

EFFECTS OF A WASTEWATER OUTFALL IN A ROCKY INTERTIDAL
COMMUNITY

by

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We certify that we have read this study and that it conforms to acceptable standards of scholarly presentation and is fully acceptable, in scope and quality, as a thesis for the degree of Master of Arts.

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ABSTRACT

Effects of a wastewater outfall in a rocky intertidal community

Karen M. Warburton

The Crescent City wastewater treatment facility services a small community on the northern coast of California, where treated wastewater is discharged into a rocky intertidal community. A biological assessment of the habitat was conducted to determine if the released effluent had a negative impact on the intertidal community. I predicted the community composition at the outfall site would differ when compared to a site not subjected to an outfall. I also expected to detect a gradient in community composition moving away from the outfall.

Enderts Beach was selected as a control site because it is in close geographic proximity and is similar to the discharge site in elevation, wave exposure and micro-topography.

Three sampling events occurred in August 2002, April 2003, and August 2003, during which, I obtained thirty samples at each site. Descriptive statistics comparisons showed no significant differences between sites. However, differences in community composition were detected between the two sites.

A number of confounding factors may have influenced the statistical results of the study. The type of sampling design utilized during this study provided no means of determining why the differences occurred. However, there was no obvious

impoverishment of the community composition at the outfall site. Furthermore, there was no detectable gradient in community composition moving away from the outfall.

Conditions at the outfall site made it difficult to locate more than one reference site. Therefore, results of the study were statistically inconclusive. In order to provide a less ambiguous conclusion, more control sites would be required.

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INTRODUCTION

The effects of anthropogenic influences on ecological processes can be profound (Nixon 1998, Burridge *et al.* 1999, Crowe *et al.* 2000, Soltan *et al.* 2001, Arvai *et al.* 2002). In aquatic environments, where water is the mechanism by which contaminants are spread, the effects can be far reaching (Smith 1996, Chisholm *et al.* 1997, Costanzo *et al.* 2001, Johnston and Keough 2002). This phenomenon has been well-documented, showing that chronic pollution can be quite influential on shoreline communities of plants and animals (Foster *et al.* 1988, Raffaelli and Hawkins 1999, Crowe *et al.* 2000, Soltan *et al.* 2001, Johnston and Keough 2002). Disposal of treated and non-treated sewage wastewater into near-shore and offshore marine environments has facilitated shifts in community structure (Raffaelli and Hawkins 1999). Biological assessments of the effects of coastal wastewater outfalls are important in determining the levels and impacts of pollution. In ocean environments, a shift in community structure may be a biological indicator of water quality in the region (Bilyard 1987, Smith 1996, Chisholm *et al.* 1997, Crowe *et al.* 2000). Water containing high nutrient loads may be toxic to nearby human populations, whether they are in direct contact with it, or utilizing resources from within the contaminated region (Nixon 1998).

Crescent City is a small town on the northern coast of California (latitude 41°44.7' N, longitude 124°11.0' W) with a population of approximately 7,500. The small wastewater treatment facility that serves this community currently discharges on average between 1.5 and 1.7 million gallons per day of secondary treated sewage wastewater

effluent into the rocky intertidal region at Battery Point Lighthouse, on the west side of the city. The effluent is a combination of treated domestic wastewater and discharge from the Crescent City Harbor, which consists of waste from commercial fisheries processing facilities (S. Li, Crescent City Wastewater Treatment Facility, personal communication).

Since 1994, the wastewater treatment facility has been operating at capacity, particularly during the winter months with the influx of winter storm runoff. The city is in the process of completing plans to build a new treatment facility or expand the current one so that it can better serve the community (Zabinsky 2002). A new or larger facility will continue to use the existing outfall and possibly increase the amount of effluent as growth occurs in the community. As of the year 2002, the maximum amount of effluent being discharged from the facility is as high as 4 million gallons per day (S. Li, Crescent City Wastewater Treatment Facility, personal communication).

An assessment was necessary to provide insight into how the site may be impacted in the future by the increase in discharged wastewater. This was accomplished by investigating the effects of the effluent prior to enlarging the facility. The purpose of my study was to determine how the rocky intertidal biota near the discharge pipe compared to the biota at a site similar in wave exposure and elevation.

Some previous studies have reported a small or minimal negative impact on areas exposed to sufficiently treated wastewater effluent (Smith 1994, Underwood and Chapman 1996). Diener and Fuller (1995) were able to show evidence of enhancement of a benthic community from a secondary treated wastewater outfall. Other studies, however, have indicated that wastewater outfalls can detrimentally impact the ocean

environments into which they are discharged (Littler and Murray 1975, Fairweather 1990, Gappa *et al.* 1990). Changes in water quality can often influence the kind and abundance of organisms that are present in a given location (Myslinski and Ginsburg 1977). As nutrient loads increase, so does eutrophication of green ephemeral algae and phytoplankton, increasing algal blooms that can decrease oxygen supply in the water (Nixon 1998). Previous studies have documented near-shore habitats subjected to sewage effluents of varying treatment methods exhibit changes in invertebrate community structure and decreases in species richness, density, diversity, and total abundance (Littler and Murray 1975, Bilyard 1987, Smith 1996, Chisholm *et al.* 1997, Crowe *et al.* 2000, Johnston and Keough 2002). Spatial gradients of species density or diversity can also occur, which change with decreasing influence from the effluent source (Kennish 1998, Soltan *et al.* 2001, Je *et al.* 2003).

This evidence suggests that a comparison of community compositions between the two sites in combination with an analysis of community composition moving away from the outfall, was an appropriate means of detecting effects of treated effluent from the outfall at the discharge site.

Because the treated wastewater is discharged in close proximity to the intertidal rocks that serve as substrate and shelter to many types of organisms, it was my hypothesis that the effluent released into the vicinity would demonstrate a negative impact on the community structure. I expected that the impact would manifest itself by exhibiting differences in community composition between the outfall site and a site not subjected to the wastewater discharge. Furthermore, I expected to be able to detect a noticeable

gradient in community composition when analyzing samples taken nearer to the outfall when compared to those taken further away.

MATERIALS AND METHODS

Study Area

Outfall Site

The outfall is located at Battery Point, below the lighthouse in Crescent City (latitude N 41° 44.632' longitude W 124° 12.162'). Effluent is discharged into a modified surge channel at approximately 10 feet below MLLW in the base of the rock. The surge channel faces the direction of prevailing wave impact, which results in intense wave activity. There is substantial agitation and mixing of the effluent into the receiving waters. The pipeline, which feeds from the wastewater treatment plant to the outfall, is covered by concrete, resulting in an intertidal bench that is flat and rough in texture. This bench is located just to the south of the surge channel (Figure 1).

Control Site

Enderts Beach is located approximately 4.3 miles south of Battery Point Lighthouse, within Redwood National Park (latitude N 41° 42.27', longitude W 124° 08.66'). This site is similar in micro-topography to Battery Point Lighthouse. There are rocky benches at this location which provide horizontal surfaces supporting an intertidal community at approximately the same intertidal elevation, a necessary condition at the control site for comparison purposes (Figure 2). Furthermore, Enderts Beach is an exposed location, subjected to the same type of wave activity observed at the study site.

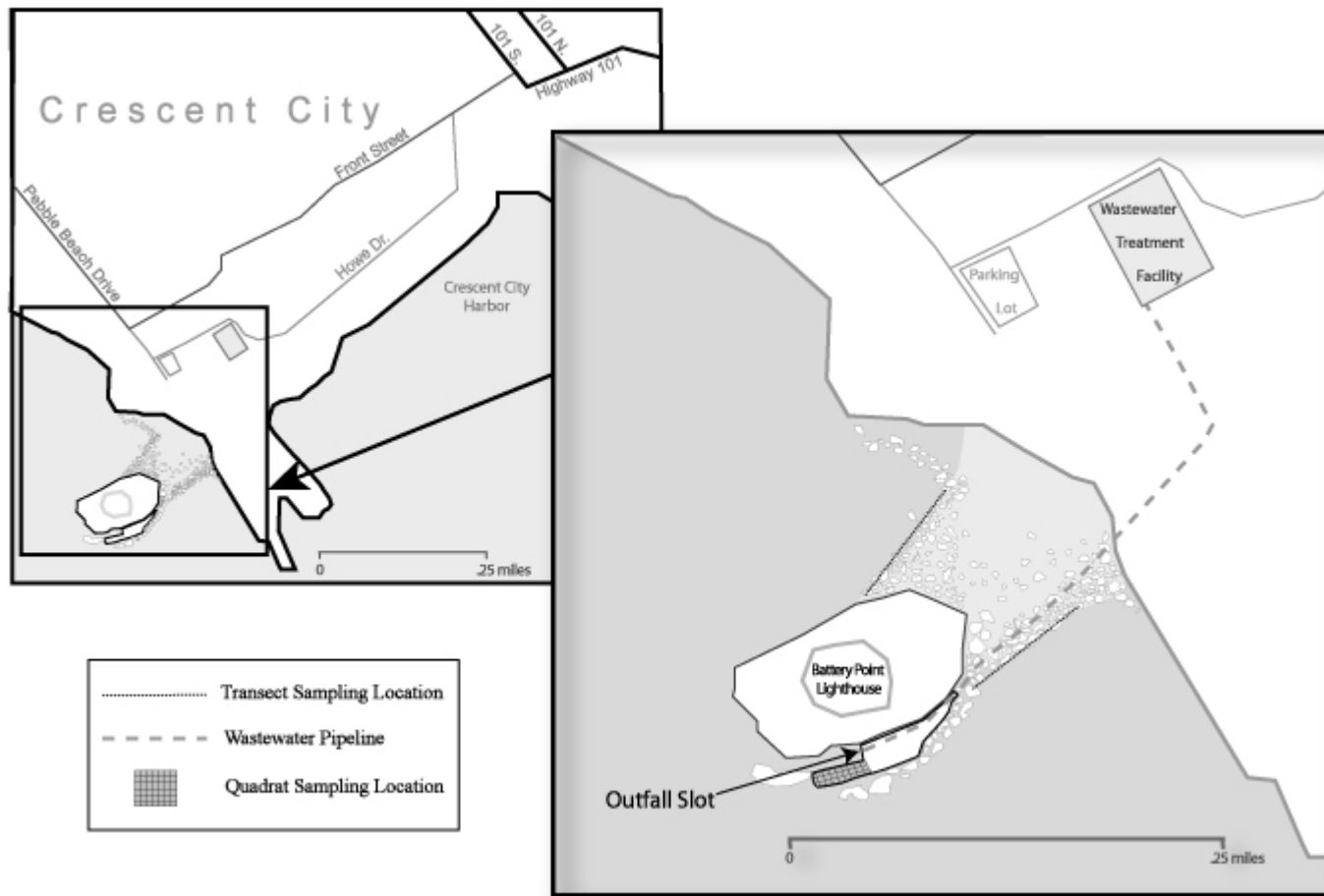


Figure 1. Location of Crescent City, California wastewater treatment facility, ocean outfall, and study area.

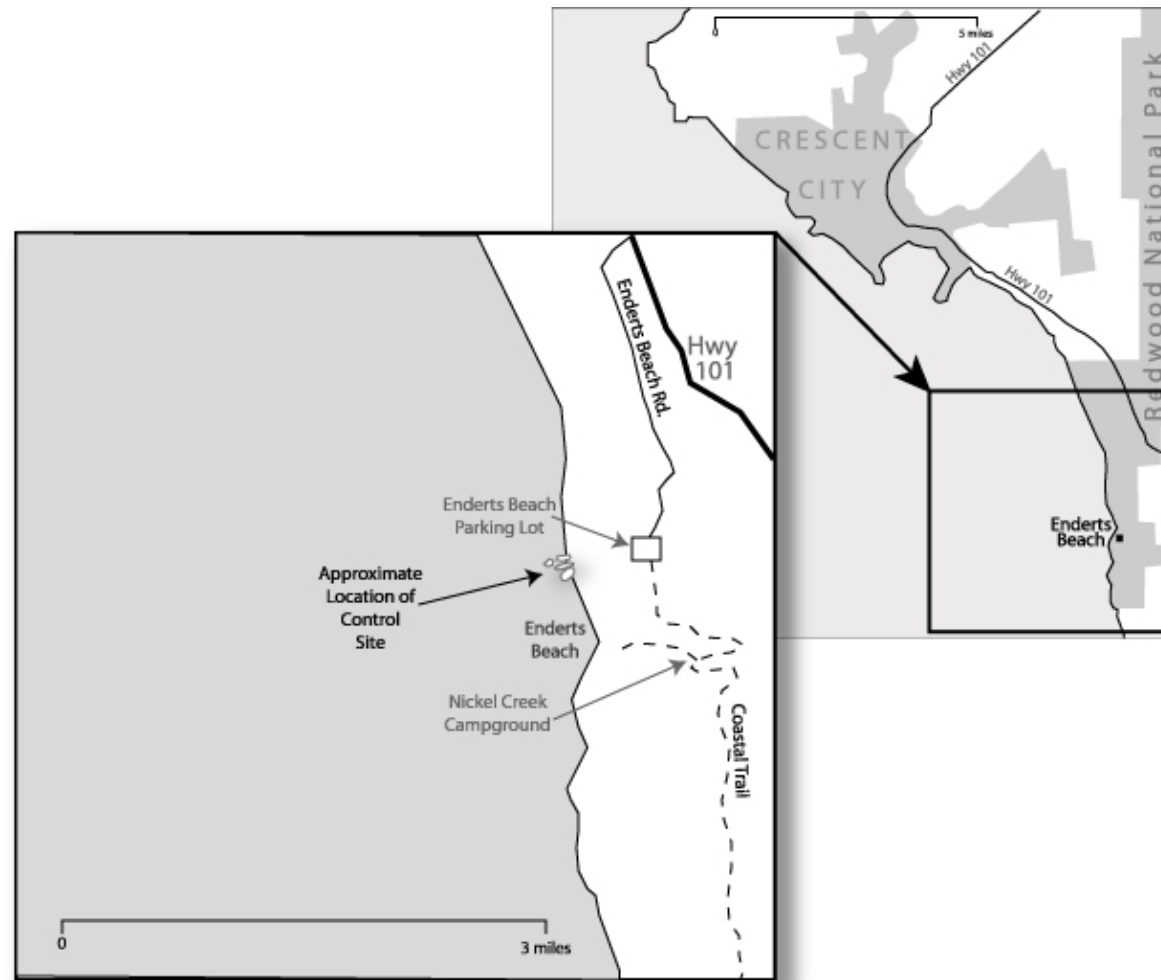


Figure 2. Location of control site at Enderts Beach inside Redwood National Park in Northern California.

Sampling Methods

An initial site visit was necessary to determine the exact location of the outfall and the adjacent community likely to be within the zone of initial dilution of the treated wastewater. A salinity meter was used to establish that the salinity of the water in the surge channel near the outfall was 26.0 to 27.7 parts per thousand. A second surge channel bordering the south side of the concrete and rock surfaces adjacent to the outfall had a measured salinity of 33.8 to 33.9 parts per thousand. These measurements suggest that the effluent is well mixed into the receiving waters because of the high agitation from wave action. It also indicated that the concrete and rock surfaces adjacent to the surge channel containing the outfall would be an appropriate region for an examination of the community structure.

The total available sampling area near the outfall encompassed 25 m². At the control site, three adjacent flat rocky benches comprising 25 m² were selected for comparison.

A grid was established covering the rocky surfaces at each site and encompassing the sample areas, the corners of which were marked by four screws drilled into the rocky substrate. Thirty sampling points were randomly selected and examined within the grid at each site. A 0.25 m² sampling quadrat was placed on the rock at each determined location, and populations of invertebrates and algae were identified and enumerated within the area of the sampling quadrat. These sampling

episodes took place once in August 2002, April 2003, and August 2003. The seasonal and yearly sampling events were conducted in order to account for any differences resulting from seasonal fluctuations and to permit comparison of data over an annual cycle.

In order to investigate the spatial distribution of organisms in relation to a pollution gradient, samples were taken along a transect line moving away from the outfall. Wave exposure and rock formations would not allow for an examination of the communities immediately adjacent to the outfall site, so this sampling occurred at approximately 100 meters north and south of the outfall. A 100 meter transect line was established at these locations (Figure 1, indicated by arrows). The transect lines were placed at the same tidal elevation as the outfall site, and ten quadrat samples were examined along each line. A 0.25 m² quadrat was placed on the rocks every 10 meters, and populations of invertebrates and algae were identified and counted within the area of the sampling quadrat. These additional sampling locations at the outfall site were presumably outside of the zone of initial dilution, or the area where the effluent first mixes with the receiving water. Because of time and resource constraints, this additional sampling took place once, in August 2003. I collected these supplementary data in order to provide some additional insight not detected with the initial study design and allow for comparisons of potential graduated changes moving away from the outfall site. This type of comparison has been suggested as a means to reduce the effects of pseudoreplication on the results of study designs similar to this one (Eberhardt and Thomas 1991, Wiens and Parker 1995).

Any organisms that were unidentifiable in the field were returned to the lab at Humboldt State University for identification. Samples were kept in seawater and identified immediately so that preservation was not necessary. I identified invertebrate specimens using *Light's Manual: Intertidal Invertebrates of the Central California Coast* (Smith and Carlton 1975) and *Marine Invertebrates of the Pacific Northwest* (Kozloff 1987). Algal specimens were identified using *Keys to the Benthic Marine Algae and Seagrasses of British Columbia, Southeast Alaska, Washington and Oregon* (Gabrielson *et al.* 2000) and *Marine Algae of California* (Abbott and Hollenberg 1976).

Data Analysis

An initial examination of the grid data took place to determine if the number of samples obtained was adequate enough to detect differences or similarities. This was accomplished using a simple species richness estimator, by grouping species occurrences into geometric classes by abundance (Gray 1981). Geometric classes were assigned by the percentages of organisms having abundance values within the limit of defined classifications (Class I = 1-2 individuals, Class II = 2-3 individuals, Class III = 4-7 individuals, Class IV = 8-15 individuals, Class V = 16-31 individuals, Class VI = 32-63 individuals, Class VII = 64-127 individuals, Class VIII = 128-255 individuals, Class IX = greater than 255 individuals). The classes were graphed versus a logarithmic axis representing the cumulative percentage of species. This procedure produces a graphical representation of the percentage of species likely to be encountered at each site

The observed species richness ($R = s$, where s is the number of species) for each location was determined. In order to verify the accuracy of the simple species richness method, another estimation of species richness was calculated with the use of EstimateS software using the Chao2 estimator ($S_{\text{Chao2}} = S_{\text{obs}} + Q_1^2/2Q_2$, where S_{obs} is the observed number of species, Q_1 is the number of uniques, and Q_2 is the number of duplicates) (Colwell 2005). Because the estimation values of the Chao2 calculation were different from the observed values for species richness ($r^2 = 0.69$), I used the estimated values in my analysis. Since the Chao2 estimate was very similar to that of the previous richness estimate, I opted to use the values from the simple species richness estimator.

In addition, a Shannon-Wiener diversity index (H') was determined, in order to take relative abundances into account ($H' = - \sum p_i \ln(p_i)$, where p_i = proportion of the i^{th} species, and $\ln(p_i)$ = natural logarithm of p_i). An index value was ascertained for each sampling episode, at each site.

Species evenness ($E = H'/\ln(R)$) also indicates diversity, but has the added component of expressing the Shannon-Wiener index as a proportion of the maximum possible diversity (Zar 1984). Evenness values were determined for each sampling episode, at each site.

Data were examined using the statistical software package PC-Ord, version 4.25 (McCune and Mefford 1999). A 2-axis Bray-Curtis ordination technique, with Sorenson (Bray-Curtis) Distance Measure, was utilized to analyze quadrat data (McCune and Mefford 1999).

A Multi-Response Permutation Procedures (MRPP) comparison was used to assess differences in community composition between the two sites during each individual sampling event, and to detect seasonal and annual differences within each site.

An analysis of each technique described above was conducted for the original, raw quadrat data collected. Because raw data appeared to strongly weigh the most abundant species, statistical analyses were applied to the data using multiple methodologies. Analytical methods were applied to data indicating presence-absence only, to examine similarities or differences of species occurrence without taking abundance into account. In addition, these analyses were performed on transformed [$\log(x + 1)$] data to reduce the influence of very abundant species, such as *Semibalanus cariosus*, on those species represented by a single individual.

Since the ordination graphs compared quadrat data only, univariate comparisons of mean abundance were produced for specific organisms that were most highly or negatively correlated with axis 1 in Pearson correlation coefficients generated by the ordination analysis. The univariate graphs were used to illustrate patterns exhibited by specific species not illustrated by the ordination graph. A two-factor ANOVA was conducted to analyze differences over time and by location.

Data obtained from the transect line were examined using cluster analyses. A dendrogram graph was generated to illustrate any potential gradation pattern. The graph was obtained with a cluster analysis using the Ward's Method group linkage method.

In addition, because eutrophication of green ephemeral algae is common in areas with increased nutrient loads, a critical examination of *Ulva* sp. identified along the

transect lines was conducted to determine the possibility of a reduction in abundance of this green alga, moving away from the outfall (Graham and Wilcox 2000). This was accomplished by graphing abundance versus distance from the outfall and a regression analysis was used to see if there was a downward trend in abundance with increasing distance.

RESULTS

The results of abundance lognormal distribution encompass all three sampling episodes and show trendlines that, if extended to intersect with the y-axis, indicate the likely percentage of species not detected during the survey. This analysis indicates that at the outfall and control sites 90% and 88% of the respective species likely to be encountered were identified (Figures 3, 4).

There were 23, 25, and 26 estimated species at the outfall site in summer 2002, spring 2003, and summer 2003, respectively. At the control site, species richness was estimated at 13 in summer 2002, 27 in spring 2003, and 20 in summer 2003 (Figure 5). There was no significant difference in comparison of the number of species between treatment ($p = 0.42$), between sampling episodes ($p = 0.48$) or between an interaction of treatment and sampling episodes ($p = 0.80$).

The Shannon-Wiener diversity index for the outfall site was 0.331 in summer 2002, 0.524 in spring 2003, and 0.244 in summer 2003. At the control site, the diversity index was 0.560 in summer 2002, 0.329 in spring 2003, and 0.585 in summer 2003 (Figure 6). No significant differences were detected in diversity indices between treatment ($p = 0.52$), between sampling events ($p = 0.88$) or in an interaction between treatment and time sampled ($p = 0.80$).

Evenness at the outfall site was 0.016 in summer 2002, 0.023 in spring 2003, and 0.010 in summer 2003. Evenness values for the control site were 0.047 in summer 2002, 0.014 in spring 2003, and 0.033 in summer 2003 (Figure 7). Species evenness did not

differ significantly between treatments ($p = 0.38$), sampling event ($p = 0.60$) or the interaction of treatment and time sampled ($p = 0.81$).

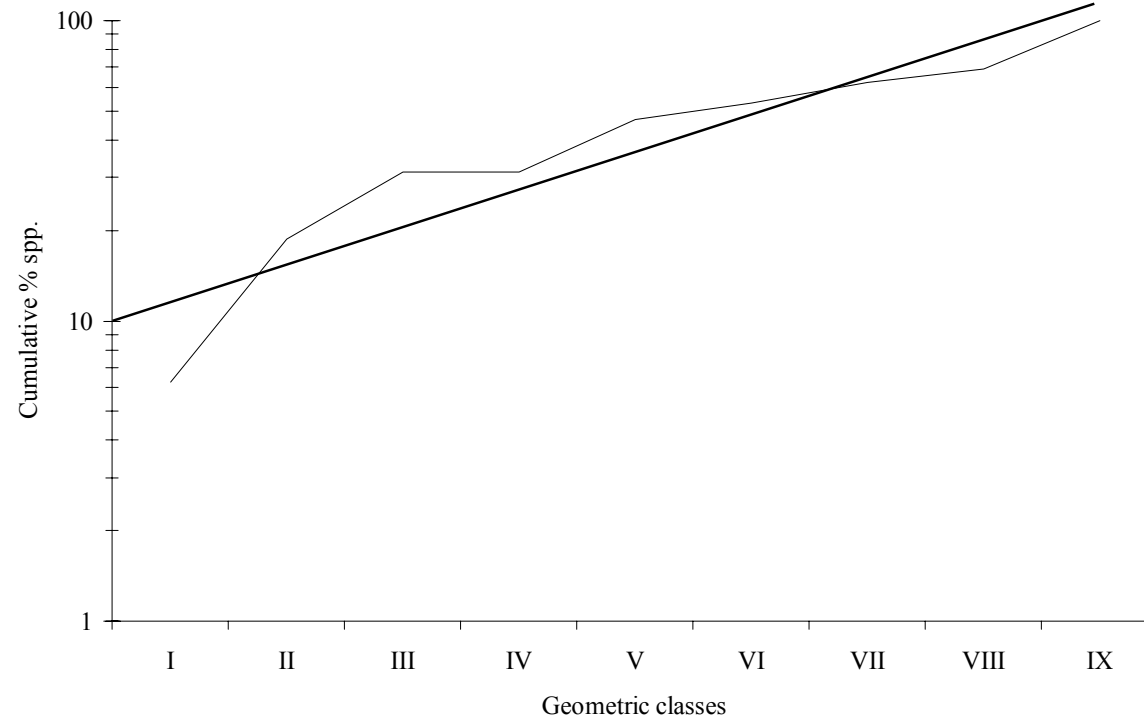


Figure 3. Species richness estimation at the discharge site for all sampling events. Data include sampling episodes in summer 2002, spring 2003, and summer of 2003, and a total of 90 samples. The trendline of abundance by geometric class indicates that approximately 90% of the species likely to be encountered were identified.

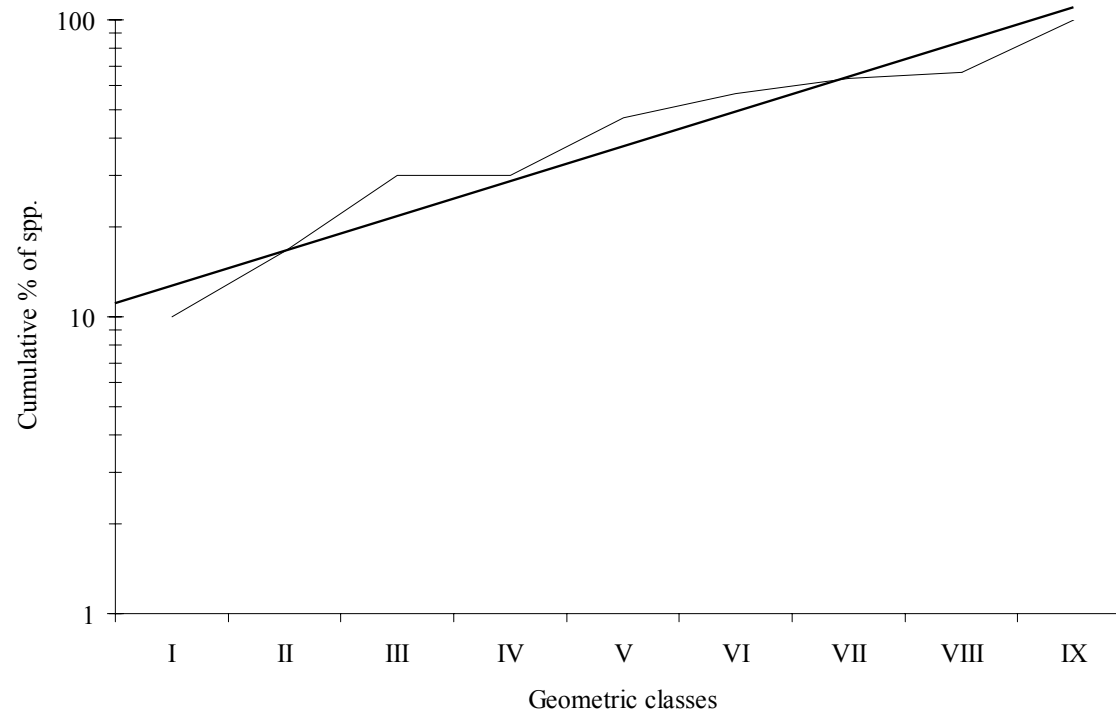


Figure 4. Species richness estimation at the reference site for all sampling events. Data include sampling episodes in summer 2002, spring 2003, and summer 2003, and a total of 90 samples. The trendline of abundance by geometric class indicates that approximately 88% of the species likely to be encountered were identified.

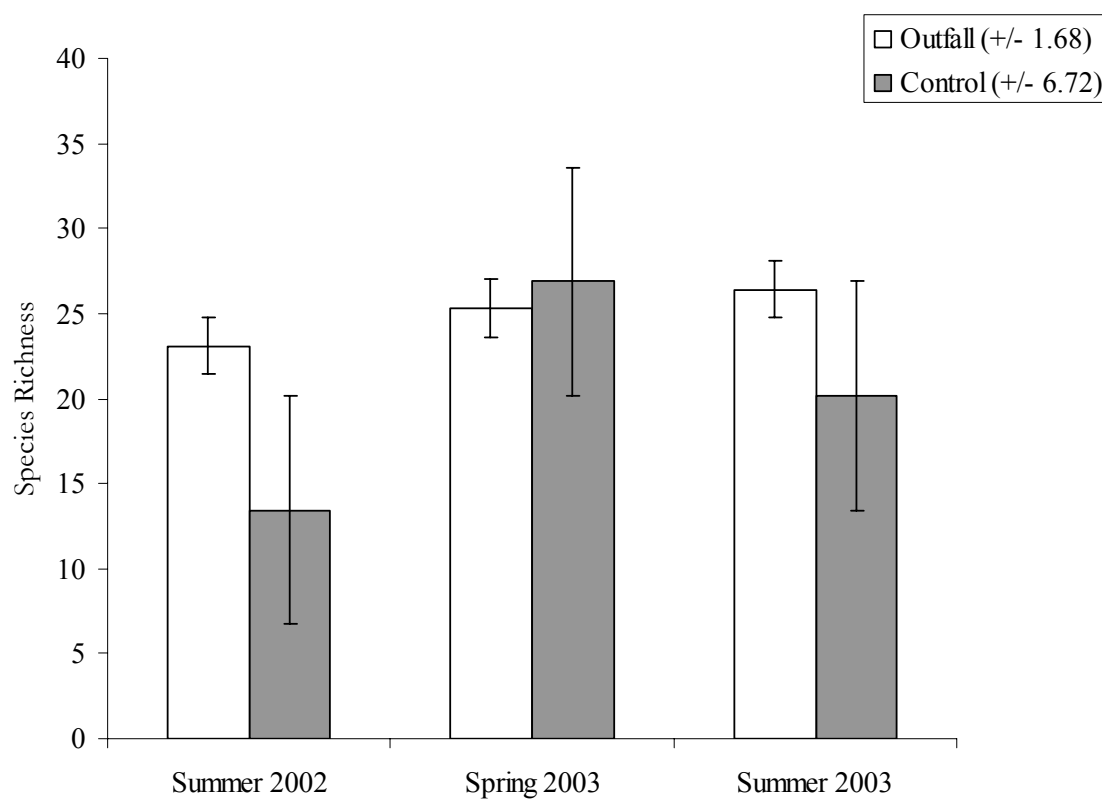


Figure 5. Comparison of estimated species richness at each site during three sampling events. No significant differences were detected between treatments ($p = 0.42$), time sampled ($p = 0.48$) or between interactions with treatment and time sampled ($p = 0.80$).

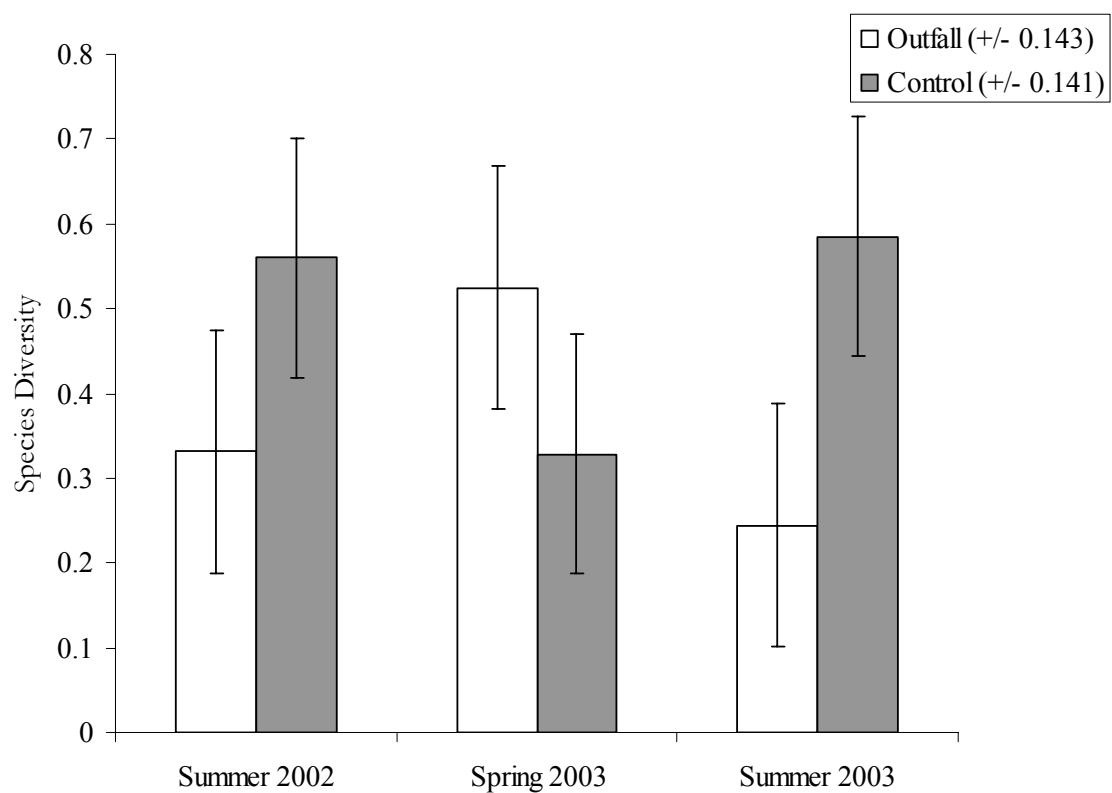


Figure 6. Comparison of Shannon-Wiener diversity index at each site, during three sampling events. No significant differences were detected between treatments ($p = 0.52$), time sampled ($p = 0.89$) or between interactions of treatment and time sampled ($p = 0.80$).

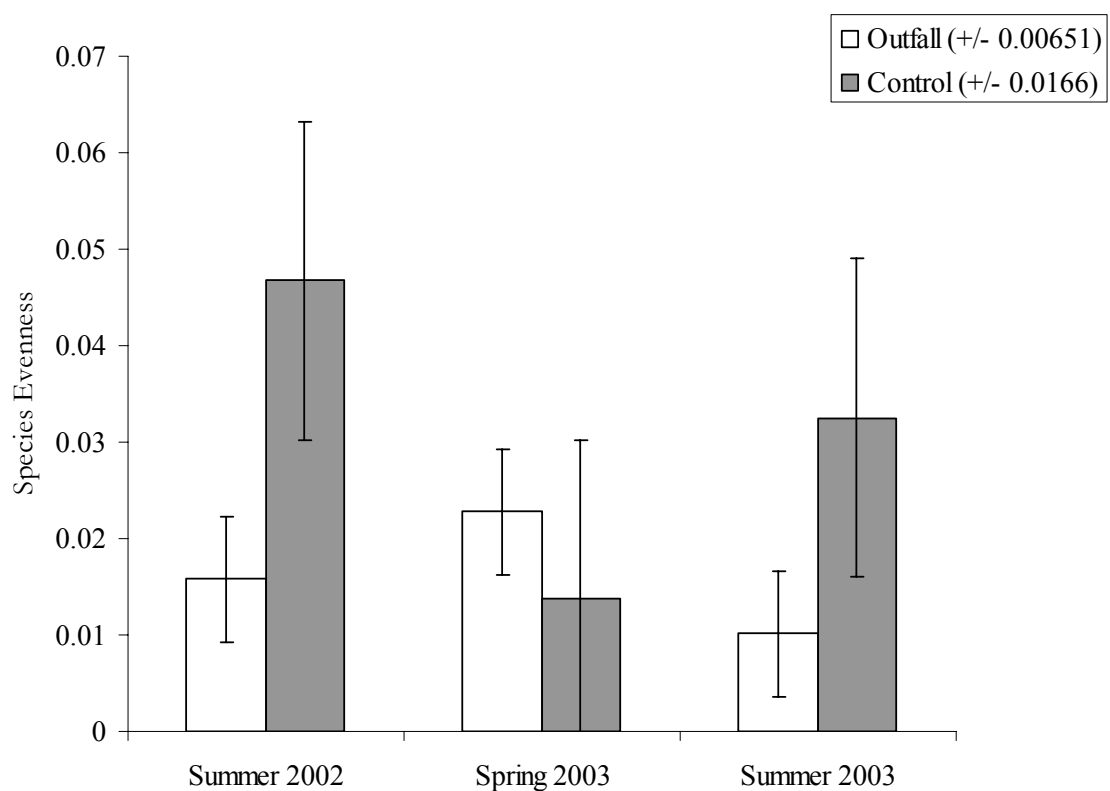


Figure 7. Comparison of species evenness at each site, during three sampling events. No significant differences were detected between treatments ($p = 0.38$), time sampled ($p = 0.60$) or between interactions of treatment and time sampled ($p = 0.81$).

Bray-Curtis ordination techniques yielded similar results for both raw and transformed data. The transformed data were used as the focus of the ordination analysis because the transformation removed variation that resulted from the extreme abundances of some species.

The ordination graph for summer 2002 sampling showed control samples were clustered on the mid-right of the graph (Figure 8). The outfall samples display a tendency to spread over a wider region indicating dissimilarity in the composition of samples from each location. Pearson correlation coefficients (Table 1) of species highly correlated with axis 1 include species such as the goose barnacle *Pollicipes polymerus*, the limpet *Notoacmea scutum*, and the snail *Nucella emarginata*. Univariate graphs (Figures 9, 10, 11) illustrated mean abundance for these organisms were significantly higher at the control site than the outfall site ($p < 0.01$). In contrast, the red algae *Mastocarpus papillatus* and *Neorhodomela larix*, and the barnacle *Balanus glandula* were shown to have a negative correlation with axis 1. Univariate graphs (Figures 12, 13, 14) indicated mean abundances for organisms negatively correlated with axis 1 were significantly higher at the outfall site ($p < 0.01$). No pattern could be determined for the species most highly or negatively correlated with axis 2.

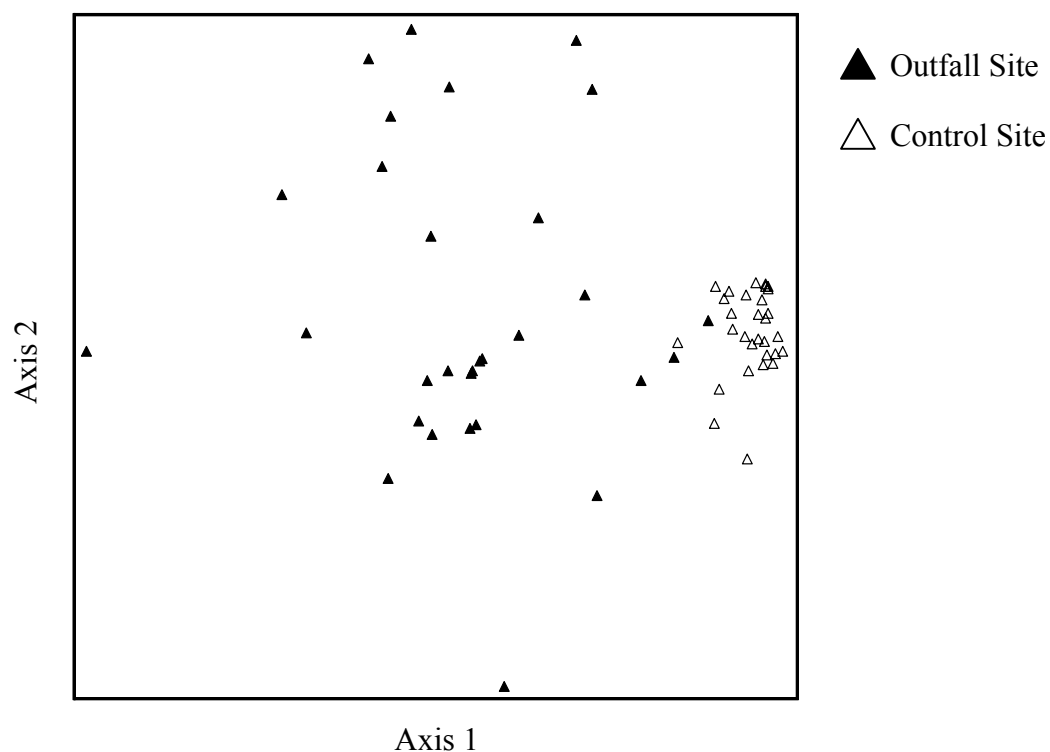


Figure 8. Bray-Curtis ordination graph illustrating the $[\log(x + 1)]$ transformed data collected at the outfall and control sites during summer 2002. Distances between sampling quadrats depicted on the graph are representative of the dissimilarity of the samples.

Table 1. Pearson correlation coefficients generated from an ordination analysis to determine species correlation with ordination axes during summer 2002 (r = Pearson correlation coefficient).

Axis 1	r	Axis 2	r
<i>Pollicipes polymerus</i>	0.82	<i>Semibalanus cariosus</i>	0.748
<i>Notoacmea scutum</i>	0.652	<i>Mastocarpus papillatus</i>	0.733
<i>Nucella emarginata</i>	0.623	<i>Amphiporus</i> sp.	0.406
<i>Mytilus californianus</i>	0.565	<i>Megalorchestia californiana</i>	0.351
<i>Anthopleura xanthogrammica</i>	0.391	<i>Ulva</i> sp.	0.351
<i>Lottia strigatella</i>	0.352	<i>Neorhodomela larix</i>	0.214
<i>Pisaster ochraceus</i>	0.322	<i>Collisella digitalis</i>	0.045
<i>Semibalanus cariosus</i>	0.304	<i>Lottia strigatella</i>	0.023
<i>Anthopleura elegantissima</i>	0.228	<i>Balanus glandula</i>	0.007
<i>Collisella digitalis</i>	0.17	<i>Collisella pelta</i>	-0.021
<i>Collisella pelta</i>	0.106	<i>Notoacmea scutum</i>	-0.033
<i>Mazzaella splendens</i>	-0.01	<i>Mazzaella splendens</i>	-0.04
<i>Endocladia muricata</i>	-0.047	<i>Pisaster ochraceus</i>	-0.056
<i>Fucus gardneri</i>	-0.074	<i>Nucella emarginata</i>	-0.062
<i>Diadumene leucolena</i>	-0.082	<i>Analipus japonicus</i>	-0.071
<i>Analipus japonicus</i>	-0.141	<i>Cladophora columbiana</i>	-0.09
<i>Amphiporus</i> sp.	-0.143	<i>Endocladia muricata</i>	-0.105
<i>Bossiella cretacea</i>	-0.163	<i>Fucus gardneri</i>	-0.109
<i>Idotea wosnesenskii</i>	-0.172	<i>Pollicipes polymerus</i>	-0.11
<i>Cladophora columbiana</i>	-0.183	<i>Anthopleura xanthogrammica</i>	-0.153
<i>Megalorchestia californiana</i>	-0.237	<i>Anthopleura elegantissima</i>	-0.165
<i>Ulva</i> sp.	-0.246	<i>Prionitis lanceolata</i>	-0.177
<i>Prionitis lanceolata</i>	-0.425	<i>Idotea wosnesenskii</i>	-0.185
<i>Mastocarpus papillatus</i>	-0.479	<i>Bossiella cretacea</i>	-0.399
<i>Neorhodomela larix</i>	-0.699	<i>Diadumene leucolena</i>	-0.427
<i>Balanus glandula</i>	-0.701	<i>Mytilus californianus</i>	-0.652

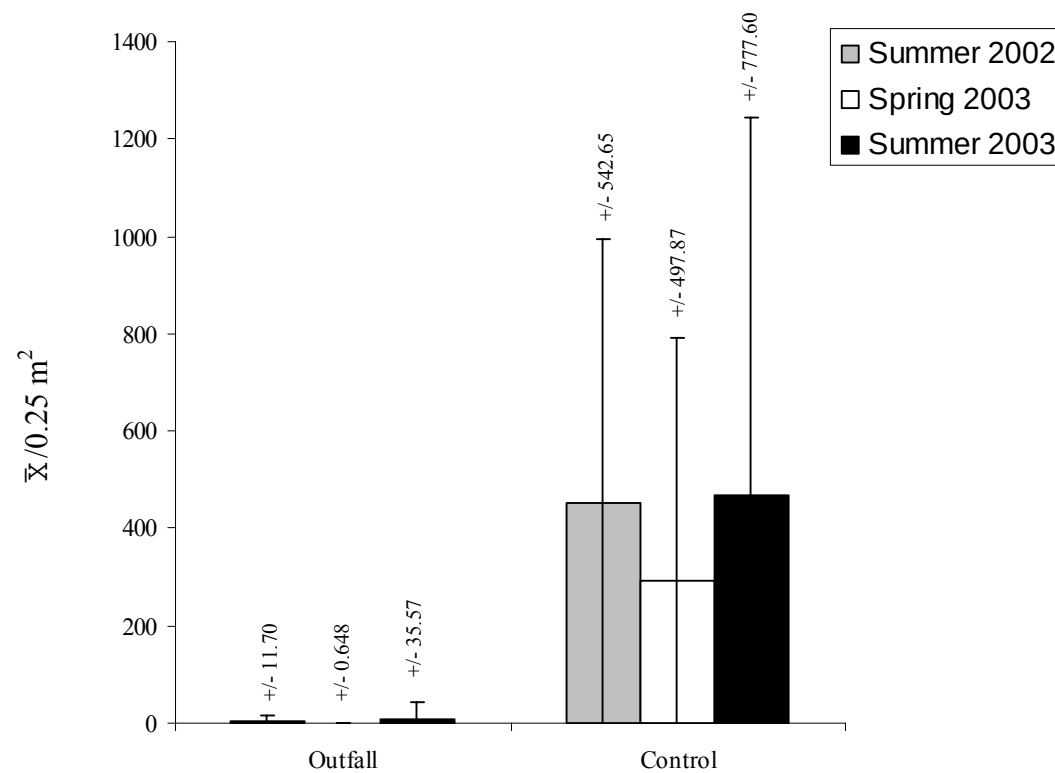


Figure 9. Univariate comparison of mean abundance for *Pollicipes polymerus*. ANOVA comparisons detected a significant difference between sites over all sampling events ($p < 0.01$). There were no significant differences between sampling times ($p = 0.10$) or an interaction between sites and sampling times ($p = 0.30$).

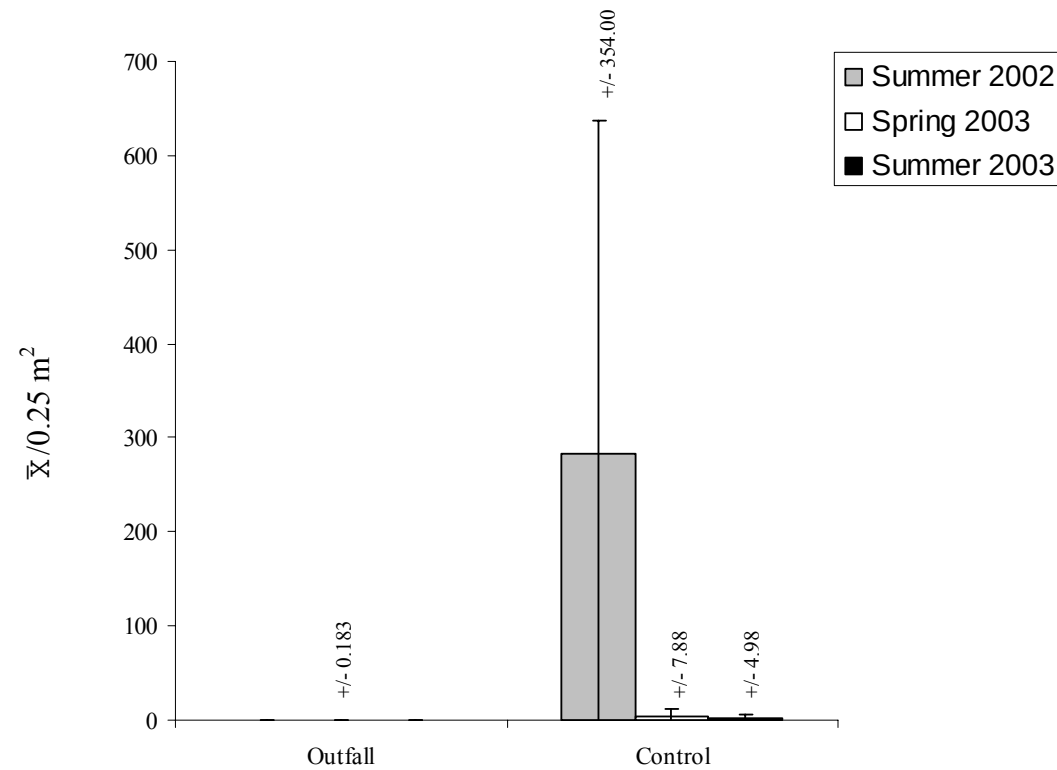


Figure 10. Univariate comparison of mean abundance for *Notoacmea scutum*. ANOVA comparisons detected a significant difference between sites over all sampling events ($p < 0.01$), between all sampling events ($p < 0.01$) and in the interaction between location and time sampled ($p < 0.01$).

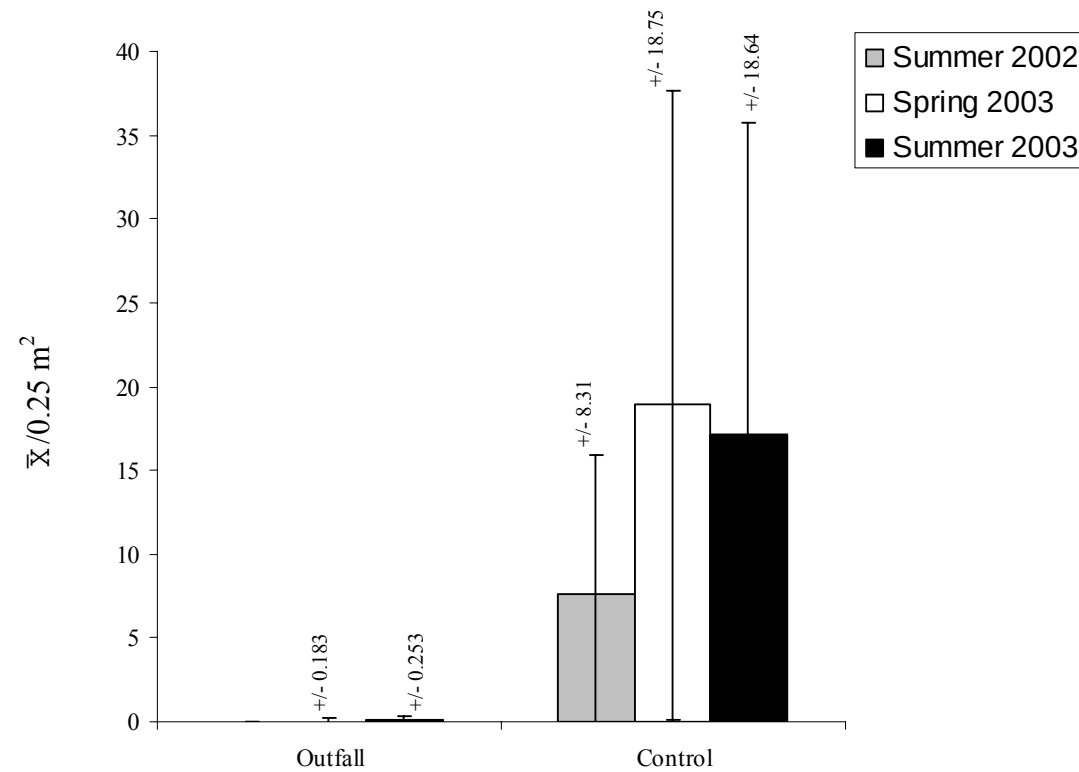


Figure 11. Univariate comparison of mean abundance for *Nucella emarginata*. ANOVA comparisons detected a significant difference between sites over all sampling events ($p < 0.01$), between all sampling events ($p < 0.05$) and in the interaction between location and time sampled ($p < 0.05$).

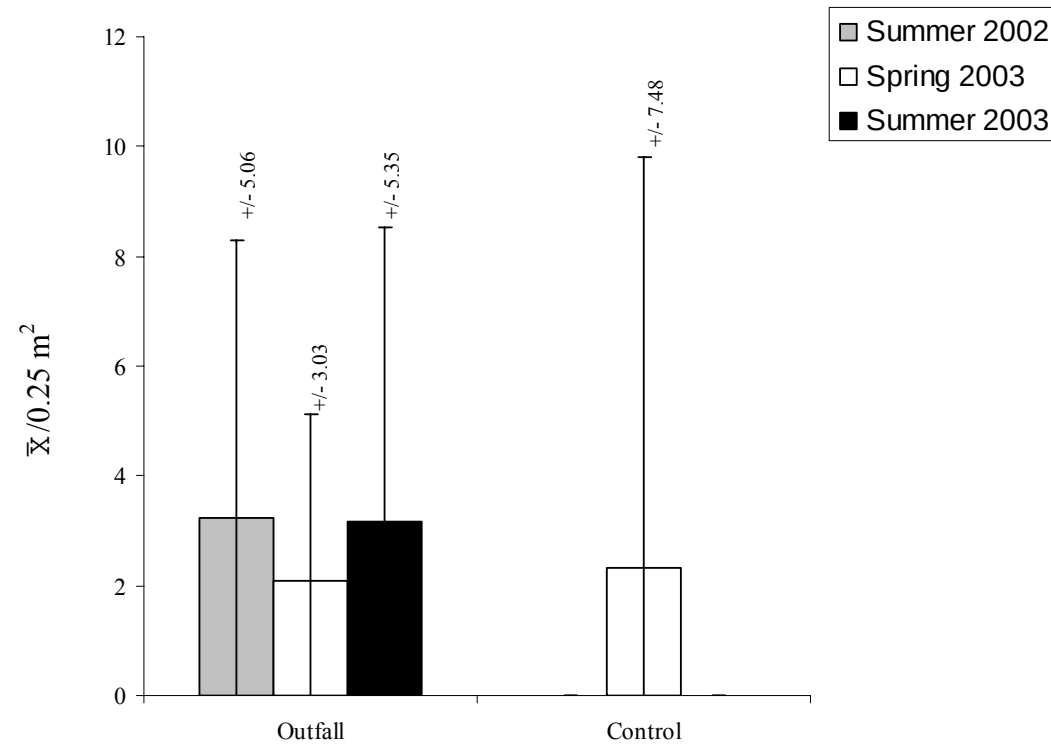


Figure 12. Univariate comparison of mean abundance for *Mastocarpus papillatus*. ANOVA comparisons detected a significant difference between sites over all sampling events ($p < 0.01$). There were no significant differences between sampling times ($p = 0.88$) or an interaction between sites and sampling times ($p = 0.88$).

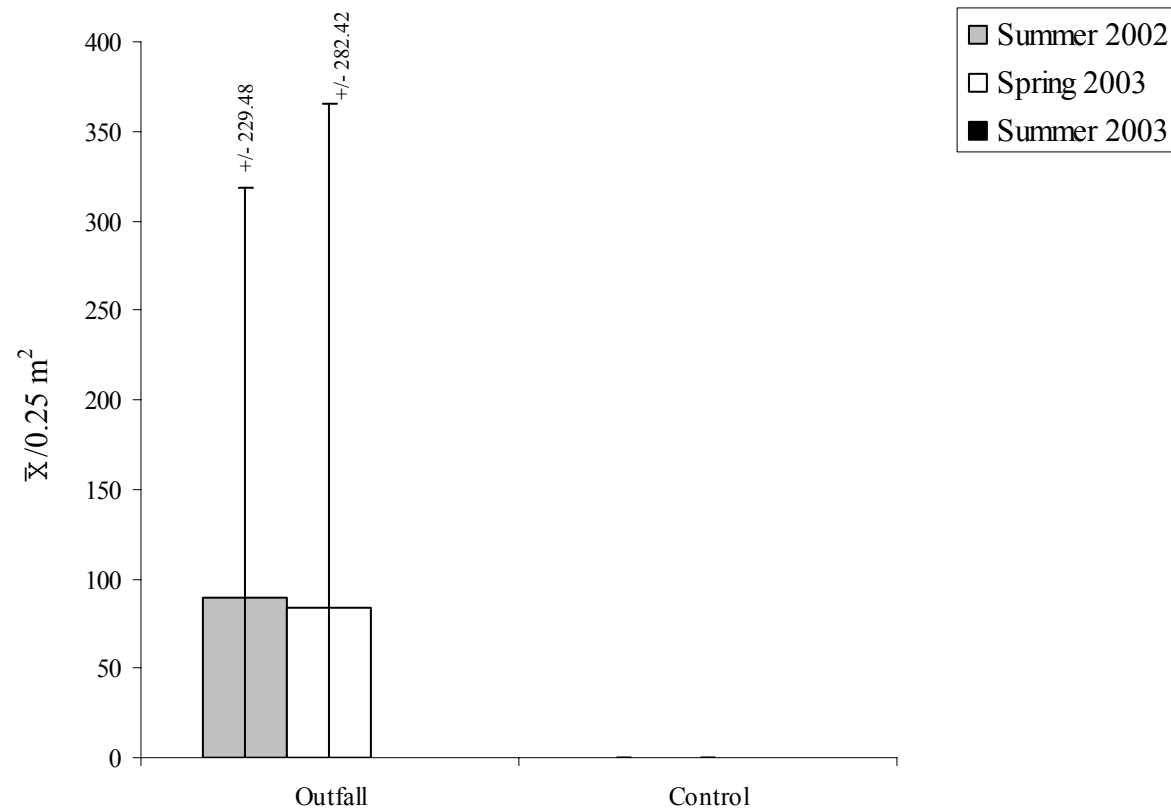


Figure 13. Univariate comparison of mean abundance for *Neorhodomela larix*. ANOVA comparisons detected a significant difference between sites over all sampling events ($p < 0.01$), between all sampling events ($p < 0.01$) and in the interaction between location and time sampled ($p < 0.01$).

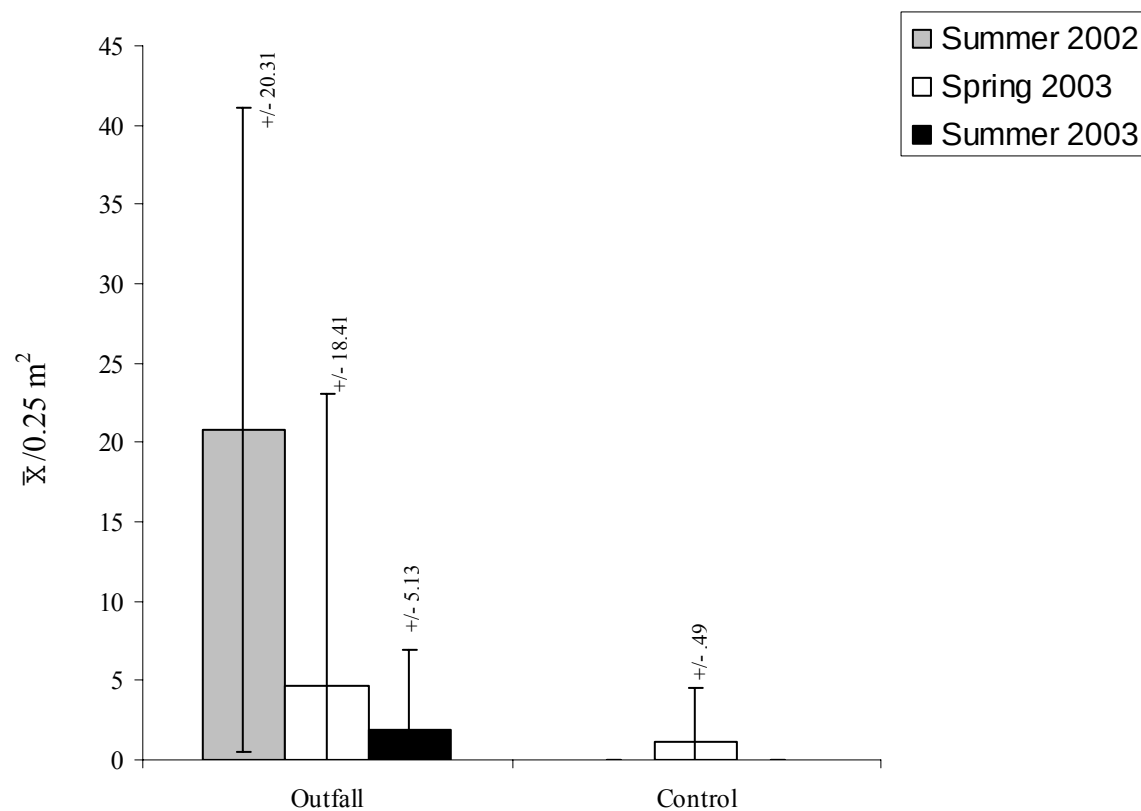


Figure 14. Univariate comparison of mean abundance for *Balanus glandula*. ANOVA comparisons detected a significant difference between sites over all sampling events ($p < 0.01$), between all sampling events ($p < 0.01$) and in the interaction between location and time sampled ($p < 0.01$).

The results from spring 2003 sampling also indicated differences between the two sites along axis 1 (Figure 15). Though there appears to be a decrease in dissimilarity between samples from either site, the control site samples remain clustered on the right side of the ordination graph, whereas outfall site samples are primarily on the left side of the graph. The species most highly correlated with axis 1, the barnacle *Semibalanus cariosus* (Figure 16), *N. emarginata* (Figure 11), and *P. polymerus* (Figure 9) were significantly ($p < 0.01$) more abundant at the control site during this sampling event (Table 2). Species negatively correlated with axis 1 were the brown algae *Fucus gardneri* and *Analipus japonicus*, and the red alga *Bossiella cretacea* and were significantly ($p < 0.01$) more abundant at the outfall site (Figures 17, 18, 19).

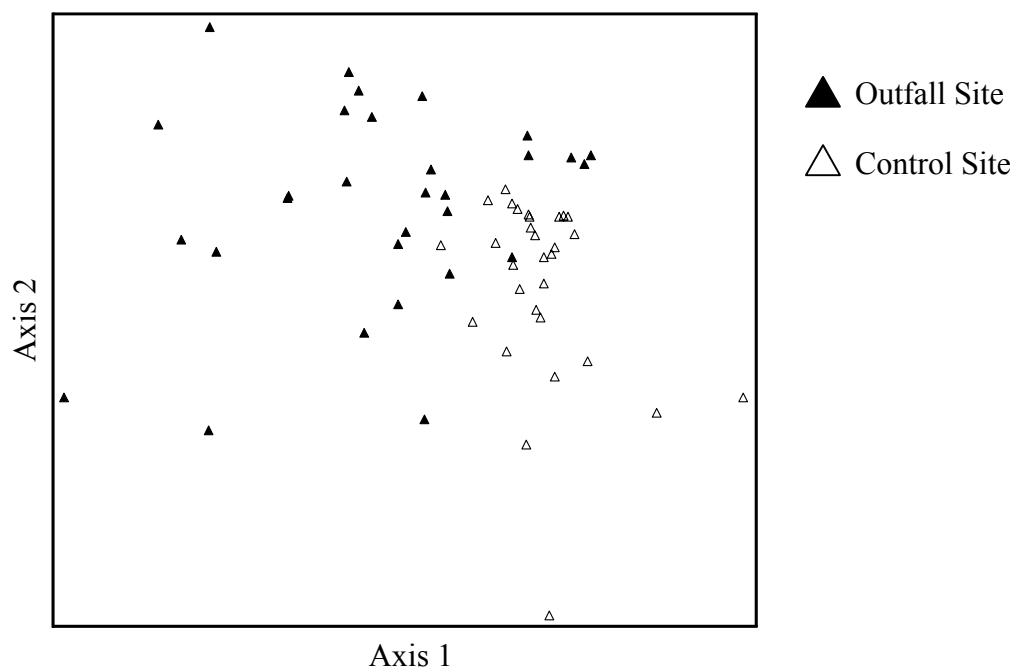


Figure 15. Bray-Curtis ordination graph illustrating the $[\log(x + 1)]$ transformed data collected at the outfall and control sites during spring 2003. Distances between sampling quadrats depicted on the graph are representative of the dissimilarity of the samples.

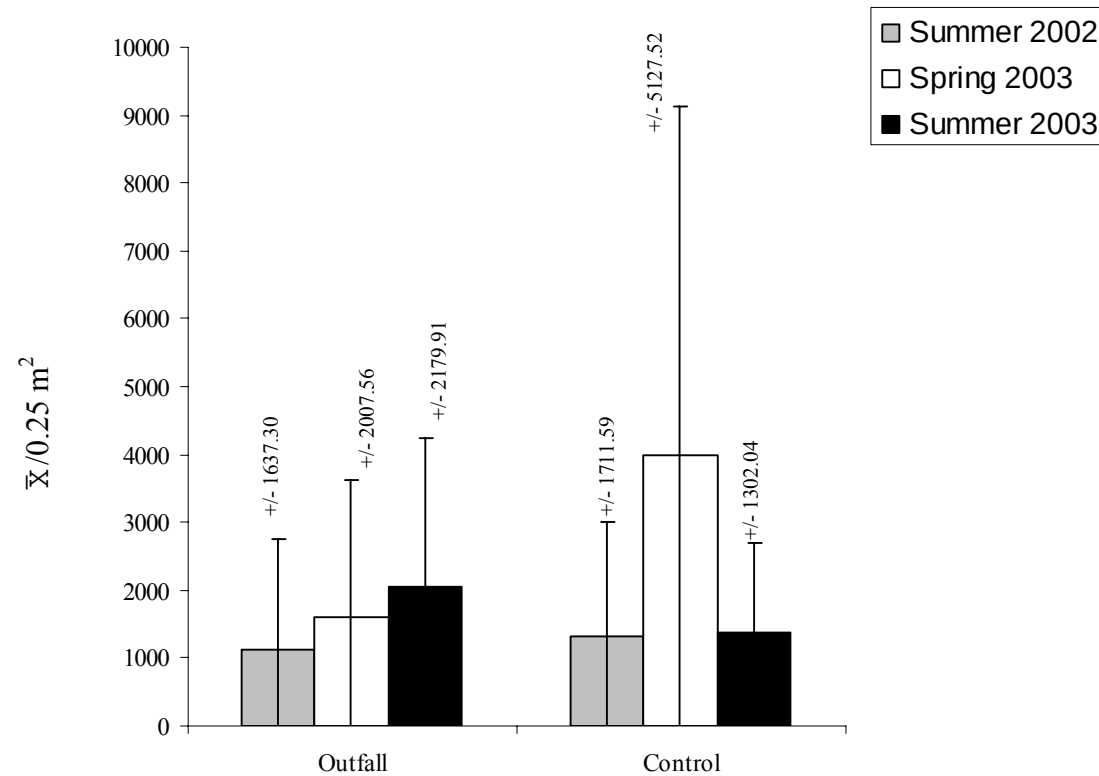


Figure 16. Univariate comparison of mean abundance for *Semibalanus cariosus*. ANOVA comparisons detected a significant difference between sites over all sampling events ($p < 0.01$), between all sampling events ($p < 0.01$) and in the interaction between location and time sampled ($p < 0.05$).

Table 2. Pearson correlation coefficients generated from an ordination analysis to determine species correlation with ordination axes during spring 2003 (r = Pearson correlation coefficient).

Axis 1	r	Axis 2	r
<i>Semibalanus cariosus</i>	0.765	<i>Endocladia muricata</i>	0.595
<i>Nucella emarginata</i>	0.564	<i>Mytilus californianus</i>	0.302
<i>Pollicipes polymerus</i>	0.53	<i>Strongylocentrotus purpuratus</i>	0.188
<i>Anthopleura elegantissima</i>	0.316	<i>Fucus gardneri</i>	0.135
<i>Collisella digitalis</i>	0.314	<i>Balanus glandula</i>	0.113
<i>Callithamnion pikeanum</i>	0.262	<i>Halosaccion glandiforme</i>	0.06
<i>Notoacmaea scutum</i>	0.26	<i>Phyllospadix</i> sp.	0.026
<i>Phyllospadix</i> sp.	0.224	<i>Notoacmaea scutum</i>	0.019
<i>Mastocarpus papillatus</i>	0.166	<i>Microcladia borealis</i>	0.015
<i>Pisaster ochraceus</i>	0.143	<i>Prionitis lanceolata</i>	-0.004
<i>Ulva</i> sp.	0.129	<i>Ahnfeltiopsis</i> sp.	-0.015
<i>Cladophora columbiana</i>	0.128	<i>Laminaria sinclarii</i>	-0.025
<i>Flustrellidra corniculata</i>	0.112	<i>Megalorchestia californiana</i>	-0.025
<i>Ahnfeltiopsis</i> sp.	0.108	<i>Leptasterias hexactis</i>	-0.049
<i>Anthopleura xanthogrammica</i>	0.101	<i>Cladophora columbiana</i>	-0.063
<i>Idotea wosnesensii</i>	0.089	<i>Idotea wosnesensii</i>	-0.072
<i>Leptasterias hexactis</i>	0.086	<i>Analipus japonicus</i>	-0.096
<i>Laminaria sinclarii</i>	0.055	<i>Collisella digitalis</i>	-0.109
<i>Megalorchestia californiana</i>	0.055	<i>Mastocarpus papillatus</i>	-0.14
<i>Prionitis lanceolata</i>	0.014	<i>Pisaster ochraceus</i>	-0.153
<i>Mazzaella splendens</i>	-0.089	<i>Katharina tunicata</i>	-0.192
<i>Microcladia borealis</i>	-0.125	<i>Flustrellidra corniculata</i>	-0.196
<i>Balanus glandula</i>	-0.206	<i>Neorhodomela larix</i>	-0.207
<i>Mytilus californianus</i>	-0.271	<i>Pollicipes polymerus</i>	-0.237
<i>Neorhodomela larix</i>	-0.335	<i>Semibalanus cariosus</i>	-0.252
<i>Halosaccion glandiforme</i>	-0.36	<i>Nucella emarginata</i>	-0.349
<i>Endocladia muricata</i>	-0.386	<i>Anthopleura xanthogrammica</i>	-0.353
<i>Strongylocentrotus purpuratus</i>	-0.39	<i>Bossiella cretacea</i>	-0.365
<i>Katharina tunicata</i>	-0.4	<i>Ulva</i> sp.	-0.402
<i>Fucus gardneri</i>	-0.476	<i>Mazzaella splendens</i>	-0.464
<i>Analipus japonicus</i>	-0.533	<i>Callithamnion pikeanum</i>	-0.485
<i>Bossiella cretacea</i>	-0.644	<i>Anthopleura elegantissima</i>	-0.651

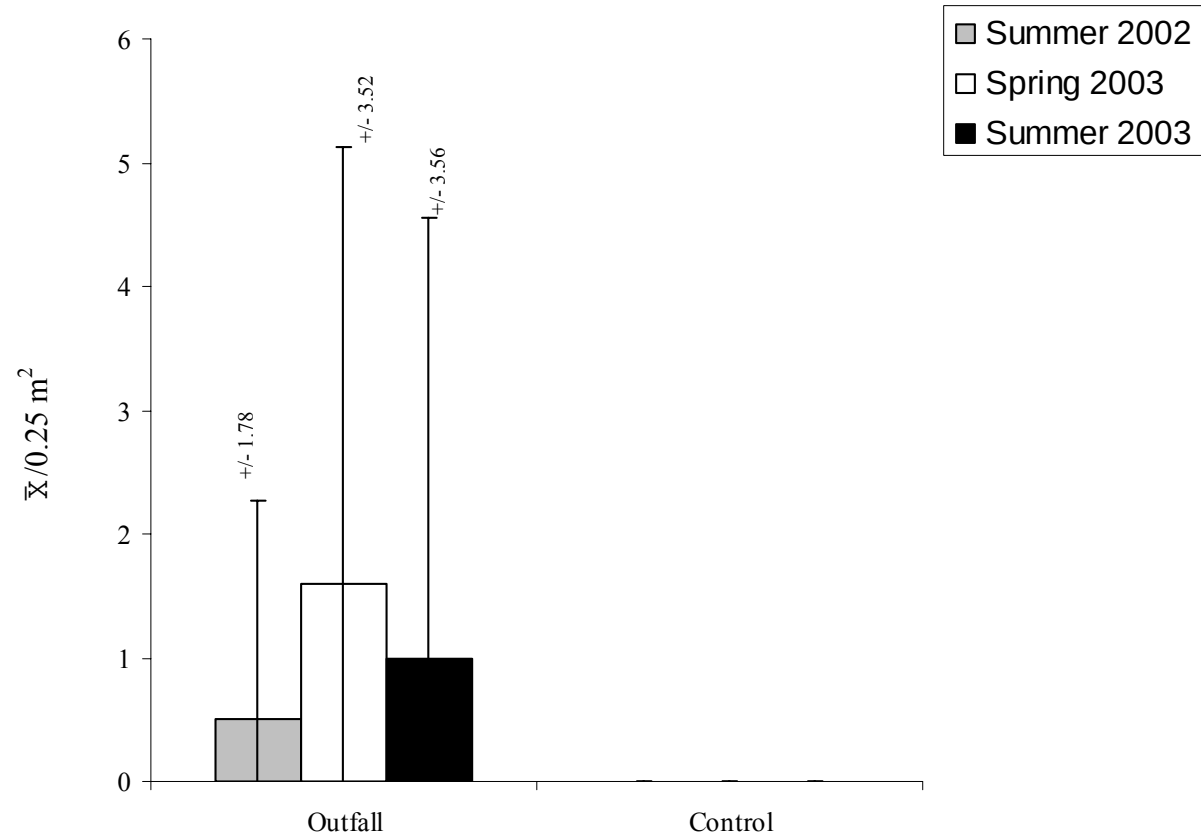


Figure 17. Univariate comparison of mean abundance for *Fucus gardneri*. ANOVA comparisons detected a significant difference between sites over all sampling events ($p < 0.01$). There were no significant differences between sampling times ($p = 0.70$) or an interaction between sites and sampling times ($p = 0.70$).

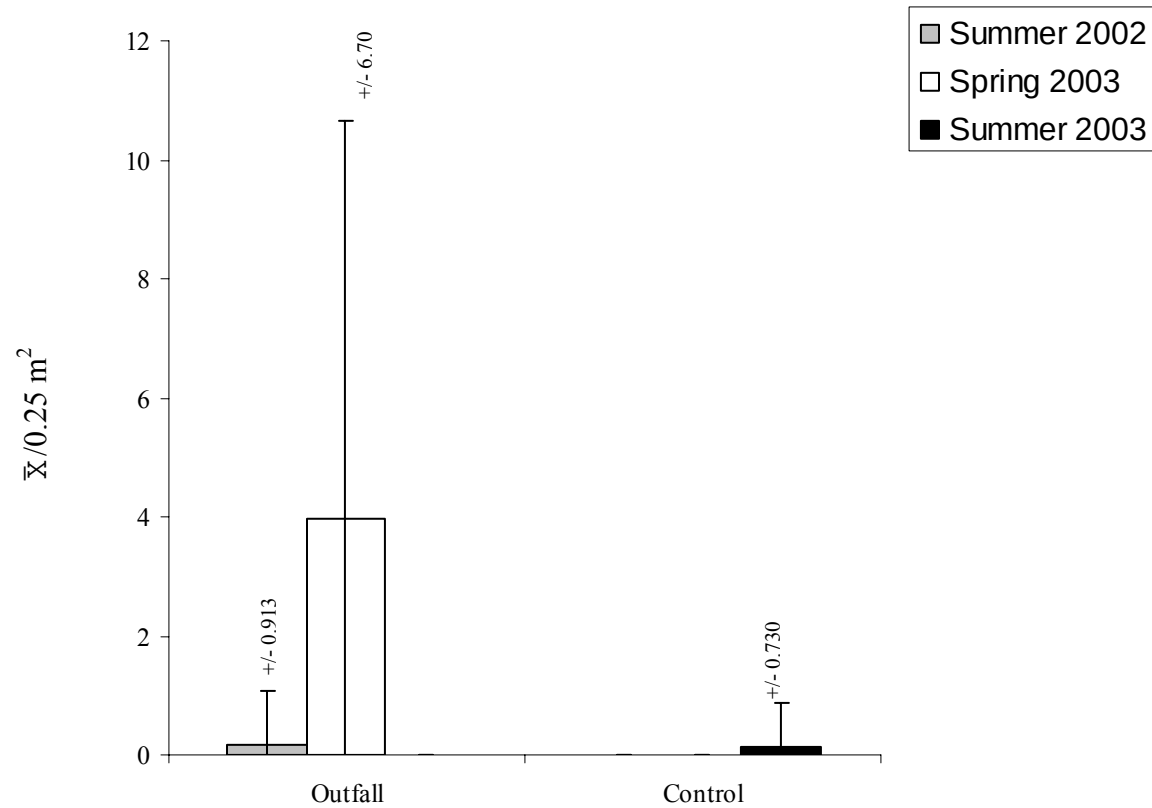


Figure 18. Univariate comparison of mean abundance for *Analipus japonicus*. ANOVA comparisons detected a significant difference between sites over all sampling events ($p < 0.01$). There were no significant differences between sampling times ($p = 0.98$) or an interaction between sites and sampling times ($p = 0.59$).

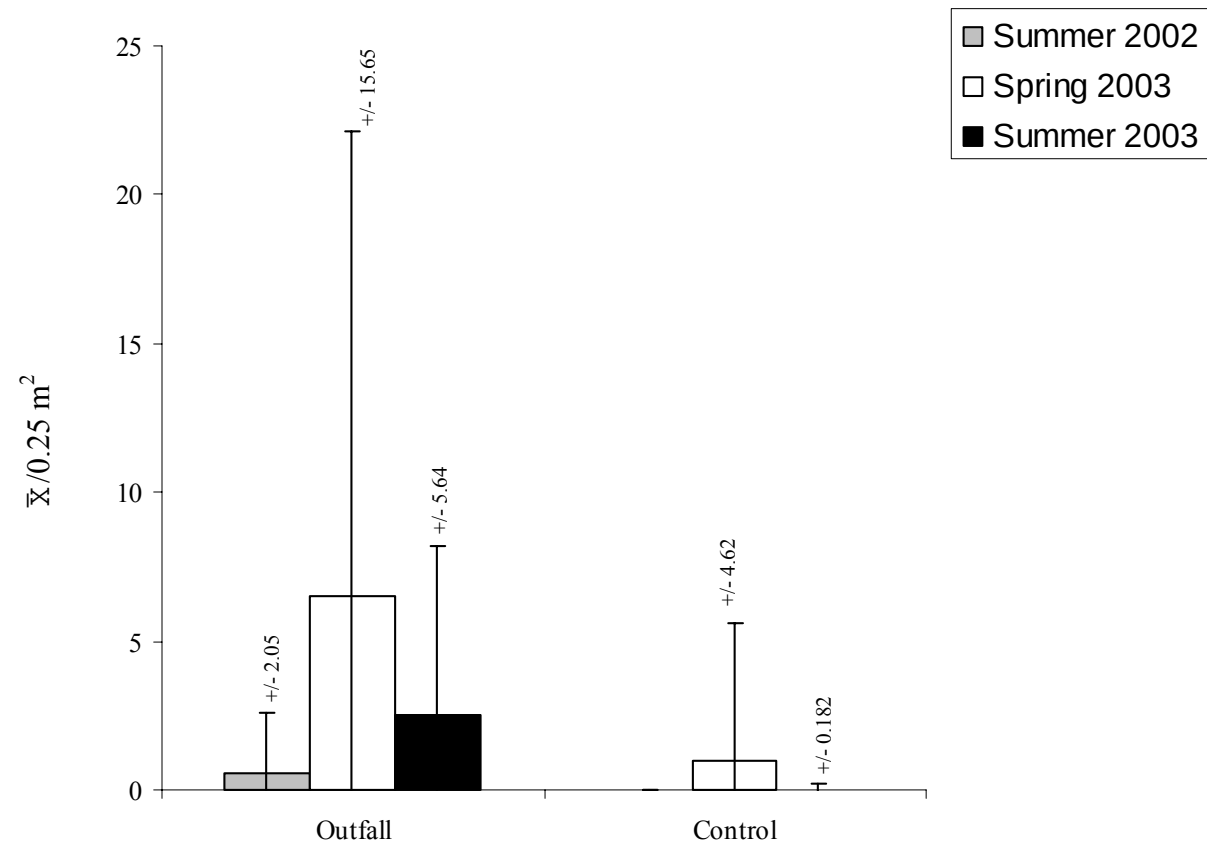


Figure 19. Univariate comparison of mean abundance for *Bossiella cretacea*. ANOVA comparisons detected a significant difference between sites over all sampling events ($p < 0.01$). There were no significant differences between sampling times ($p = 0.14$) or an interaction between sites and sampling times ($p = 0.19$).

Variation in summer 2003 samples also appeared to be related to site. Control site samples clumped on the right side of the ordination graph illustrated differences from the outfall site samples, which appeared more spread out over the left region of the graph (Figure 20). Species with the most extreme positive correlation to axis 1 were *P. polymerus*, the mussel *Mytilus californianus*, and the limpet *Collisella digitalis* (Table 3). These were all significantly more abundant at the control site (Figures 9, 21, 22) in comparison to the outfall site ($p < 0.01$). Species exhibiting a negative correlative value to this axis were the nemertean *Amphiporus* sp. (Figure 23), and the red algae *Microcladia borealis* (Figure 24) and *M. papillatus* (Figure 12), each being significantly more abundant at the outfall site ($p < 0.01$).

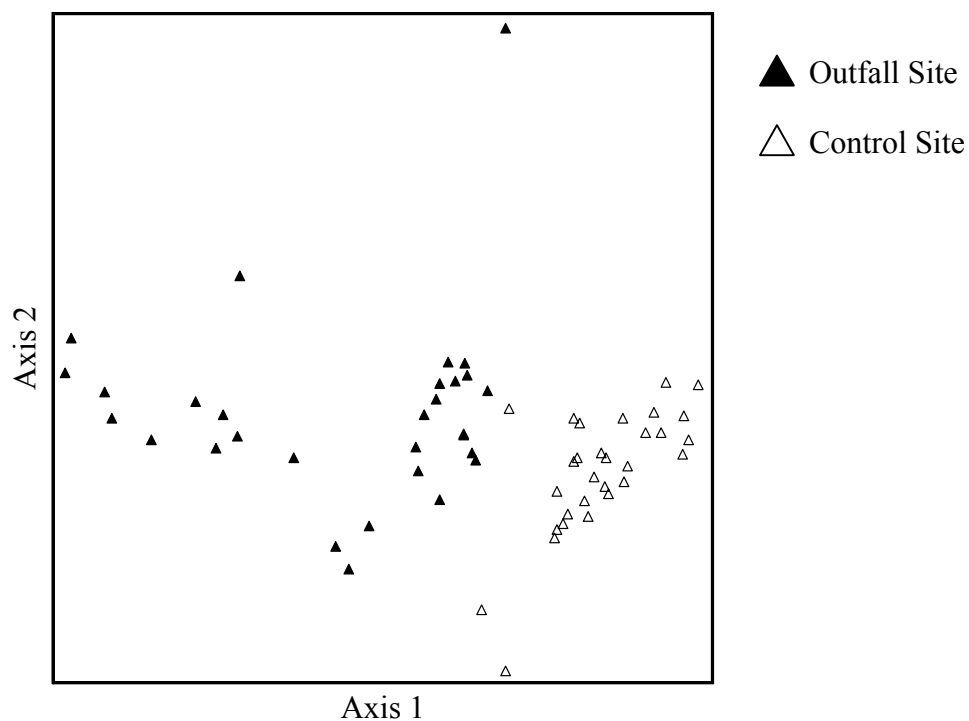


Figure 20. Bray-Curtis ordination graph illustrating the $[\log(x + 1)]$ transformed data collected at the outfall and control sites during summer 2003. Distances between sampling quadrats depicted on the graph are representative of the dissimilarity of the samples.

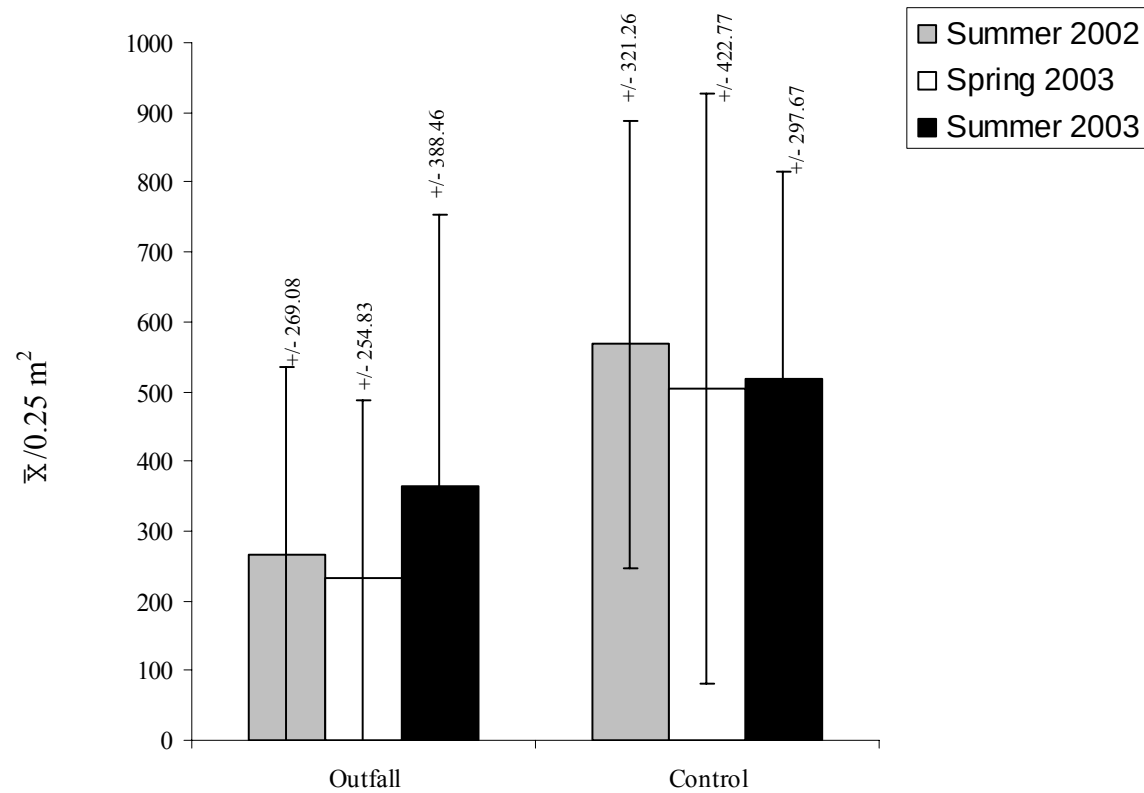


Figure 21. Univariate comparison of mean abundance for *Mytilus californianus*. ANOVA comparisons detected a significant difference between sites over all sampling events ($p < 0.01$). There were no significant differences between sampling times ($p = 0.52$) or an interaction between sites and sampling times ($p = 0.45$).

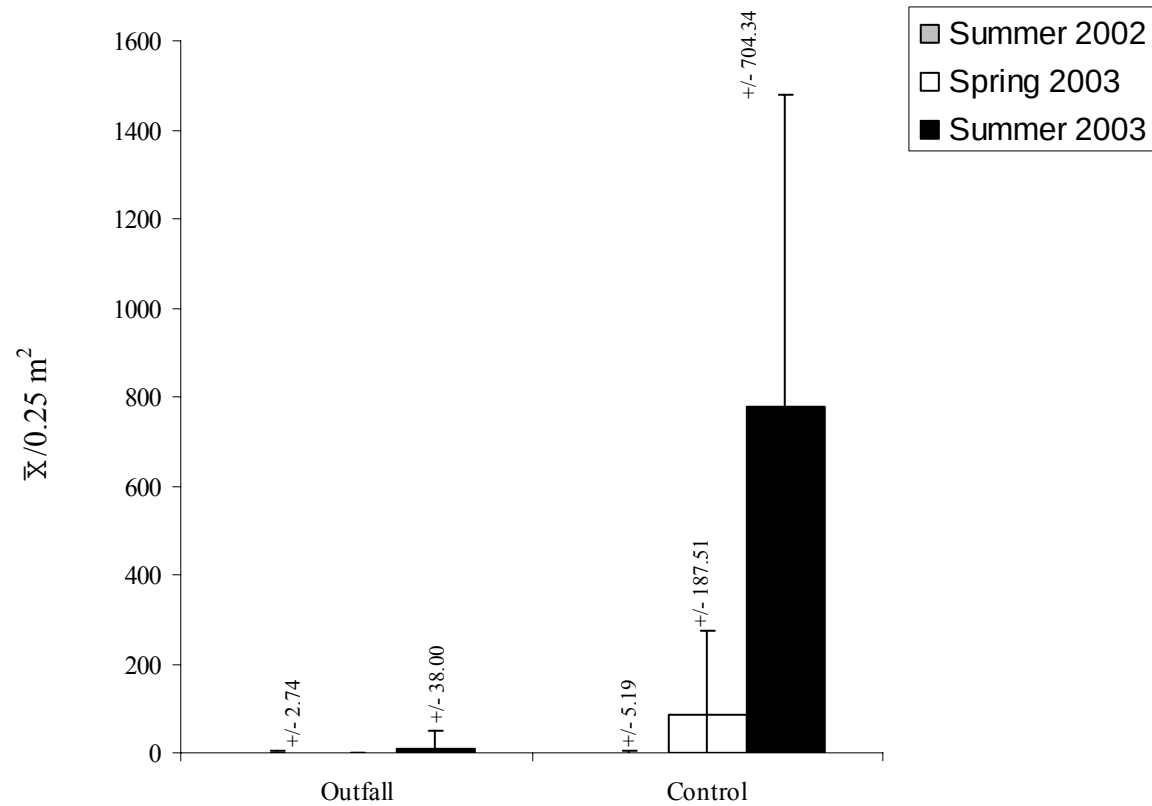


Figure 22. Univariate comparison of mean abundance for *Collisellis digitalis*. ANOVA comparisons detected a significant difference between sites over all sampling events ($p < 0.01$), between all sampling events ($p < 0.01$) and in the interaction between location and time sampled ($p < 0.01$).

Table 3. Pearson correlation coefficients generated from an ordination analysis to determine species correlation with ordination axes during summer 2003 (r = Pearson correlation coefficient).

Axis 1	r	Axis 2	r
<i>Pollicipes polymerus</i>	0.745	<i>Collisella digitalis</i>	0.496
<i>Mytilus californianus</i>	0.698	<i>Mytilus californianus</i>	0.377
<i>Collisella digitalis</i>	0.687	<i>Semibalanus cariosus</i>	0.368
<i>Nucella emarginata</i>	0.657	<i>Anthopleura xanthogrammica</i>	0.305
<i>Lottia strigatella</i>	0.326	<i>Pisaster ochraceus</i>	0.288
<i>Anthopleura xanthogrammica</i>	0.294	<i>Anthopleura elegantissima</i>	0.287
<i>Anthopleura elegantissima</i>	0.261	<i>Ulva</i> sp.	0.267
<i>Notoacmea scutum</i>	0.2	<i>Acrosiphonia arcta</i>	0.259
<i>Pisaster ochraceus</i>	0.175	<i>Nucella emarginata</i>	0.24
<i>Bonnemaisonia nootkana</i>	0.105	<i>Lottia strigatella</i>	0.221
<i>Analipus japonicus</i>	0.088	<i>Fucus gardneri</i>	0.104
<i>Mazzaella cornucopiae</i>	0.088	<i>Analipus japonicus</i>	0.091
<i>Ulva</i> sp.	0.042	<i>Mazzaella cornucopiae</i>	0.091
<i>Amphipolus pugetana</i>	0.012	<i>Katharina tunicata</i>	0.09
<i>Acrosiphonia arcta</i>	0.008	<i>Strongylocentrotus purpuratus</i>	0.09
<i>Collisella pelta</i>	0	<i>Mastocarpus papillatus</i>	0.051
<i>Katharina tunicata</i>	-0.024	<i>Notoacmea scutum</i>	0.042
<i>Strongylocentrotus purpuratus</i>	-0.024	<i>Bonnemaisonia nootkana</i>	0.025
<i>Cladophora columbiana</i>	-0.03	<i>Cladophora columbiana</i>	0.024
<i>Balanus glandula</i>	-0.039	<i>Prionitis lanceolata</i>	0.017
<i>Endocladia muriata</i>	-0.104	<i>Bossiella cretacea</i>	0.016
<i>Bossiella cretacea</i>	-0.111	<i>Microcladia borealis</i>	-0.006
<i>Littorina scutulata</i>	-0.134	<i>Littorina scutulata</i>	-0.03
<i>Fucus gardneri</i>	-0.137	<i>Endocladia muriata</i>	-0.066
<i>Prionitis lanceolata</i>	-0.198	<i>Megalorchestia californiana</i>	-0.073
<i>Megalorchestia californiana</i>	-0.481	<i>Amphipolus pugetana</i>	-0.079
<i>Semibalanus cariosus</i>	-0.552	<i>Pollicipes polymerus</i>	-0.103
<i>Amphiporous</i> sp.	-0.576	<i>Amphiporous</i> sp.	-0.203
<i>Microcladia borealis</i>	-0.683	<i>Balanus glandula</i>	-0.483
<i>Mastocarpus papillatus</i>	-0.699	<i>Collisella pelta</i>	-0.501

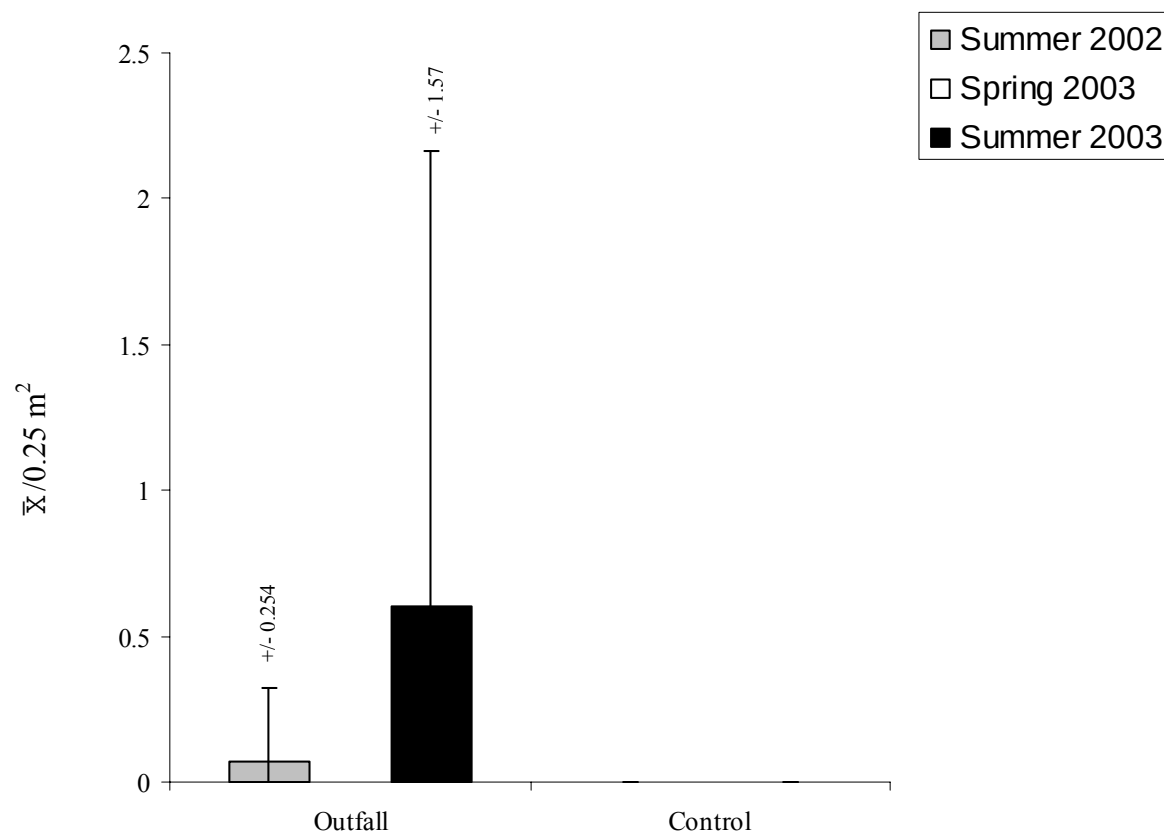


Figure 23. Univariate comparison of mean abundance for *Amphiporus* sp. ANOVA comparisons detected a significant difference between sites over all sampling events ($p < 0.01$), between all sampling events ($p < 0.05$) and in the interaction between location and time sampled ($p < 0.05$).

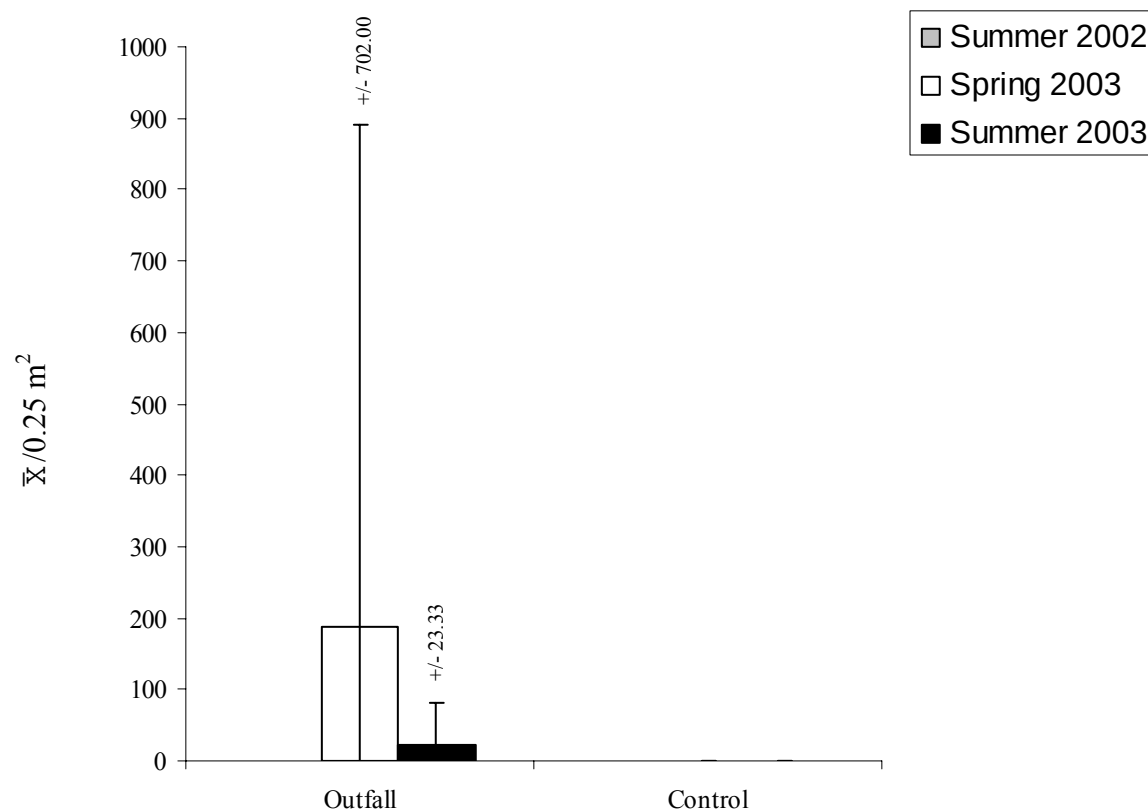


Figure 24. Univariate comparison of mean abundance for *Microcladia borealis*. ANOVA comparisons detected a significant difference between sites over all sampling events ($p < 0.01$), between all sampling events ($p < 0.05$) and in the interaction between location and time sampled ($p < 0.05$).

All three of the ordination analyses indicated that the samples were clearly distributed into two distinct groups according to one factor: one group contained the control site samples and the other contained the outfall samples. These groups appeared to be separated in a horizontal direction along axis 1.

A more complex distribution separated samples in a vertical direction along axis 2. Summer sampling episodes showed that control site samples were more cohesive along this axis than in the spring sampling episode. The outfall samples showed a lack of similarity to each other with respect to this axis. Samples at this site during the summer 2002 sampling event fell out at opposite extremes, and the other two sampling events displayed a comparable degree of dissimilarity.

MRPP analysis indicated that the community compositions between sites were highly significantly different ($p < 0.0001$) during all sampling episodes (Table 4). In addition, seasonal and annual sampling events within each site were different to a significant degree ($p < 0.0001$).

Table 4. Multi-Response Permutation Procedure (MRPP) analyses results. Site comparisons (outfall site vs. control site) indicated significant differences for all sampling events. Seasonal (summer 2003 vs. spring 2003) and annual comparisons (summer 2002 vs. summer 2003) within sites resulted in significant differences for both the outfall and the control site. (δ = change [delta], T = test statistic, p = probability, A = chance corrected within-group agreement)

	δ under null hypothesis		
	T	p	A
Site Comparison (outfall site vs. control site) – Summer 2002	-28.2	<0.0001	0.221
Site Comparison (outfall site vs. control site) – Spring 2003	-31.4	<0.0001	0.219
Site Comparison (outfall site vs. control site) – Summer 2003	-31.3	<0.0001	0.246
Outfall Site			
Seasonal Comparison (Spring 2003 vs. Summer 2003)	-20.3	<0.0001	0.141
Annual Comparison (Summer 2002 vs. Summer 2003)	-8.37	<0.0001	0.0623
Control Site			
Seasonal Comparison (Spring 2003 vs. Summer 2003)	-14.2	<0.0001	0.0784
Annual Comparison (Summer 2002 vs. Summer 2003)	-28.4	<0.0001	0.211

The cluster analysis generated a dendrogram for the samples taken during transect line sampling. This showed no obvious gradient moving away from the outfall (Figure 25). There was some indication that the species composition north and south of the outfall exhibited some differences. This difference was indicated by the grouping of a majority of south side samples on top of the cluster analysis, and the grouping of most the north side samples on the bottom of the analysis.

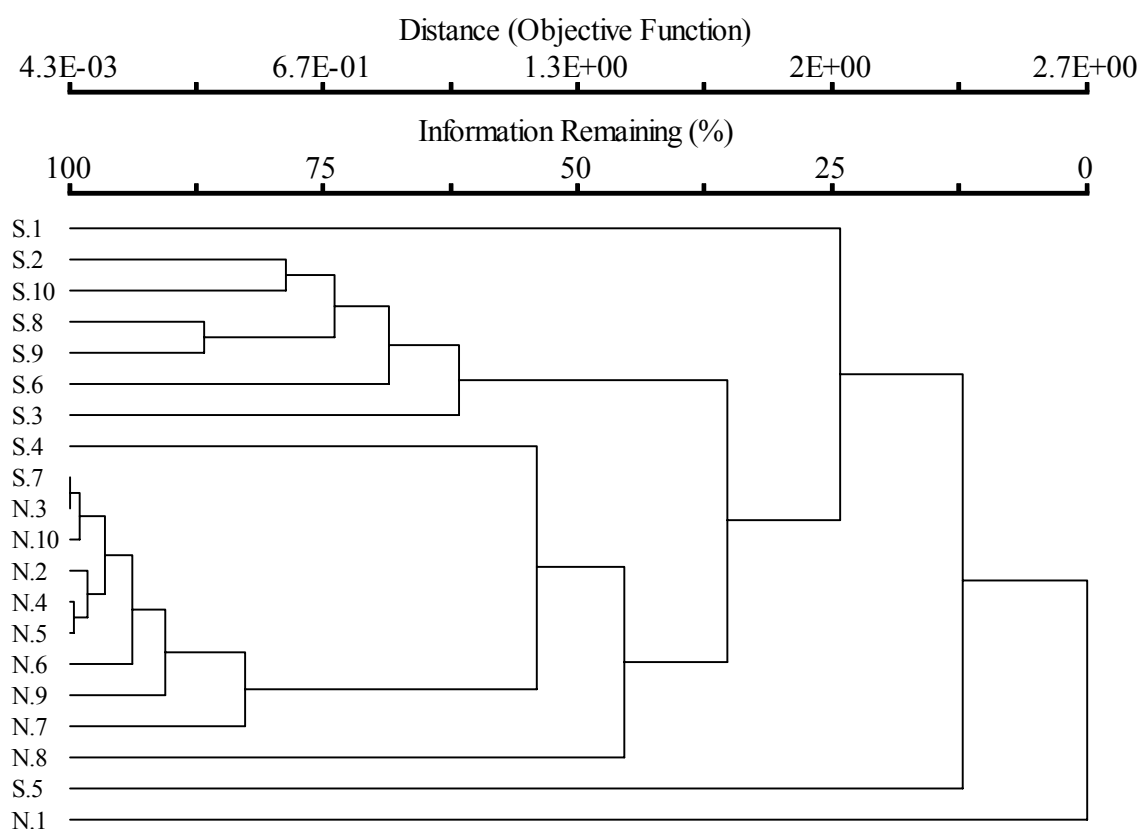


Figure 25. Cluster analysis of the transect line samples taken to the north (N) and south (S) of the outfall location. Samples were numbered 1 at the location closest to the outfall and were assigned higher numbers sequentially further from the outfall.

The result from the examination of *Ulva* sp. abundance compared with distance from the outfall did not show significant correlation ($r^2 = 0.0069$), even on a logarithmic scale (Figure 26).

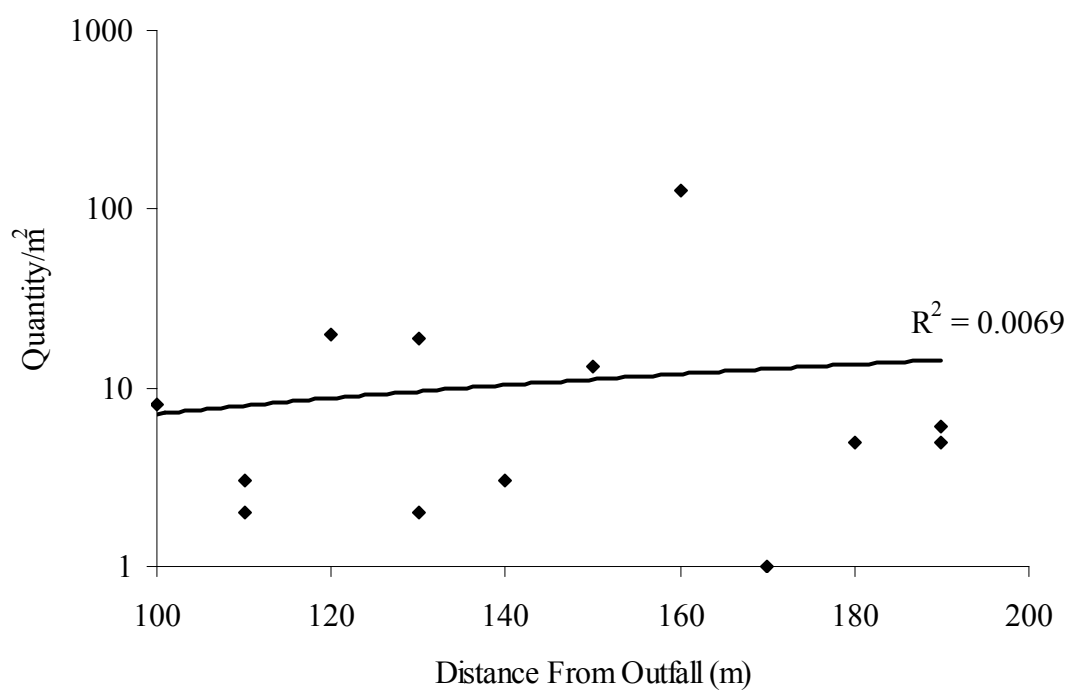


Figure 26. Logarithmic abundance of *Ulva* sp. versus distance from the outfall.

DISCUSSION

The factors which affect species composition, number of species, and abundance of assemblages in rocky intertidal habitats are highly complex. Disturbances from wastewater outfalls are typically demonstrated by changes in community structure, often manifested by decreased species richness, diversity, density, or abundance (Bilyard 1987, Smith 1996, Chisholm *et al.* 1997, Raffaelli and Hawkins 1999).

Ordination graphs did show a degree of dissimilarity between the outfall and control sites. Overall, the control site samples had higher incidences of overlap and coherency compared to the outfall site samples, regardless of seasonal or annual data. The extent of cohesion displayed on these graphs indicates that there is a distinction between the two sites. Since the Pearson correlation coefficients associated with axis 1 for all three sampling episodes seemed correlated with abundant species at the control site, and negatively correlated with abundant species at the outfall site, it can be interpreted that axis 1 may be influenced, at least partially, by sampling location (Tables 1, 2, and 3).

Univariate graphs in combination with ANOVAs illustrated site differences as well, by confirming that organisms most highly correlated with axis 1 of the ordination graphs were more highly abundant at the control site, and organisms most negatively correlated with axis 1 were more highly abundant at the outfall site.

Since there was a lack of congruence in conjunction with outliers in how samples fell out along axis 2, it was unclear what variable, or combination of variables may be the

dominating influence in dissimilarity of the samples. However, the only variables determined during the study were treatment and time sampled. Since each of the sampling events was applied to the ordination separately, the variable of time sampled was removed. Therefore, the only known variable in these analyses was treatment. Determination of additional influential factors then, is not possible.

In spite of that, MRPP analyses further supported that there were differences in community composition between the two sites. However, since there were apparent site differences, it was necessary to determine if the differences were a result of the outfall discharge. While the impact of the effluent was a possible cause, other possibilities needed to be considered.

Many of the more abundant species at the outfall site were algal species. This is an important observation, because these species may be more abundant where nutrients, in the form of nitrogen and phosphates, are readily available (Graham and Wilcox 2000). However, it is also of interest that probable grazers of these algae (limpets) were more abundant at the control site. The decreased abundance of algae in the presence of an increased abundance of herbivores indicates that there may be a food-web interaction occurring at the control site. The amount of grazing that occurs at the control site may reduce the abundance of algae in that location.

The relatively small rocky intertidal platforms at both sites restricted the ability to obtain non-overlapping quadrats during the three sampling episodes. It is important to point out in this scenario, that samples used for all statistical analyses may not have been independent of one another if a sampling quadrat was randomly selected more than once.

Because the sampling area was confined to a small space, correcting for non-independence was not possible.

Also, the difference in substrate that exists between the two sites may have been a factor. There was a large section of the sampling area near the outfall that included a concrete slab near the outfall pipe. This concrete area was connected to a small rocky area to the west of the outfall. Conversely, the entire Enderts Beach control site sampling area consists of rocky substrate.

This difference in substrate was investigated by discarding quadrat data taken from the concrete substrate, then reanalyzing and comparing all data sets. The results of the MRPP analysis still indicated a significant difference in community composition between the two sites, even without the samples from the concrete substrate.

Furthermore, the control site was selected as a comparison to the outfall site because it is similar in elevation, wave exposure, and micro-topography. This does not necessarily mean that they are precisely comparable in all these attributes. The differences found may be a result of the fact that the sites are simply different in any or all of these characteristics.

It is also important to note that ecologists and statisticians have scrutinized this type of sampling design and subsequent analysis because it does not adequately replicate experimental units (Hurlbert 1984, Chapman *et al.* 1995, Underwood 2000). The use of inferential statistics on such data sets can lead to either the error of acceptance of the hypothesis when it should be rejected (Type I error) or the error of acceptance of the null

hypothesis when it should be rejected (Type II error). Therefore, in this situation, it is impossible to determine if the differences found in comparison of the sites can be attributed to the influence of the outfall. Furthermore, geographical variation between the outfall and control sites cannot be differentiated.

Suggested sampling design methods for projects such as this one include an increase in the number of sampling sites, and an analysis of conditions prior to disturbance compared to conditions after the disturbance (Osenberg *et al.* 1992, Stewart-Oaten *et al.* 1992, Underwood 1994, 2000). It is obviously not possible to complete a Before-After-Control-Impact (BACI) type study to compare conditions before and after the outfall existed. Conditions prior to the release of the effluent are not available and the outfall is currently in use.

A complex set of variables exists at the outfall site, including elevation, wave exposure, and the unique, flat micro-topography of the concrete substrate overlying the outfall pipe. The circumstances surrounding this investigation suggest a “single-time” analysis is necessary, because sufficient acceptable sites to appropriately replicate the experimental units required for statistical analyses are not available (Wiens and Parker 1995).

In order to reduce the influence of pseudoreplication on the results obtained for the study, additional data were collected to determine if differences discovered in community composition between the outfall and control sites could be attributed to the outfall. This type of comparison has been suggested as a means to reduce the effects of

pseudoreplication on the results of study designs similar to this one (Eberhardt and Thomas 1991, Wiens and Parker 1995).

As effluent is released into ocean environments, it is diluted by water currents and wave action. Therefore, the presence of wastewater discharge in the receiving water decreases as the effluent is moved away from the source. The phenomenon of effluent being most evident at the source and least evident or non-detectable as it moves away from the source, is defined as continuous variability (Zar 1984). Since the released effluent is a continuous variable, it is sometimes possible to detect a gradient of influence through an examination of the intertidal community structure as it moves away from the outfall (Foster *et al.* 1988).

However, the cluster analysis of the transect line samples moving away from the outfall showed no clear pattern of gradation in the community structure. This indicates that although differences between the areas north and south of the outfall were detected, this distinction did not appear to be related to the effluent. In addition, results from the examination of *Ulva* abundance did not show that the effluent was influential in increasing green ephemeral algae near the outfall source. But, because it was not physically possible to place the end of the transect line immediately next to the outfall pipe, the possibility exists that these data were taken from an area outside the initial zone of dilution.

In spite of the statistical differences revealed in data collected during this study, there is no clear evidence to implicate the outfall effluent as the cause.

Species considered pollution indicators have been noted in situations where marine habitats are exposed to excessive nutrients (Crowe *et al.* 2000). Cyanobacteria growth may be pronounced, and populations of typical dominant animals become replaced by ephemeral green algae and filter feeders (Fairweather 1990). Ephemeral green algae populations remained low throughout the study at either site. Filter feeders were present at either site in large numbers and similar densities.

In addition, there appeared to be no obvious impoverishment of community composition resulting from the effluent discharge at the outfall site. This was supported by the descriptive aspects of this study, which indicated no differences between the two sites.

Additionally, previous studies suggest that the extent to which wastewater is treated, in combination with the rate of mixing, may be negatively correlated with the rate of degradation to the aquatic environments it is released into (Kellogg *et al.* 1997, Soltan *et al.* 2001, Diener and Fuller 1995). In this study ecological observations suggest that the secondary treated wastewater released at the study site is sufficiently treated, and well-mixed enough into the receiving waters to minimize any effects on the surrounding rocky intertidal community.

Furthermore, water quality, mussel, and sediment monitoring took place throughout the year of 2003 in a program implemented by the City of Crescent City. The monitoring was to determine the quality of the receiving waters surrounding the effluent discharge. In all cases, the parameters and nutrient levels met the guidelines of the National Pollutant Discharge Elimination System (NPDES) permit, and did not indicate

that any detected elevation of nutrients or metals was a result of the effluent (Brown and Caldwell 2003).

The subtleties of interactions of ecological assemblages with their surrounding environments can be difficult to detect. Unique attributes of the outfall site made it impossible to complete an analysis without the possibility of statistical errors. Without more conclusive results, I am unable to accept or reject the null hypothesis. In order to determine a less ambiguous conclusion to this project, a different sampling design would be crucial, and require much more time and resources. Specifically, the addition of more sampling stations and replicates would improve the integrity of the results.

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