

The effects of metal and nutrient addition on Ribbed Mussels, *Geukensia demissa*, in the Great
Sippewissett Salt Marsh and Eel Pond

18 December, 2006

Will Daniels

Semester in Environmental Science, Marine Biological Laboratory,
Woods Hole, MA

Lawrence University, Appleton, WI

Abstract

To determine the potential effects of nutrient loading and heavy metal pollution on salt marshes, researchers established long term experiments in the Great Sippewissett Marsh, Massachusetts, in which sewage-derived fertilizer (containing high concentrations of metals), and nutrient-only fertilizer was added to plots within the marsh. I investigated the effects of these alterations on the populations of ribbed mussels, *Geukensia demissa*, in the experimental plots. I also compared these mussels to those from Eel Pond, MA, a site highly contaminated with heavy metals.

Nutrient additions did not promote or deter the health of mussels in the Sippewissett plots. Mussel tissues were elevated in copper and chromium content compared to background levels. However, they were not at high enough levels in the Sippewissett plots to have significant impacts on the mussels. Mussels from Eel Pond contained higher levels of Cu than the sludge-fertilized plots; they exhibited reduced filtration rates, were slightly smaller, and grow slightly slower than the Sippewissett Marsh plots, perhaps a result of metal contamination.

Keywords

Salt marsh, nutrients, heavy metals, copper, mussels, Massachusetts, *Spartina*

Introduction

As a result of coastal land use changes worldwide, coastal aquatic ecosystems are subject to changes in their basic structure and function (Valiela et. al. 1992;). The largest threat to coastal communities is eutrophication due to land-derived nutrient addition. Since primary production in these systems is often limited by nitrogen, increased N-loading leads to increased plant productivity and, in some cases, shifts in plant communities (Valiela 1995). Another threat, which has decreased significantly since the 1970's, is heavy metal-contamination. Although metals (copper, lead, chromium, zinc, etc.) exist naturally in ecosystems, if they are in high enough concentrations, then metals can have detrimental effects on the health and behavior of animals. Common sources of metal pollution include sewage, lead paint and gasoline, boat paint, and pressure-treated wood.

Salt marshes are examples of highly productive coastal systems (Valiela 1995) that are affected by land-derived nutrient loading and metal pollution. Salt marshes along the coast of New England are a buffer between land and sea - acting as a sink for anthropogenic nutrient and metal pollution and, since they are low-energy systems, by protecting organisms and land from the turbulence of the ocean.

In 1970, researchers established a series of experimental plots in the Great Sippewissett Salt Marsh to study the effects of both nutrient addition and metal addition on the structure of a salt marsh ecosystem (Valiela et. al. 1975). They added nutrient- and metal-rich fertilizer to some plots and nutrient-only fertilizer to other plots. Although the concentrations of various trace metals in the fertilizer have decreased significantly since the 1970's, they are still at levels above their natural occurrence. Early studies of these plots determined that nitrogen addition increased the production of *Spartina* marsh grass (Valiela et. al. 1975). The metals are bioavailable – they are taken up in the tissues of plants and animals inhabiting these experimental plots (Giblin 1980, Greenbaum 1999).

Ribbed mussels, *Geukensia demissa*, are a common species of salt marshes, found in the intertidal zone of marshes. These are filter-feeding bivalves, which feed on a mixture of suspended *Spartina* detritus, phytoplankton, and to a lesser extent bacteria (). Because of their numbers and behaviors, these organisms play a critical role in the nutrient cycling of salt marshes (Jordan 1980; Jordan and Valiela 1982). During the peak of metal addition in the plots, the mussels contained elevated levels of Cu, Cd, and Cr (Giblin 1983).

Nitrogen addition to marshes boosts the growth and production of these animals as a result of increased food availability (Evgenidou and Valiela 2002). In contrast, trace heavy-metal contamination is detrimental to mussels. Metals are taken into the tissue either through food assimilation, or it is absorbed directly from the water (). In San Francisco Bay, another species of bivalve, *Macoma balthica*, exhibited significantly reduced reproductive output as a result of high concentrations of copper and silver (Hornberger et. al. 2000). For *M. balthica*, the effects range-median (ERM) of copper in the sediment is 270 µg/g; the ERM of zinc is 410 µg/g. In the presence of enhanced metal concentrations, marine bivalves also keep their shells closed longer (Doherty et. al. 1983), are less resistant to infection (Pipe and Coles 1995), and slow or stop their feeding (filtration) rates (Grace and Gainey 1987; Redpath and Davenport 1988).

Although the concentrations of trace metals within the tissue of *G. demissa* have been measured, the health of the mussels has not been studied. I returned to the plots to compare the populations of ribbed mussels from plots of various treatments within the Great Sippewissett Marsh. The plots are receiving both nutrient and metal addition; it is difficult, but necessary to study both counteracting drivers simultaneously.

I also chose a site for comparison to the Great Sippewissett Marsh. This site, Eel Pond, MA, is known to be highly contaminated with the trace metals Pb, Cu, and Zn (Chappell 1998), but is not thought to be receiving excessive nutrient loading. The mussels at this reference site are predicted to be less abundant and less 'healthy' than mussels from the Sippewissett sites because of the chronic contamination with heavy metals.

Site Description

The experimental plots in the Great Sippewissett Marsh were established in 1970 by Valiela and Teal (Valiela et. al. 1975). They are 20 m in diameter and each is divided by a small creek that drains into a larger stream (Fig. 1). The high marsh in each plot is dominated by the marsh grasses *Spartina patens* and *Distichlis spicata*, while the low marsh and creek banks are dominated by tall *Spartina alterniflora*. I sampled from two control plots (C), two plots that receive a urea-phosphate fertilizer (UP), and two plots that receive metal-bearing sewage-derived fertilizer (HF). Since the abundance and growth of mussels is dependent on the time they are submerged by the tides (Evgenidou and Valiela 2002), I used vegetation type as a proxy to control for the elevation of my sampling sites – all surveys and collections were performed along the creek banks among the tall *S. alterniflora* grass.

I also sampled from the *S. alterniflora* marsh area along the east side of Eel Pond, MA. This harbor is historically home to many boats, docks, and wastewater from laboratories and perhaps septic tanks; I chose this site because it is known to be contaminated with heavy metals, especially lead and copper (Chappell 1998).

Methods

I performed a survey of the *Geukensia demissa* population at each site. I estimated the abundance of mussels by counting individuals in eight 0.25 m² - four random quadrats on each

side of the creeks that run through the plots. In order to construct size distributions for the populations, I measured the length of every mussel within these quadrats.

From each plot, I collected 8 individual mussels for analysis of filtration rates, growth rates, lipid content, metal content, and stable isotopes. Since most of the mussels were between 4 and 6 cm in length I collected four mussels in the 4-5 cm range (Figure x) and 4 mussels in the 5-6 cm range. Half of the mussels from each plot were randomly chosen for metal content analysis, the remaining were used for lipid content analysis. I also gathered detrital surface sediments (0-2 cm) for analysis of metal content, carbon and nitrogen content, and stable isotopes.

Relative filtration rates were estimated by measuring the loss of suspended microalgae over time in the presence of a mussel (Culbertson pers. comm.). I placed each individual, chosen from a random plot, into a 1 L beaker with 600 mL of sea water containing *Isochysis sp.* (T-iso) algae. Each beaker was aerated to keep the mussel alive and to keep the algae well-mixed in the water. Once the individuals began filtering, I withdrew a 10 mL subsample of water from the center of the beaker with a syringe. Samples were collected at times 0, 5, 10, 20, and 30 minutes; these were stored on ice until chlorophyll a determination. Two controls were run using the same procedure but without mussels in the beakers. Chlorophyll concentrations were measured *in vivo* on a fluorometer; note that I did not do use the standard acetone extraction in order to a) save time and supplies and b) because the *in vivo* method is reliable for determining the relative loss of chlorophyll. Since the initial chlorophyll concentrations for each run were not the same, I normalized each reading to the initial chlorophyll values to obtain the percent chlorophyll remaining. Overall, the loss of chlorophyll follows an exponential decay, but it is fairly linear for the first 20-30 minutes, so I considered the linear slope of the % chlorophyll remaining vs. time as the relative filtration rate.

After allowing the guts of the animals to clear during the filtration experiment, I sacrificed the animals, randomly picked half of the mussels from each plot and size class, removed the shells, and then digested the mussel tissue with 10 mL of nitric acid heated to 70 C for one approximately two hours. I also digested ~0.3 g of sediment from each plot. I exposed each sample to 5 mL of nitric acid heated to 70 C for one hour, followed by 5 mL of hydrochloric acid at 70 C for one hour. The sediment digestion was filtered using glass fiber filters and diluted to 100 mL. I measured lead, copper, zinc, and chromium in these digests using the Perkin-Elmer flame atomic absorption spectrophotometer.

I measured the lipid content of entire mussels using a modified version of the method described by Bligh and Dyer (1959). The method utilizes the ability of chloroform to extract lipids, while the non-lipids enter a methanol layer; the mixture separates into a biphasic state, allowing determination of lipid content. The mussel tissue fit the method's criteria of containing approximately 80% water, but I had to scale the procedure down to match the weight of individual mussels.

I counted the growth rings after first breaking the shells along the longest line through the umbo, sanding the cross sections smooth, and shining them with water. The growth rate was determined by relating the length of shells to the number of growth rings.

Isotope samples were prepared by drying a portion of the adductor muscles of the same animals used in metal analysis. Tissue from animals of the same plot was compiled, then ground using a mortar and pestle. Sediment samples were dried, ground, and acidified using 10% HCl to dissolve any carbonates present. Samples were analyzed for ^{13}C and ^{15}N on a mass spectrophotometer by Marshall Otter of the MBL ecosystem center.

I used the CHN analyzer to measure the carbon and nitrogen content of the sediment. These samples were prepared by drying and grinding sediment from each site with a mortar and pestle.

Results

The surface 2 cm of sediment in the control (C) and urea-phosphate (UP) treatment plots contain similar amounts of each metal that was measured (Table 1). These concentrations are similar to those determined by Giblin et. al. (1982), except for Cr, which appears >10x higher now than it did 25 years ago. The sewage-fertilizer plots (HF) are not elevated in Pb in the sediment. They are elevated in Zn, Cu, and Cr. The concentration of Cr in the HF plots is 250 µg/g, which is more than an order of magnitude lower than was observed previously in the XF plots. Eel Pond sediment is highly elevated in Pb and Cu compared to the Great Sippewissett sediments. It contains more Zn than the C and UP plots, but less than the HF plots. The concentration of Cr at Eel Pond is similar to that of the C and UP plots in the marsh

The concentration of Cu and Cr in the mussels reflects the concentrations in the sediment (Table 1). The highest value of Cu is 32 µg/g, seen in at Eel Pond, while the HF mussels were the most elevated in Cr at 2.2 µg/g. The Pb content of the mussel tissue is more erratic because the concentrations are low enough that precise measurements are difficult with the technique I used. The Pb in the Eel Pond mussel samples was not markedly higher than the other sites as was seen in the sediment. The amount of Zn in the mussel tissues was not different between any of the sites.

The population dynamics of the mussels show little differences between sites as a result of fertilization and/or metal load. The abundance of animals is roughly the same, 25-35 individuals/m² (Fig. 2) and the size distributions do not vary greatly between sites (Fig. 3). It is difficult to discern cohorts within the population based on the size histograms. The median size of the individuals ranges from 58 mm at Eel Pond to 65 mm in the C plots. Figure 4 shows that the length vs. age distributions are similar between the Sippewissett plots, but perhaps, for a given size range, the mussels at Eel Pond are older than those at Sippewissett. This would suggest that they either grow slower or have a shorter maximum length, and it would support the size distribution having a slightly lower median.

Using lipid content of the mussel as a condition index, there was little difference in health of the mussels between the sites (Fig. 5). Lipids comprised between 3% and 5% of the mussel dry mass, which is comparable to previous studies of the lipid content of *G. demissa*. The HF and Eel Pond mussels are slightly elevated in lipid content compared to the C and UP plots, which is contrary to the hypothesis that increased metal contamination would result in decreased lipid content.

All but nine of the mussels exhibited feeding during the filtration experiment. The change in chlorophyll vs. time had a significant regression for each treatment ($p < 0.01$). The relative rates of water filtration performed by the mussels were the same for each treatment in the Sippewissett Marsh (Fig. 6). The Eel Pond mussels filtered approximately half as fast as the others.

The carbon isotope results show that mussels from different sites feed on roughly the same mixture of *Spartina* and algae (Table 2); the tissue samples fall between -16.3 and -16.7. The $\delta^{13}\text{C}$ of the sediment at Sippewissett ranges from -15.2 to -16.6 which suggests high

Spartina contribution; $\delta^{13}\text{C}$ is -23.4 in the Eel Pond sediment, suggesting a large algae constituent.

The isotopic nitrogen signal of the C plots is $\delta^{15}\text{N} = 2.4$. It is slightly lower than this in the UP plots, as expected since the source of the urea fertilizer is atmospheric nitrogen (Table 3). The HF sediment is enriched in ^{15}N compared to the C plot, and the Eel Pond signal is the heaviest, at 8.8. Assuming that the sediment detritus is an important food source for the mussels, there is high fractionation during assimilation by the animals. The ^{15}N isotope signals in the mussel tissue reflect the ^{15}N isotope signals in the sediment – it is lower in the UP than the control, higher in the HF, and highest in the Eel Pond mussels.

The carbon:nitrogen molar ratio is lower at the HF plots and Eel Pond than it is at the C and UP plots (Table 4).

Discussion

In the Great Sippewissett Marsh plots, nutrient addition did not positively affect the ribbed mussel populations. In Waquoit Bay, mussel growth was enhanced by nutrient loading as a result of increased phytoplankton growth (Evgenidou and Valiela 2002); in the Sippewissett plots, nitrogen loading did not increase phytoplankton availability but rather *Spartina* availability. Although the mussels were eating *Spartina* detritus from within their plots, as suggested by stable isotope signatures, the size of the plots and the scale of the increased *Spartina* productivity were not large enough to boost mussel growth.

The mussels appear to be consuming approximately the same proportions of algae and *Spartina*. The $\delta^{13}\text{C}$ signature of the mussels from Eel Pond is slightly lower than the signature of the Sippewissett mussels suggesting that algae or terrestrial inputs play a slightly larger role in their diets than is true for the Sippewissett mussels. Phytoplankton, with a typical $\delta^{13}\text{C}$ signature of -21 (Peterson and Howarth 1987) is probably more important because if terrestrial inputs were significant, one would expect lower $\delta^{15}\text{N}$ values for the Eel Pond sediment and mussel tissue. Algae and *Spartina* comprise different proportions of mussel diets depending on their location in the marsh (Peterson et. al. 1985). The Eel Pond marsh is located on the front edge of the marsh where it has access to an abundance of phytoplankton when submerged; the Sippewissett plots are set back from the main tidal channel where they see less algae and more *Spartina*.

The signals of ^{15}N from the sediment of various Sippewissett plots are as expected. The $\delta^{15}\text{N}$ signature is lower in the UP plots than the control plots because the source of nitrogen in the fertilizer is atmospheric N, which has a $\delta^{15}\text{N}$ of zero (Peterson and Howarth 1987). The signal of ^{15}N is higher in the HF plots because the fertilizer is derived from sewage sludge which is typically ^{15}N enriched. The Eel Pond sediment is the most enriched, suggesting that there is some septic runoff entering the harbor; further research needs to be done regarding this.

As nutrient addition did not promote the mussels in the experimental plots, metal addition in the HF plots did not seem to harm the animals in any way that I measured. The length-age relationship was no different between the Sippewissett Marsh plots. There is much fuzz in this data set, but the growth appears similar to that found by Evgenidou and Valiela (2002) in Waquoit Bay. Although I only sampled from the middle range of sizes, the growth of mussels can be modeled with a standard von Bertalanffy curve. Lipid content was less than 5% of dry weight in all plots, which is comparable to other studies on New England ribbed mussels (Bergen et. al. 2001), but it is difficult to see differences in lipid content, if any, at such low levels.

The Eel Pond mussels may be slightly affected by metal contamination. The ERM of copper in sediment for bivalves is 270 µg/g; I observed 200 µg/g in the Eel Pond sediment. Thus, although they are not exposed to toxic levels, they are well above the background (~20 µg/g in the control plots) and may be in the low range of harmful concentrations. Mussels and sediment of Eel Pond are also elevated in Cr.

The most notable difference observed in these mussels is the filtration rates. Laboratory conditions held equal, the Eel Pond mussels cleared the water roughly half as fast as Sippewissett mussels. They also are slightly smaller as was indicated by a shorter median length for a given year class. Individuals from Eel Pond also appear to grow slightly slower, although more sampling must be performed to verify this. In terms of growth rates, younger mussels are more responsive to nutrient additions than old mussels (Evgenidou and Valiela 2002). Thus, the range of growth rate analysis that I used should be expanded to the smaller size classes.

In effect, modern levels of heavy metal pollution may be having a slight impact on *G. demissa* in Eel Pond. The findings of this study merit further analysis. It would be useful to study gamete production of the various populations.

Nutrient addition to experimental plots in the Great Sippewissett Marsh did not affect the local populations of ribbed mussels. However, these plots were small and we might expect that if the entire marsh were to receive additional nutrients, population dynamics of the mussels would be altered because of widespread increases in primary productivity. It would be worthwhile to collect suspended particulates from each site during high tide to analysis the food sources of the animals.

Works Cited

Bergen, B. J. et. al. 2001. Relationships among total lipid, lipid classes, and polychlorinated biphenyl concentrations in two indigenous populations of ribbed mussels (*Geukensia demissa*) over an annual cycle. *Environ. Toxic. Chem.* **20** (3): 575-581.

Bligh, E. G. and Dyer, W. J. 1959. A Rapid Method of Total Lipid Extraction and Purification. *Can. J. Biochem. Physiol.* **37** (8): 911-917.

Chappell, D. 1998. Heavy Metal Mud: A Comparison of Heavy Metal Profiles and Metal Lability in the Sediments of Harbors around Cape Cod. SES project.

Doherty, F. G. et. al. 1987. Valve closure responses of the Asiatic clam *Corbicula fluminea* exposed to cadmium and zinc. *Hydrobiologia* **153**: 159-167.

Evgenidou, A. and Valiela, I. 2002. Response of growth and density of a population of *Geukensia demissa* to land-derived nitrogen loading, in Waquoit Bay, Massachusetts. *Estuarine, Coastal, and Shelf Science* **55**: 125-138.

Giblin, A. E. et. al. 1980. Uptake and losses of heavy metals in sewage sludge by a New England Salt Marsh. *Amer. J. Bot.* **67** (7): 1059-1068.

Giblin, A. E. et. al. 1983. The fate of metals introduced into a New England salt marsh. *Water, Air, and Soil Pollution* **20**: 81-98.

Grace, A. L. and Gainey Jr., L. F. 1987. The effects of copper on the heart rate and filtration rate of *Mytilus edulis*. Mar. Polut. Bull. **18** (2): 87-91.

Greenbaum, A. 1999. Long term trends in heavy metal inventories in experimental plots. SES project.

Hornberger, M. I. et. al. 2000. Linkage of bioaccumulation and biological effects to changes in pollutant loads in South San Francisco Bay. Environ. Sci. Technol. **34**: 2401-2409.

Jordan, T. E. 1980. A nitrogen budget of the ribbed mussel, and nitrogen flow in a New England salt marsh. Ph. D. thesis, Boston University Graduate School, Boston, MA.

Jordan, T. E. and Valiela, I. 1982. A nitrogen budget of the ribbed mussel, *Geukensia demissa*, and its significance in the nitrogen flow in a New England salt marsh. Limnol. and Oceanogr. **27** (1): 71-90.

Peterson, B. J. et. al. 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. Science **227**: 1361-1363.

Peterson, B. J. and Howarth, R. W. 1987. Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt marsh estuaries of Sapelo Island, Georgia. Limnol. Oceanogr. **32** (6): 1195-1213.

Pipe, R. K. and Coles, J. A. 1995. Environmental contaminants influencing immune function in marine bivalve mollusks. Fish and Shellfish Immunol. **5** (8): 581-595.

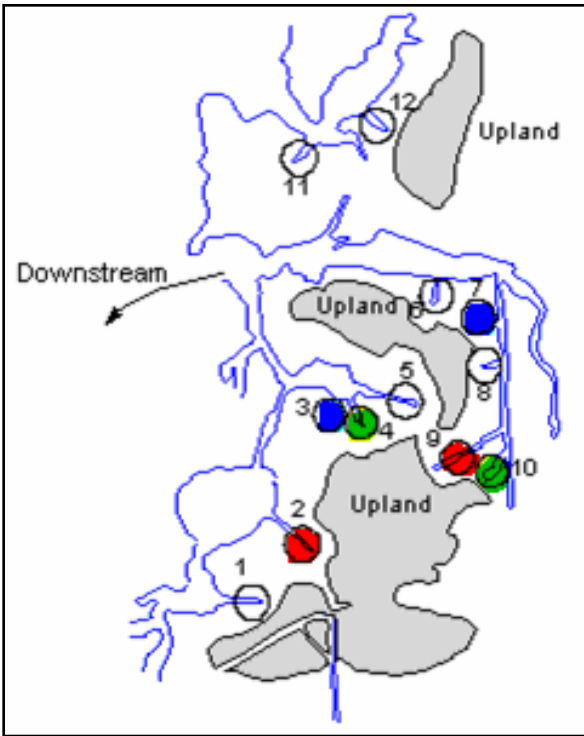
Redpath, K. J. and Davenport, J. 1988. The effect of copper, zinc, and cadmium, on the pumping rate of *Mytilus edulis*. Aquatic Toxicol. **13**: 217-226.

Valiela, I. 1995. Marine Ecological Processes 2nd ed. Springer, New York, USA.

Valiela, I. et. al. 1975. Production and Dynamics of Salt Marsh Vegetation and the Effects of Experimental Treatment with Sewage Sludge: Biomass, Production and Species Composition. J. App. Eco. **12** (3): 973-981.

Valiela, I. et. al. 1992. Couplings of watersheds and coastal waters: sources and consequences of nutrient enrichment in Waquoit Bay, Massachusetts. Estuaries **15**: 443-457.

Tables and Figures



Plot	Treatment
C	Control
UP	Urea-Phosphate addition
HF	Sewage-fertilizer addition
Eel Pond	

Figure 1: Map of the Great Sippewissett Marsh.

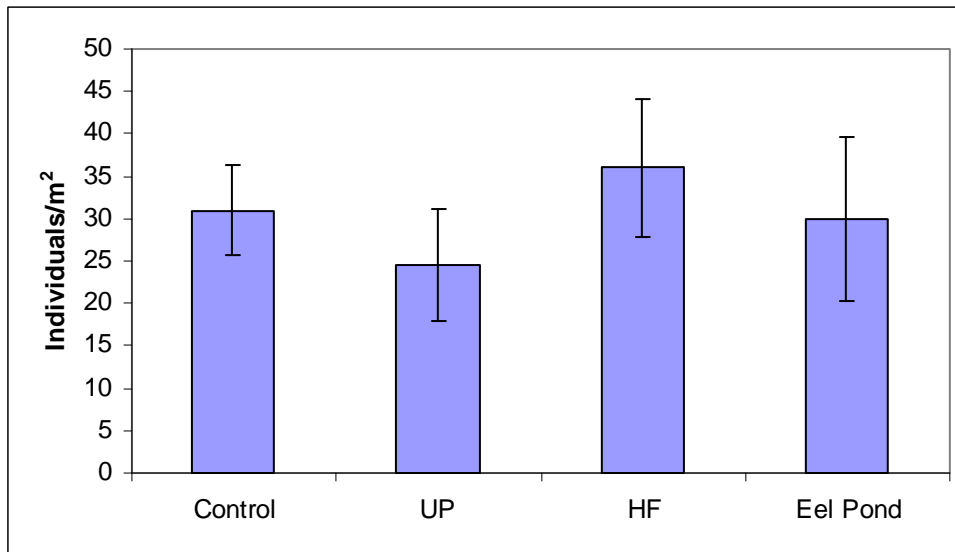


Figure 2: The abundance of ribbed mussels at the various sites.

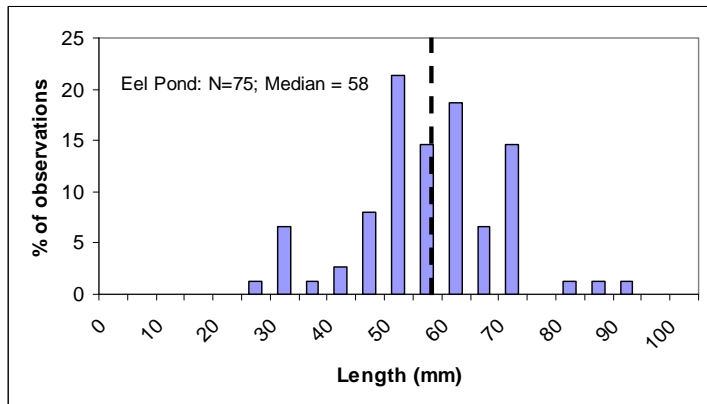
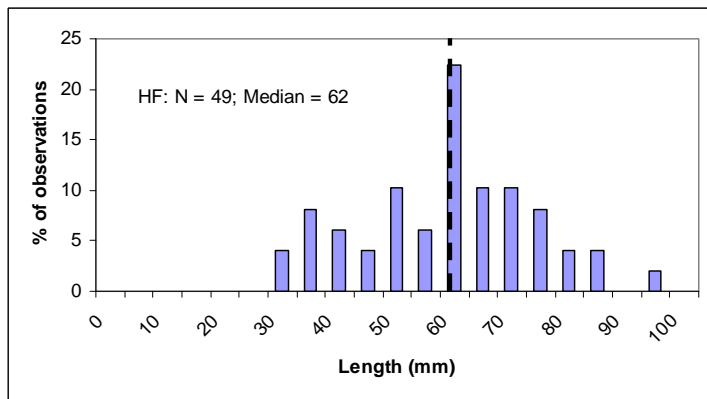
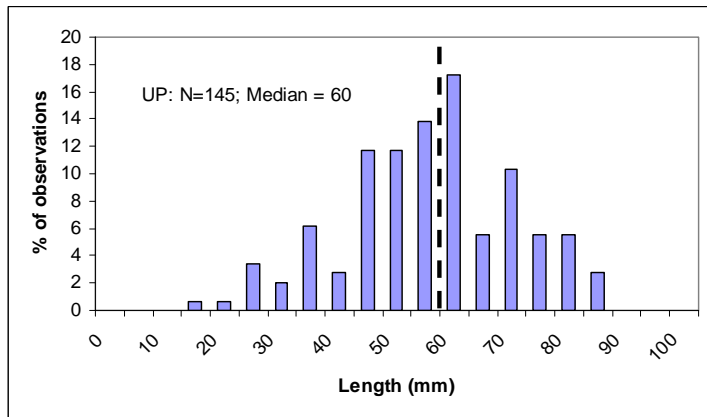
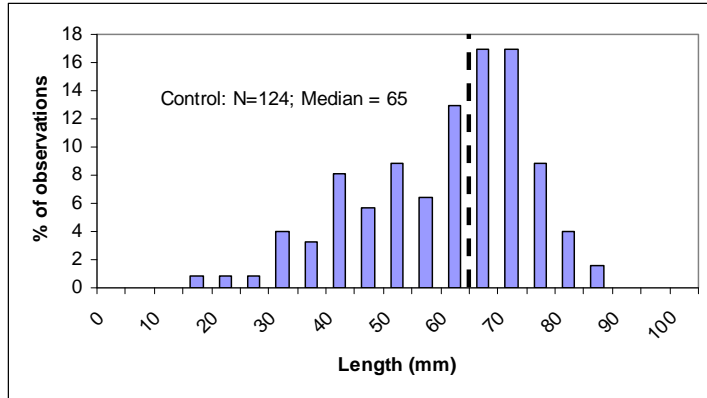


Figure 3: Size distributions of mussels from the four sites. Dotted lines are the medians.

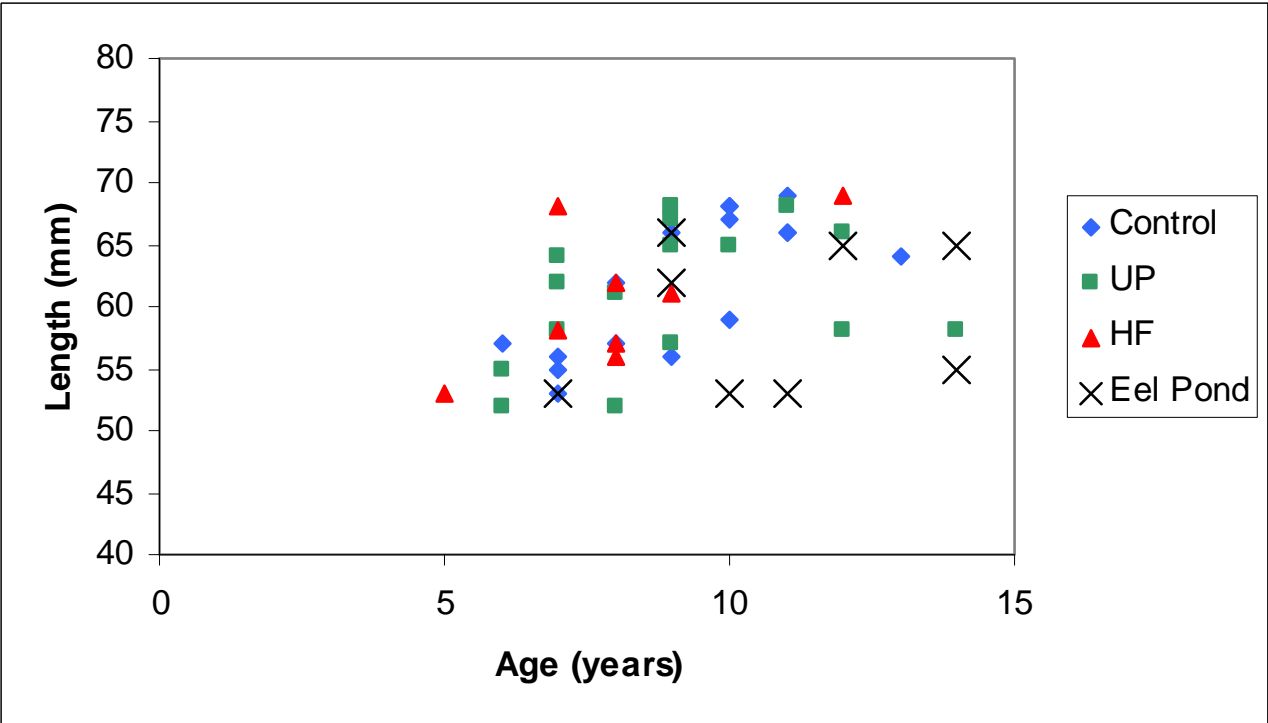


Figure 4: Age length relationship of mussels from the four sites. This tells us about the growth rates of the mussels.

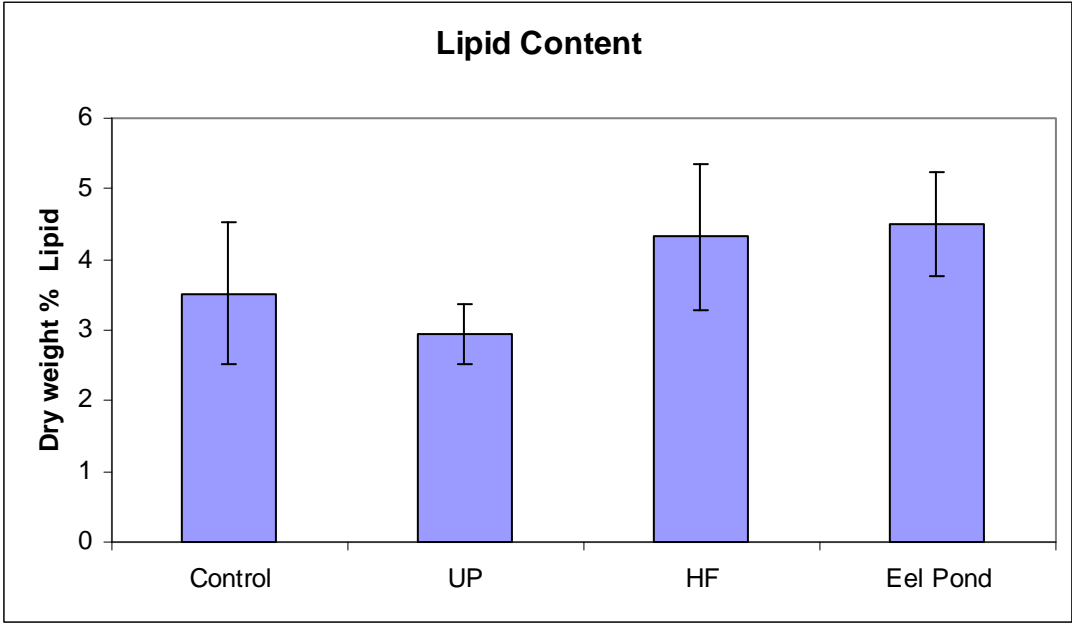


Figure 5: The lipid content of the mussel tissue.

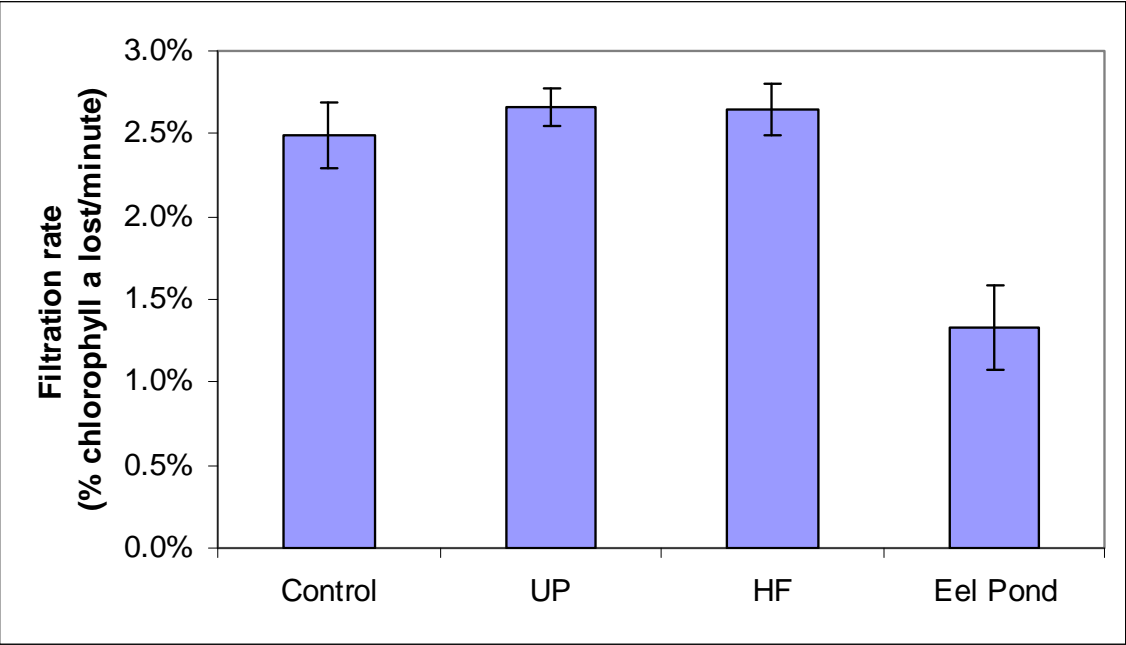


Figure 6: Relative filtration rates of mussels from different treatments.

		Control	UP	HF	Eel Pond
Pb	Sediment	45.4	35.9	44.6	214.2
	Mussel	1.0	0.0	1.9	1.1
Zn	Sediment	60.9	65.4	164.2	91.0
	Mussel	58.8	47.3	62.0	53.1
Cu	Sediment	18.0	27.0	101.9	201.3
	Mussel	8.5	6.4	12.5	32.5
Cr	Sediment	21.8	41.5	249.8	34.1
	Mussel	0.6	0.3	2.2	0.8

Table 1: Concentration of Pb, Zn, Cu, and Cr in the sediment and mussel tissue. Units are $\mu\text{g/g}$ dry sediment or dry tissue.

	Sediment	Mussels
Control	-15.2	-16.7
UP	-15.2	-16.5
HF	-16.6	-16.3
Eel Pond	-23.4	-17.6

Table 2: $\delta^{13}\text{C}$ signatures of sediment and mussel tissue.

d15N	Sediment	Mussels
Control	2.4	7.6
UP	1.8	7.4
HF	4.0	8.4
Eel Pond	5.6	8.8

Table 3: $\delta^{15}\text{N}$ signatures of sediment and mussel tissue.

	C:N
Control	14.7
UP	14.9
HF	12.0
Eel Pond	12.0

Table 4: Carbon to nitrogen ratio in the sediments from the study sites.