 State Ave NE 1056	Date Received:	12/17/15	Reveiwed by Brent Thyssen, CPSSc
360-754-2741			Amount: $ 120.00

Nutrients

	Method	As Rcvd.	Dry Wt.	Units	Low	Normal	High	Typical Range
Moisture	70 C	**43.4**		%	**********			15 to 40
Solids	70 C	**56.6**		%	**********			60 to 85
pH	1:5	**6.7**	**NA**	SU	**********			5.5 to 8.5
E.C	1:5	**4.14**	**7.31**	mmhos/cm	**********			below 5.0
Total N	TMECC 04.02D	**0.68**	**1.20**	%	**********			1 to 5
Organic C	TMECC 04.01A	**15.3**	**27.0**	%	**********			18 to 45
Phosphorus	TMECC 04.12B/04.14A	**0.14**	**0.25**	%				
P_2O_5		**0.33**	**0.58**	%	**********			1 to 8
Potassium	TMECC 04.12B/04.14A	**0.43**	**0.75**	%				
K_2O		**0.51**	**0.90**	%	**********			3 to 12
Calcium	TMECC 04.12B/04.14A	**2.81**	**5.0**	%	**********			0.5 to 10
Magnesium	TMECC 04.12B/04.14A	**0.19**	**0.33**	%	**********			0.05 to 0.7
Sodium	TMECC 04.12B/04.14A	**0.18**	**0.32**	%	**********			0.05 to 0.7
Sulfur	TMECC 04.12B/04.14A	**0.12**	**0.21**	%	**********			0.1 to 1.0
Boron	TMECC 04.12B/04.14A	**7**	**12**	mg/kg	**********			25 to 150
Zinc	TMECC 04.12B/04.14A	**29**	**52**	mg/kg	**********			100 to 600
Manganese	TMECC 04.12B/04.14A	**213**	**376**	mg/kg	**********			250 to 750
Copper	TMECC 04.12B/04.14A	**15**	**27**	mg/kg	**********			100 to 500
Iron	TMECC 04.12B/04.14A	**6126**	**10820**	mg/kg	**********			1000 to 25000
C/N ratio			**23**	ratio	**********			18 to 24

WAC 173-350-220

	Method	Dry Wt.	Units	Low	Normal	High	WAC Limit
Arsenic	TMECC 04.12B/04.14A	**4.3**	mg/kg	**********			20
Cadmium	TMECC 04.12B/04.14A	**0.1**	mg/kg	**********			10
Chromium	TMECC 04.12B/04.14A	**19.1**	mg/kg				-
Cobalt	TMECC 04.12B/04.14A	**5.5**	mg/kg				-
Copper	TMECC 04.12B/04.14A	**27**	mg/kg	**********			750
Lead	TMECC 04.12B/04.14A	**6.1**	mg/kg	**********			150
Mercury	TMECC 04.12B/04.14A	**0.03**	mg/kg	**********			8
Molybdenum	TMECC 04.12B/04.14A	**2.1**	mg/kg	**********			9
Nickel	TMECC 04.12B/04.14A	**14.5**	mg/kg	**********			210
Selenium	TMECC 04.12B/04.14A	**0.1**	mg/kg	**********			18
Zinc	TMECC 04.12B/04.14A	**52**	mg/kg	**********			1400
		Pass					

Sample was received, handled and tested in accordance with TMECC procedures

Appendix E: Environmental Education Photo Montage

Students from Marshall Middle School's Citizen Science Institute program visit a nutrient bioextraction site to observe live plankton, collect mussel growth data, measure water quality parameters, measure biodiversity on mussel straps and create an underwater Go-Pro video, October 2015.

Recent graduate student assisting with data collection and mussel harvest at West Bay Marina, October 2015.

Students from TESC's Organic Farm mixing ingredients to make mussel compost, October 2015.

Evergreen State College students touring the Organic Farm and learning about the mussel compost project.

Komachin Middle School students observe mussels improving water clarity by filtering plankton from the water.

Komachin Middle School students collect mussel length and weight data.

Komachin Middle School students observe and sketch live phytoplankton.

Students learn about various water quality equipment including depth gauges, secchi disks, plankton nets, and YSI probes.

Young scientists collecting plankton during the What's Blooming in Budd program, Summer 2016.

Kids observing live plankton during the What's Blooming in Budd program, Summer 2016.

Community members measuring water depth in Budd Inlet.

Community members learning about nutrient pollution sources including dog waste at the Great Yards Get Together Event, September 2016.

One of the lovely giveaway items offered to responsible dog owners at the Great Yards Get Together - Rice Crispies Treat Doggie Doos.

Appendix F

Budd Inlet Water Quality

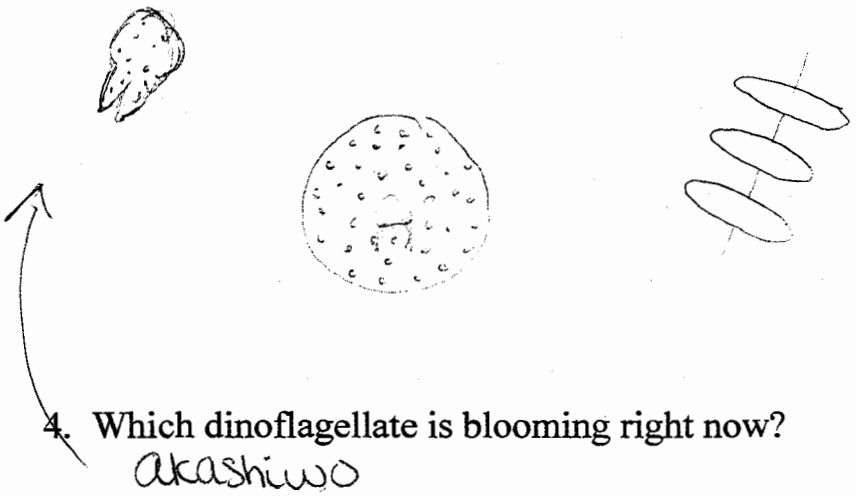
Heidi Kirk
Environmental Studies
September 13, 2013

Station A. Mussel Filtration Display

1. What do mussels filter out of the water column?
Planktonic plants and animals for a food source.
2. How does mussel filtration impact the surrounding marine environment?
They impact their surroundings by processing & recycling natural materials. cleans the water so other things can grow.

Station B. Phytoplankton

3. Draw 1-2 phytoplankton species from the Budd Inlet water sample.



4. Which dinoflagellate is blooming right now?
Akashiwo

Station C. Nutrient Sources

This station displays various sources of nutrients that can flow into lakes, streams, groundwater and ultimately Puget Sound where they fuel phytoplankton growth. As blooms die, bacterial decomposition leads to depleted oxygen levels which can be stressful to marine life.

5. Which products contain phosphates?
Miracle-Gro
6. Which product is phosphate-free?
Dishwashing liquid
7. List at least 2 nutrients found in Miracle Grow.
Nitrogen & phosphate

Station D. Mussel Growth Measurements (work in small groups)

This strap contains thousands of native blue mussels from Boat Works Marina in Budd Inlet. Randomly select 5 mussels and record their lengths in cm.

Mussel 1 2.1 CM
Mussel 2 3.2 CM
Mussel 3 2.4 CM
Mussel 4 2.7 CM
Mussel 5 3.0 CM

8. What is the average mussel length?
   2.72 CM
9. Compare your length to the graph. Are the mussels still growing?
   NO.

Station E. Seasonal Water Quality Data

The following graphs depict seasonal water quality data (temperature, salinity, pH and dissolved oxygen) from the 4 sites: BHM = Boston Harbor Marina, WBM = West Bay Marina, HF = Hearthfire Restaurant, STM = Swantown Marina

10. Which station is the coldest and saltiest?
    Boston Harbor Marina
11. Does pH and oxygen tend to increase or decrease as the summer progresses? Why?
    It decreases, because the plankton begin to decay.

Bonus Question!!!!

Department of Ecology is offering $100,000 to the organization with the best plan for reducing nutrient levels in the Deschutes River/Budd Inlet watershed. What's your plan?

Budd Inlet Water Quality

Per. 3 Sept. 13th 2013

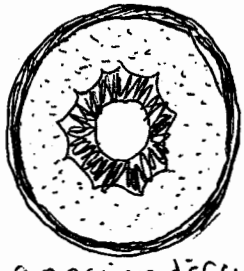
Heidi Kirk
Environmental Studies
September 13, 2013

Station A. Mussel Filtration Display

1. What do mussels filter out of the water column?
   small and free floating plants and animals for food
2. How does mussel filtration impact the surrounding marine environment?
   Filter feeding shellfish process and recycle natural materials which make them available for other living things.

Station B. Phytoplankton

3. Draw 1-2 phytoplankton species from the Budd Inlet water sample.



Coscinodiscus

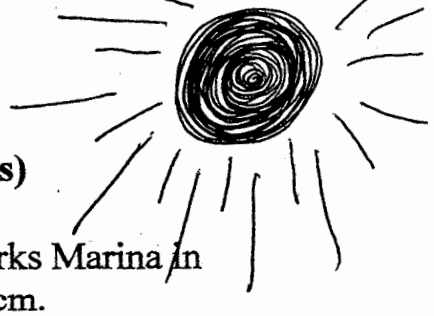
Thalassionema

4. Which dinoflagellate is blooming right now?
   Acoshuo

Station C. Nutrient Sources

This station displays various sources of nutrients that can flow into lakes, streams, groundwater and ultimately Puget Sound where they fuel phytoplankton growth. As blooms die, bacterial decomposition leads to depleted oxygen levels which can be stressful to marine life.

5. Which products contain phosphates?
   Miracle-gro ~~and FINISH~~ and FINISH tablets
6. Which product is phosphate-free?
   Dishwashing ~~liquid~~ soap
7. List at least 2 nutrients found in Miracle Grow.
   Nitrogen and zinc



Station D. Mussel Growth Measurements (work in small groups)

This strap contains thousands of native blue mussels from Boat Works Marina in Budd Inlet. Randomly select 5 mussels and record their lengths in cm.

Mussel 1 3 1/2 cm
Mussel 2 4 1/2 cm
Mussel 3 3 cm
Mussel 4 1.8 cm
Mussel 5 2.3 cm

8. What is the average mussel length?
3.02 cm
9. Compare your length to the graph. Are the mussels still growing?
yes they are. growing.

Station E. Seasonal Water Quality Data

The following graphs depict seasonal water quality data (temperature, salinity, pH and dissolved oxygen) from the 4 sites: BHM = Boston Harbor Marina, WBM = West Bay Marina, HF = Hearthfire Restaurant, STM = Swantown Marina

M: tonharbor Marina

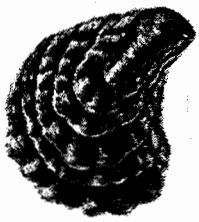
10. Which station is the coldest and saltiest?
BHM is the coldest ~~and~~ and saltiest
11. Does pH and oxygen tend to increase or decrease as the summer progresses? Why?
PH levels decrease, while water temperature rises. ~~Bacteria die~~

Bonus Question!!!!

Department of Ecology is offering $100,000 to the organization with the best plan for reducing nutrient levels in the Deschutes River/Budd Inlet watershed. What's your plan?

P.1

Budd Inlet Water Quality Worksheet



Pacific Shellfish Institute
Olympia, WA
www.pacshell.org

Station A. Phytoplankton

1. Draw 1-2 phytoplankton species from the Budd Inlet water sample.

Archshiwo sanguinea

ceratium fusus

2. Can you tell which are zooplankton or phytoplankton? Diatoms or dinoflagellates? Species? If so, label as such.

Station B. Mussel Growth Measurements (work in small groups)

3. Select 5 mussels and record their lengths in cm. What is the average mussel length?

Mussel 1 4cm

Mussel 2 4 1/2cm

Mussel 3 3.2 cm

Mussel 4 3.4 cm

Mussel 5 4.6cm Average Mussel Length 3.94 (cm)

Press the tare button on the scale. Place the 5 mussels in the dish and record their weight in grams. Divide the weight by 5 to obtain the weight per individual mussel.

Weight of 5 mussels 28.5 (g) Weight per Mussel 5.7 (g)

4. Compare your data to the graphs. To maximize the amount of nitrogen removed, we want the average mussel length to be at least 3 cm (or 30 mm) and the weight per mussel to be at least 1.5 grams. Is it time to harvest or should we wait longer?

I think it's to harvest because all of our measurements are big.

Station C. Water Quality Sampling

5. What do you think each piece of sampling equipment (A-D) is used for?

A. Depth
B. temp.
C. filter
D. water samples

6. Use the YSI probe (or refractometer) to measure the salinity in each jar. Which jar contains seawater and which is fresh?

Fresh water	Brackish water	Saline water	Brine
< 0.5 ppt	0.5 – 30 ppt	30-50 ppt	>50.0 ppt

A B

Station D. Solutions to Nutrient Pollution

This station displays various nutrient sources that can travel from our neighborhoods into lakes, streams, and ultimately Puget Sound where they fuel phytoplankton (algae) growth. As algae die, the process can rob bottom waters of oxygen placing stress on marine life.

7. Name one product that contains phosphates and one that is phosphate free.

Phosphates: fertilizer
No Phosphate: Muscle compost

8. List several actions that you can take to prevent nutrients from flowing into Puget Sound?

Changing the fertilizer people use. pick up dog poop. using soap w/o phosphate.

Nutrient Bioextraction is the process of growing and harvesting shellfish to remove nutrients from natural water bodies. Pacific Shellfish Institute has been testing this idea as a way to improve water quality in Budd Inlet. The mussels are then harvested and turned into nutrient rich, organic compost.

9. What are these mussels filtering out of the water?

Nitrogen from the algea.

10. How can shellfish filtration impact the surrounding marine environment?

Cleaner water and more oxygen.

11-21-16

What I learned today was very intresting. First, we learned about how nutriens go to the Oceans, lakes, and ponds. Then we got to observe many fastinating animals. For example, the jelly-fish, it was very soft, and it was clear.

Now that I heared you talk about that, I will try to be more aware of my sorroundings. For example, I will always pick up my dogs poo.

11-21-16

Today I learned about eutrophication. Eutrophication is when too much nutrients gets into the water. Then, the plankton feast on the nutrients. Once they're done, they die. Next, bacteria comes in and clean up the dead plankton - and the oxygen. These places with no oxygen are called dead zones. When the weather gets cold, and storms happen, the water mixes up, and the oxygen returns.

w/ Hang & Aimee

11-21-2016

Today I learned about the plankton and the life cycle of planktons. I also learned that too much nutrients in the water also called eutrophication.

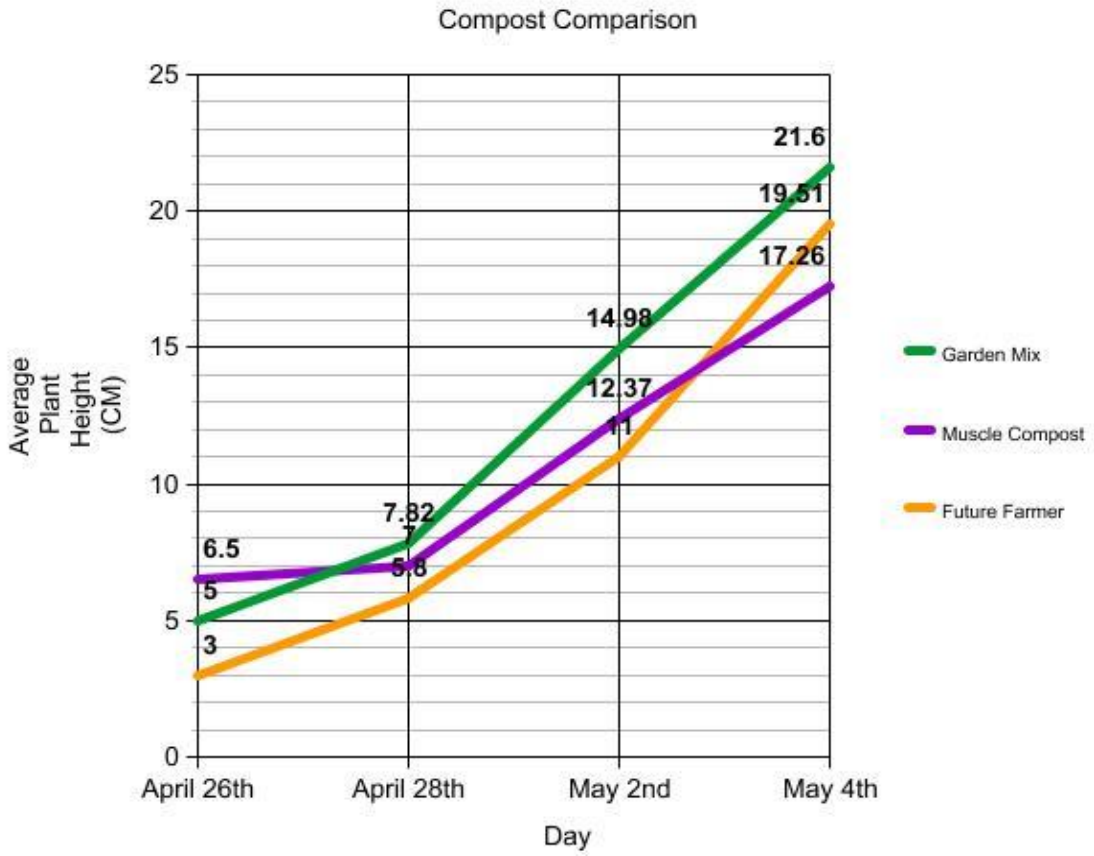
One way I could help the enviroment is to fertilize on the lawn and not get any on the sidewalk.

11/21/16

I learned that there are different kinds of plakton, like plakton that grow into plants and plakton that grow into sea animals. Like clams for example. I also learned that etrophication means extra nutrients. Another thing that I learned is that bioextraction means to remove extra nutrients. One thing I can do to keep extra nutrients is when I walk my aunts dog I can pick up its poop.

Appendix G

Marshall Middle School Mussel Compost Growth Trials Spring 2016



As one can see from this graph, by the end of the experiment, the Mussel Compost produced the shortest plant height average. Although, there is only a 4.34 CM difference between Garden Mix, the compost that produced the tallest plant average, and Muscle Compost. In the beginning of the experiment, the Muscle Compost had the tallest plant average, but later it was overtaken by the Garden Mix and the Future Farmer composts.

Although the Mussel Compost proved least effective by the end, it can still be used as a reliable compost material. All plants need nitrogen for growth and photosynthesis. Nitrogen is something this compost has a lot of. However, different plants consume different amounts of nitrogen. This compost would be very useful for plants that are particularly heavy nitrogen consumers such as roses, corn, lettuce, tomatoes, squash, cucumbers and cabbage. There is such a thing as too much nitrogen, which can be just as harmful to a plant as to little. The amount of nitrogen that is too much varies from plant to plant. This compost will be beneficial to plants like mentioned before but not to others.

All in all, this compost works pretty well but will really thrive with gardens that are in need of lots of nitrogen. It could however be too much for certain gardens. A nitrogen heavy compost can be harmful to plants that do not need as much nitrogen. More experiments could be done to find the perfect amount of nitrogen that could be used on a larger variety of plants. Making this compost material is a great way to use the excessive amount of muscles in the Puget Sound.

Appendix H

What's Blooming in Budd?

With the onset of spring comes blooming crocus, Indian plum and red-flowering currant. Did you know Puget Sound blooms as well? Spring marks a time of plentiful nutrients, sunshine and good mixing conditions in Puget Sound—perfect ingredients for fueling the microscopic plants of the sea, phytoplankton.

Last year marked the fourth year of Stream Team's plankton monitoring events in Budd Inlet near downtown Olympia. Between June and September, volunteers gathered at the Port Plaza dock to collect weekly information about the weather, tides, temperature, salinity and water clarity. Plankton samples were taken to the LOTT WET Science Center where they were projected onto a large screen for viewing, analyzed for species composition, and screened for harmful algal bloom (HAB) species. This ongoing data set allows the tracking of seasonal changes as well as the detection of changes over time.

Why study plankton?

Volunteers collect phytoplankton samples and view under microscopes to discover what's blooming in Budd.

Besides being fascinating to observe under the microscope, plankton are the life force of the ocean. Phytoplankton and zooplankton, the microscopic plants and animals of the sea, are the basis of the marine food web. The food web, which is a delicate balance between species and the environment, responds to human pollution and pressures in ways we are only beginning to understand. For example, Christopher Krembs, Washington Department of Ecology (WDOE), hypothesizes that Noctiluca, the bioluminescent dinoflagellate responsible for painting surface waters bright orange, may be blooming more frequently and intensely than in the past. The voracious appetite of this organism for phytoplankton, protozoans, copepods and fish eggs may be having an impact on important species such as diatoms and copepods. Copepods are not only a critical food source for many fish and invertebrates, but their sinking fecal pellets transfer nutrients to deposit-feeding organisms below. As you can see, a simple shift in plankton composition could have profound and unexpected impacts on the surrounding environment.

Phytoplankton also influence dissolved oxygen levels in seawater. They produce oxygen while photosynthesizing and are believed to be responsible for over half of the oxygen that we breathe today. However, in late summer and early fall, bacterial decomposition of plankton that have settled to the bottom can cause dissolved oxygen levels to plummet to dangerously low levels. This is especially true in lower Budd Inlet, where excess nutrients from a multitude of sources result in plankton-rich waters. Oxygen is critical to the health of all marine organisms and, when concentrations are low, fish and invertebrates become stressed. Moderation is key—too little or too much phytoplankton are both cause for concern.

Finally, phytoplankton is monitored because several species are capable of producing harmful biotoxins that can accumulate in filter feeding organisms such as shellfish. Washington Department of Health regularly tests shellfish for biotoxins to ensure that those harvested commercially and recreationally are safe to eat. Sound Toxins, a phytoplankton monitoring program managed by NOAA and Washington Sea Grant, relies on volunteers to collect weekly water samples throughout Puget Sound, screening them for HAB species that produce biotoxins. The "What's Blooming in Budd?" program participates in this program by entering weekly data onto the Sound Toxins database.

Noctiluca bloom captured by WDOE's Eyes Over Puget Sound program.

Did you know?

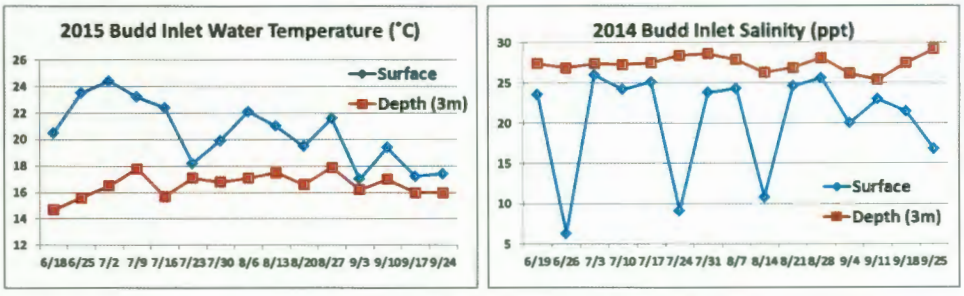
The weight of all the plankton in the oceans is greater than that of all the dolphins, whales and fish put together. Amazing when you consider that most plankton are microscopic in size!

Citizen Plankton Monitoring

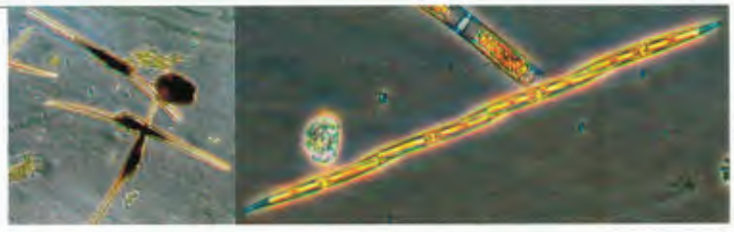
What have we discovered?

Over the past four years, volunteers have observed several interesting findings. First, it was hard not to notice the unusually warm surface water temperatures in Budd Inlet during the summer of 2015. Since 2014, researchers have identified a persistent warm water mass, nicknamed "the blob", in northeast Pacific waters. Extending into Puget Sound, "the blob" has raised water temperatures by 1.5–2.0°C. Since "What's Blooming in Budd?" was initiated, volunteers recorded peak surface temperatures reaching a high of around 21°C (70°F). Last summer, however, temperatures reached 24.4°C, or 75.2°F, by early July!

Volunteers were also fascinated by the enormous fluctuations in surface salinity occurring after rain events or Capitol Lake dam releases. While salinity remains fairly constant at depth (27–29 ppt), surface values can drop as low as 6 ppt during dam releases. Volunteers have also witnessed interesting changes in water clarity throughout the summer using an instrument called a Secchi disk. Water clarity is influenced by the amount of particulates in the water column such as suspended sediments and plankton. Too many particles can restrict light availability and visibility for submerged vegetation and marine life. Poor water clarity can also represent an overabundance of plankton, which could lead to subsequent drops in dissolved oxygen upon decomposition. According to the data collected, water clarity typically ranges from 2–5 meters in depth in lower Budd during the summer, but at times dropped to less than 1 meter, when Akashiwo and Ceratium were blooming!



Graphs displaying water temperature and salinity collected from the surface and depth.



Akashiwo sanguinea and Ceratium fusus—two of the most common dinoflagellates found in south Puget Sound Inlets during summer (left) and Pseudo-nitzschia, the diatom responsible for amnesic shellfish poisoning (right).

Finally, volunteers have detected HAB species such as the diatom Pseudo-nitzschia (responsible for amnesic shellfish poisoning) and dinoflagellate Dinophysis (responsible for diarrhetic shellfish poisoning) over the past several years. This is not unusual, and their presence does not necessarily indicate that they are producing toxins. However, one unusually large bloom of Dinophysis was detected in July of 2013. Simultaneously, Washington Department of Health posted the first closure to recreational shellfish harvesting in Budd Inlet's history based on elevated DSP toxins in tested mussel tissue.

Akashiwo sanguinea bloom in lower Budd Inlet, September 2014. Photo by Kelsey Browne, LOTT Clean Water Alliance.

How can I get involved?

Join Stream Team and biologists from Pacific Shellfish Institute at the dock this summer, starting June 23, to collect water quality data and discover what's blooming in Budd. Join us and be amazed as a drop of water comes to life right before your eyes! For more information, check the Stream Team website at www.streamteam.info

Don't Feed the Phytoplankton!

Phytoplankton are critical to the marine world, but too many nutrients can fuel large blooms that negatively impact water clarity and dissolved oxygen levels. Keep excess nutrients out of Puget Sound with these easy steps!

1. Minimize your use of synthetic lawn fertilizers. Use slow-release organic options instead.
2. Properly dispose of pet waste. Scoop It, Bag It, Trash It....every poop, every time.
3. Have your septic system inspected every year and pumped every 3–5 years.

Additional Resources

WDOE's Eyes Over Puget Sound: www.ecy.wa.gov/programs/eap/mar_wat/surface.html Learn more about algal blooms,"the blob," jellies, and Puget Sound water quality.

SoundToxins: http://www.soundtoxins.org/ Learn about this Puget Sound-wide HAB monitoring program.

Stream Team: www.streamteam.info/actions/lawncare/ Learn ways to keep your lawn healthy while keeping nutrients out of Puget Sound.

Pacific Shellfish Institute: www.pacshell.org Discover what's blooming in Budd. Also learn how PSI is removing nutrients in Budd Inlet by growing mussels and turning them into surf-to-turf compost.

Article courtesy of Aimee Christie, Pacific Shellfish Institute

Appendix I

AMELIA DICKSON adickson@theolympian.com

The West Central Park Project demonstrated a gardening style called hugelkultur at the Great Yards Get Together on Saturday. The process involves layering wood and other organic matter to create a spongy, nutrient-rich soil.

GREAT YARDS GET TOGETHER

Event guides gardeners to eco-friendly approach

BY AMELIA DICKSON
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Where in Olympia can you find compost made from ground-up Budd Inlet mussels?

At the Great Yards Get Together, of course.

The Pacific Shellfish Institute gave away the surprisingly-not-stinky compost by the tubful at the Saturday event, hosted at Heritage Park on Capitol Lake.

The event was devoted to providing gardeners with yard solutions that aren't harmful to humans, animals or the water supply, said organizer Susan McCleary, who works as a senior program specialist for the city of Olympia. The event was hosted by Stream Team, Thurston County and the cities of Lacey, Olympia and Tumwater.

She said her best advice is for people to practice integrated pest management — using solutions other than pesticides and fertilizers to improve a plant's health. These options include proper pruning techniques, placing plants in the right place and using good-quality soil.

Mussel compost is an example of a healthy solution.

Mary Middleton said the Department of Ecology-funded project is a version of nutrient bioextraction. The live mussels removed excess nutrients from Budd Inlet, and turning them into compost allows them to be used in other parts of the watershed.

SEE GARDEN, 9A

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GARDEN

The process of creating the compost was relatively simple, she said. The organization hung seatbelts in Budd Inlet and mussels attached themselves to the fabric.

Five months later, the mussels were harvested and put through a wood chipper. The concoction, mixed with wood chips, makes a great compost.

Aimee Christy tested the compost and said the plants grown in it did as well as those grown in a commercial, store-bought compost — but without the harmful chemicals.

After the compost was left outside and tended to by worms, it worked even better.

"No other compost compared," Christy said.

Dave Humphries and Alicia Elliott, of the West Central Park Project, also provided examples of healthy yard solutions.

Elliott said the park, located at the corner of Harrison Avenue and Division Street, features several sample gardens — including an example of hugelkultur. Humphries explained hugelkultur, in German, means "mound culture."

He said gardeners start by building a trench, which they then fill with rotting wood. Maple, alder and fruit woods work well — but gardeners should stay away from cedar.

On top, they place straw compost, soil and other organic materials. Plants are grown on top of the layers.

"It creates a spongy effect," Humphries said. "It adds nutrients to the soil for up to 20 years."

McCleary said gardeners who missed the Great Yards Get Together can learn more about yard solutions through the Master Gardeners Foundation of Thurston County.