# Evaluation of ASBS assessments in rocky intertidal communities for the State Water Board

Peter Raimondi
March 6, 2009

## SUMMARY

The reports reviewed below come from two sets of ASBS assessments. The first include those assessments that provided dataset's sufficient to perform independent analysis of potential impacts resulting from discharges. The second set included specific assessments of ASBS or ancillary documents provided in support of data adequacy. The common feature of the second set is that datasets were not independently evaluated. The specific review of all the assessments follows but there are some general conclusions that come from this review. First, the methods used in the assessments differ dramatically. They range from careful design leading from specific questions to almost a naturalist perspective on a site. Second, all the assessments were done either by the discharger or consultants to the discharger. Third, the basis for determining if a discharge is causing an impact differed dramatically among assessments. Fourth, and most important; it is clear that most dischargers are not clear about what the basis for determination of impact should be. One clear recommendation is that there should be a general basis for determination of impact that is consistently applied. There should also be a general assessment design that would produce information sufficient to produce a rigorous determination of impact. Finally, the reporting requirements for assessments should be standardized including data and metadata reporting, transfer and storage.

## Table of contents

## ASSESSMENTS THAT PROVIDED DATASET'S SUFFICIENT TO PERFORM INDEPENDENT ANALYSIS OF POTENTIAL IMPACTS RESULTING FROM DISCHARGES

This set of assessment included sites at San Clemente and San Nicolas Islands and at Sea Ranch and Trinidad. The approach taken at San Clemente and San Nicolas Islands are very similar and were done by the same consulting firm. These differ substantially from the approaches taken at Sea Ranch and Trinidad, which themselves differ from each other. The variety of approaches presents one of the major obstacles in: (1) evaluating the adequacy of the assessments and (2) understanding the basis for determination of impact.

For this set of assessments original datasets were available and were of the type that allowed a common analytical approach (described below). There is no policy guidance with respect to determination of impact. This means that it is unclear as to what would count as evidence of an impact related to a discharge. Because of this, I simply took the approach of comparing the composition of species between areas presumed to be in the zone of the discharge to that in reference areas. This assumes at least two things. First the areas considered to be in the discharge are actually in the area affected by the discharge. Second, and this is more important, the areas considered to be reference are areas unaffected by an anthropogenic discharge. This is different from considering areas to be reference areas when they do not contain discharges related to the applicant's discharge or discharges.

**Analytical approach:** I used Bray-Curtis ordination (using PRIMER 6) to compare community structure at reference and impact locations. ANOSIM was used for comparisons (mainly between reference and discharge locations). This allows a statistical assessment of the comparison (with an associated p-value) followed by SIMPER evaluation when ANOSIM comparisons were significant. SIMPER evaluation provide guidance as to the species driving the differences among levels (eg. between discharge and reference locations).

**ASBS: San Clemente Island**:

**Investigator**: Merkel and Associates

**Date of Report**: February 2007

**Design**: "*A total of ten locations were chosen for sampling around SCI, and these included five locations that were representative of areas that receive storm water discharges associated with distinct Navy operational activities, such as airfield operations, training ranges, or in one case, from underwater detonation operations. The total also included five locations chosen to represent areas that receive storm water runoff not associated with Navy activities, and thereby considered a reference condition. The five reference locations were chosen because historical data indicated that there are four ecoregions around the island that result in different reference conditions.*" In addition, sampling was done at 3 tide heights at each location. At each tidal height, the cover or count of target species was made in four .25$m^2$ quadrats spaced along a 10 meter transect line. Cover estimates were made based on point contact of 20 points in each quadrat.

There is concern about the selection of reference sites given that they are in discharge locations. No information was provided about the type of discharges at the references sites, hence it is unclear if they constitute 'reference" datasets for the purpose of estimating potential impacts resulting from Navy activities.

There is also concern about the level of replication. For each zone at each location, community characterization is by 80 points for species cover and from 1 meter square for counts. As noted in the report, natural spatial variability in such environments is high. Estimation of effect in the presence of high natural variability is made much more rigorous by increasing replication. Hence, the power of this design is likely to be low.

**Conclusion from report**: "Navy discharges do not compromise protection of ocean waters for beneficial uses."

**Basis for determination:** *"if a metric measured at a station was lower by 50% or more than the associated reference station, then that metric was flagged"*. This basis (50%) was selected to account for natural variability.

**Assessment of support for conclusion**: The stated basis would allow considerable impacts to go undetected, particularly because replication is low. In my opinion more replication would have yielded greater statistical power and the use of multivariate methods could allow enhanced discrimination of true impacts.

**Independent assessment (see analyses below)**: The data analysis done in this study (see below) using multivariate methods do not indicate an impact due to Navy activities – note this relies on the reference sites not being impaired.

**ASBS: San Nicolas Island**:

**Investigator**: Merkel and Associates

**Date of Report**: April 2007

**Design**: "*A total of six sites were chosen for sampling around SNI, and included four sites that are representative of areas that receive storm water discharges associated with distinct Navy operational activities such as airfield, water desalination, and rocket launch operations. The total also includes two locations chosen to represent areas that receive storm water runoff not associated with Navy activities (i.e., reference condition).*" In addition, sampling was done at 3 tide heights at each location. At each tidal height, the cover or count of target species was made in four .25$m^2$ quadrats spaced along a 10 meter transect line. Cover estimates were made based on point contact of 20 points in each quadrat.

There is concern about the selection of reference sites given that they are in discharge locations. No information was provided about the type of discharges at the references sites, hence it is unclear if they constitute 'reference" datasets for the purpose of estimating potential impacts resulting from Navy activities.

There is also concern about the level of replication. For each zone at each location, community characterization is by 80 points for species cover and from 1 meter square for counts. As noted in the report, natural spatial variability in such environments is high. Estimation of effect in the presence of high natural variability is made much more rigorous by increasing replication. Hence, the power of this design is likely to be low.

**Conclusion from report**: "Navy discharges do not compromise protection of ocean waters for beneficial uses".

**Basis for determination:** *"if a metric measured at a station was lower by 50% or more than the associated reference station, then that metric was flagged".* This basis (50%) was selected to account for natural variability

**Assessment of support for conclusion**: The stated basis would allow considerable impacts to go undetected, particularly because replication is low. In my opinion more replication would have yielded greater statistical power and the use of multivariate methods could allow enhanced discrimination of true impacts.

**Independent assessment (see analyses below)**: Using the design and data provided there is evidence that Impact locations are different from Reference locations based on comparison of community composition. This is based on both species that are counted and separately for those sampled by estimating percent cover.

## ASBS: Sea Ranch

**Investigator**: Vic Chow, Peter Connors, Jacqueline Sones, Sarah Ann Thompson and Matt Bracken

**Date of Report**: May 2006

**Design**: The sampling locations included two discharge locations (West and East Discharge) and two reference locations (West and East Control). The reference locations were set up at a distance (80 meters from discharge locations) that was considered free from the influence of the discharge. At each location, a single 5 meter transect was set up at each of four tide zones. Five $0.04m^2$ (20 x 20 cm) quadrats were sampled along each transect . Counts of mobile invertebrates and cover of sessile invertebrates and algae were made in each quadrat (the methods do not indicate how cover was estimated). In addition all visible species were listed (species richness).

An important assumption made in the report is that reference locations are unaffected by discharges. It is noted that they were placed at a distance that was considered to be outside the influence of the discharge, but this assumption is not supported in any way.

There is also concern about the level of replication. For each zone at each location, community characterization is by means of a 0.2 meter square area (5 x 0.04 $m^2$). As shown in the report (e.g. table 3) parameter estimates were very variable. This result is consistent with the widespread understanding that natural spatial variability in such environments is high. Estimation of effect in the presence of high natural variability is made much more rigorous by increasing replication. Hence, the power of this design is likely to be low.

**Conclusion from report**: *"An inventory of marine species at the two discharge sites and control sites does not indicate any water quality impacts to intertidal marine life"* [from letter from The Sea Ranch Association dated May 31, 2006.

**Basis for determination:** Statistical difference at the p=0.05 level.

**Assessment of support for conclusion**: Using a p-value for the basis of impact assessment is a widespread approach. This approach as implemented through GLM relies on a number of sampling assumptions including independence of samples, homoscedacity and most importantly for this assessment – independence of treatment levels, here the most important comparison is between reference and discharge locations. It is not clear that this assumption has been assessed and met. In addition, the use of p-values guard against Type I statistical error. Under-replication will always yield greater lowered statistical power (i.e. greater Type II error, which is the incorrect conclusion of no effect). Given the size and number of replicate samples, it is very likely that power in this design was low.

**Independent assessment (see analyses below)**: Using the design and data provided there is evidence that Discharge locations are different from Reference locations based on comparison of community composition (see below). This effect was complicated by the interaction between treatment (discharge vs reference) and zone. For species sampled by counts and those sampled by percent cover, 2 of four zones differed between Discharge and Reference Areas.

**ASBS: Trinidad**

**Investigator**: Sean Craig and Dean Janiak

**Date of Report**: May 2006

**Design**: The sampling locations included one discharge location and one reference location. The reference location was set up at a distance (100 meters from discharge locations), presumably that was considered free from the influence of the discharge. Each location was characterized by a series of boulders. Within each location a set of boulders was selected for sampling. Although the report indicates that there were four regions at each location, the datasets are only consistent for three: low, mid, and high. Each region represented a zone in the intertidal area. Each boulder was divided into up to three heights, again called low, mid and high that represented the height away from sand. Within each combination of location, region and height a single .25 $m^2$ quadrat was sampled for cover and counts of organisms. It is not clear how cover was estimated.

An important assumption made in the report is that the reference location is unaffected by the discharge. This assumption is not supported in any way.

There is also concern about the level of replication. The replication of quadrats used in this assessment may be adequate, but no formal evaluation of the power to detect an effect was given. Generally, when there is a conclusion of no impact, a formal power analysis should be done and reported.

**Conclusion from report**: "*It is concluded that from results obtained in this survey, the discharge from the City's drain pipe does not cause a measurable difference in species abundance of change in species composition*." [From Trinidad ASBS Exception Request dated May 29, 2006.

**Basis for determination:** Best Professional Judgement. No formal statistical analyses were done.

**Assessment of support for conclusion**: There is no way to determine whether the conclusion is supported.

**Independent assessment (see analyses below)**: Using the design and data provided there is strong evidence that the discharge location is different from the reference location based on comparison of community composition. In addition there was a strong difference in community composition based height above sand but not based on region.

## SPECIFIC ASSESSMENTS OF ASBS OR ANCILLARY DOCUMENTS PROVIDED IN SUPPORT OF DATA ADEQUACY

The reports reviewed briefly below range from specific assessments of ASBS to ancillary documents provided in support of data adequacy. Some are well done; some are clearly insufficient (in my opinion). Probably the single most obvious characteristic of the group of submissions is their lack of consistency in design. This compromises our ability to make general assessments of the network of ASBS's and also severely diminishes our ability to assess design adequacy for individual studies.

**ASBS**: Carmel Bay

**Investigator**: Richard Ford

**Date of Report**: April 30, 2005

**Design**: No original research was conducted. Study design is of two parts: survey data from other reports from Carmel Bay and a study done along Irvine property in Southern California. The latter study is relevant because it assessed runoff from a golf course (similar situation to Carmel Bay)

**Conclusion from report**: No discernable impact on marine environment

**Assessment of support for conclusion**: There is no direct support for the stated conclusion. The design is inadequate for the determination of impact (or lack of impact).

**ASBS**: Caltrans report for multiple ASBS locations: Enderts, Damnation Creek and False Klamath Cove, Fitzgerald Marine Reserve, Ano Nuevo, Point Lobos, Partington Point, Carmel Point, Stillwater Cove, Old Stairs (ASBS 24), Crystal Cove (ASBS 33)

**Investigator**: Peter Raimondi

**Date of Report**: April 26, 2006

**Design**: Use of PISCO and MARINe data sets in a multivariate (primarily) assessment of communities in ASBS and Reference areas. Only existing data were used in the analyses.

**Conclusion from report**: While certain ASBS sites differed from reference sites, there was no strong support that this was due to discharge or effluent.

**Assessment of support for conclusion**: I was the author of this report and for this reson it is inappropriate for me to comment of the quality of the assessment.

**ASBS**: County of Marin, Duxbury reef (Alder Creek)

**Investigator**: Peter Raimondi

**Date of Report**: July 17, 2008

**Design**: Use of PISCO and MARINe data sets in a multivariate (primarily) assessment of communities in ASBS and Reference areas. Existing and new data were utilized. New data were collected in using PISCO biodiversity protocols at sites arrayed in a gradient away from discharge.

**Conclusion from report**: *"There are clearly differences in the communities between Alder Creek and nearby sites. Part of this is due to differences in the geomorphology of the site, particularly the deep channel that separates the inshore from offshore reef. However, part of the difference also seems due to the presence of an input from the discharge and/or the creek that empties into the site. Based on the information collected during this survey and from the Coastal Biodiversity Surveys our assessment is that the differences seen at Alder creek are likely due to a combination of trampling (minor effects) and the geomorphological features (primarily fine sediments and freshwater) present at Alder Creek. Based on our surveys and reconnaissance, the effect of the input (natural or other) appears to be over a relatively small spatial scale, probably no larger than a few hundred meters along shore."*

**Assessment of support for conclusion**: : I was the author of this report and for this reason it is inappropriate for me to comment of the quality of the assessment.

**ASBS**: Pillar Point

**Investigator**: Tenera Environmental

**Date of Report**: September 27, 2007

**Design**: The design was to assess the rocky bench closest to the outfall and compare that area with a reference area further away from the outfall. Within the area close to the outfall, "sites" were arranged along a gradient of distance away from the discharge. As such this was a hybrid approach using a gradient design cross shore along with the use of impact/reference areas along shore. Analysis was primarily based on community level comparisons between impact and reference areas using multivariate techniques found in PRIMER software (ANOSIM, SIMPER and relate)

**Conclusion from report**: While the results of the analysis indicated that there were differences between impact and reference areas for both algae and inverts, these differences were not attributed to the discharge. Instead the differences were attributed to underlying spatial variation.

**Assessment of support for conclusion**: There is an inconsistency between the basis of the design and analysis and the conclusion. The goal of a design in the ASBS context should be to assess the possibility of impact due to discharge. This was the intent here. The conclusion of no evidence of impact, given that statistical results suggest differences between areas suggests that the design was not adequate to test the implicit hypothesis.

**ASBS**: Marine Resources of Redwood National and State Parks Comprehensive Report (2004-2005) for Humboldt and Del Norte County, California: Specific rocky intertidal sites include Enderts, Damnation Creek and False Klamath Cove

**Investigator**: Karah Cox, Cara McGary, Tim Mulligan, Sean Craig: Departments of Fisheries Biology and Biological Sciences, Humboldt State University

**Date of Report**: 2006

**Design**: This was a comprehensive assessment of coastal resources in Redwoods National Park including rocky and sandy shores. Methods used to assess the rocky intertidal within Redwood National and State Parks (RNSP) entailed both an inventory of the algal, invertebrate, and fish species present at three selected sites, as well as seasonal monitoring of abundant and /or ecologically important organisms. The species distribution in the rocky intertidal was examined on a presence/absence scale at each of the sites with a standardized Biodiversity Protocol used to map and derive a complete species list for one of the sites (Damnation Creek). Finer scale monitoring of the three sites focused on discrete plots or pools. Methods for monitoring algal and invertebrate communities were based on the design of MARINe (multi-agency rocky intertidal network) (www.marine.gov). Methods adapted from MARINe included scoring percent cover of algal species in permanent photoplots as well as enumerating mobile invertebrates within the plots, monitoring seastar plots and surfgrass transects. Select rocky tidepools were repeatedly sampled to provide a more quantitative assessment of specific resident species of tidepool fishes.

**Conclusion from report**: There were no conclusions pertaining to ASBS issues as this report was to characterize the resources and not to assess possible impacts to ASBS

**Assessment of support for conclusion**: N/A

**ASBS**: Hopkins Marine Reserve

**Investigator**: None

**Date of Report**: None

**Design**: the submission was in the form of two manuscripts Barry et al 1995 and Schiel et al, 2004. The work by Barry indicated that at Hopkins Marine Lab there had been a shift of species abundances consistent with the idea of global warming. Schiel et al found (for a different area) that changes in community structure were common but that the was no obvious link to global warming

**Conclusion from report**: The submissions noted above are not directly relevant to an assessment of the ASBS

**Assessment of support for conclusion**: N/A

**ASBS**: Pfeiffer

**Investigator**: NOAA/National Marine Sanctuaries

**Date of Report**: Unknown

**Design**: The submitted material is in the form of a pamphlet that indicates the monitoring programs that occur in the vicinity of Pfeiffer

**Conclusion from report**: None

**Assessment of support for conclusion: NA**
There have been a number of studies that have evaluated the impact of the landslide at Pfeiffer. In particular, the Monterey Bay National Marine Sanctuary funded PISCO to assess effects of erosion and landslides along the Big Sur Coastline. Within this area the landslide at Pfeiffer was catastrophic to the associated communities. While this study did not specifically address the issues of ASBS it is clear that there were pronounced and long-lasting effects of the material deposition.

**ASBS**: Crystal Cove

**Investigator**: Richard F. Ford, Ph.D, San Diego State University and Hubbs-Sea World Research Institute. Barbara B. Hemmingsen, Ph.D, San Diego State University. Michael A. Shane, MS, Hubbs-Sea World Research Institute. Eric Strecker, PE GeoSyntec Consultants, Inc.

**Date of Report**: April 2007

**Design**: This was a comprehensive report that evaluated a number topics not assessed in this review (including water quality and subtidal habitats). For intertidal communities the goal was to conduct quantitative marine ecological studies of benthic invertebrates, algae, and surfgrass epiphytes in rocky intertidal located at the best attainable reference site (Emerald Bay) and the potential impact sites at Muddy and Los Trancos Canyons. In addition effort was made to compare and evaluate these together with the corresponding water quality information to assess similarities and differences among sites.

Using photoplots, and on site surveys five species groups were sampled: 1) the *Anthopleura elegantissima* and associated species; 2) *Mytilus californianus* and associated species; 3) Anthopleura sola and associated species; 4) algal turf species; and 5) barnacles (Balanus glandula, Chthamalus dalli and C. fissus)

**Conclusion from report**: The major conclusion was that there is no evidence of impacts related to discharge.

**Assessment of support for conclusion:** This was a very difficult report to assess. In my opinion the authors did not rigorously test the hypothesis that reference and control sites differed in their biological communities. They did test whether there were long or short term trends in species numbers (cover, abundance etc) that differed between reference and impact locations. My assessment is that the underlying basis of the long term hypothesis was not supported. Here the idea was that evidence of an impact would be manifest in a trend at the impact sites relative to the reference site. This could indicate increasing degradation at the site. An alternative is that the community at the impact site(s) is in steady state yet still degraded. In such a situation no trend would occur. In addition there was no assessment of the community. Such assessments are often more sensitive than species specific assessments. Finally, this design rests on the adequacy of the references site. In southern CA selection of a reference site is difficult and an alternative approach involving a series of possible reference sites could have provided a more robust context for the results. Having said all of this, the study was professionally done among the best I have reviewed.

## Support material - results of analyses used in part 1.

### San Clemente Island

1) Sample Design:
    a. Treatment
        i. Reference: Castle Rock, Eel Point, Lost Point, East Reference, Sun Point
        ii. Impact: NW Harbor, East Airfield, West Airfield, NOTS, Horse Beach
    b. Other Strata
        i. Tide Height: 0,3,5 (Elevations were different in all tests)
2) Analytical Design
    a. Species that are counted
        i. Bray-Curtis Ordination on fourth root transformation.
        ii. Comparison of Treatments using Anosim: Reference found to be not different from Impact at **P=0.78**

```
TESTS FOR DIFFERENCES BETWEEN TTT GROUPS
(across all Elevation groups)
Global Test
Sample statistic (Global R): -0.075
Significance level of sample statistic: 78.9%
Number of permutations: 999 (Random sample from 2000376)
Number of permuted statistics greater than or equal to Global R: 788
```

b. Species measured by percent cover.
   i. Bray-Curtis Ordination: no transformation.
   ii. Comparison of Treatments using Anosim: Reference found to be not different from Impact at **P=0.45**

```
TESTS FOR DIFFERENCES BETWEEN TTT GROUPS
(across all Elevation groups)
Global Test
Sample statistic (Global R): -0.002
Significance level of sample statistic: 45%
Number of permutations: 999 (Random sample from 2000376)
Number of permuted statistics greater than or equal to Global R: 449
```

## San Nicolas Island

1) Sample Design:
   a. Treatment
      i. Reference: Corral Beach, Dutch Harbor
      ii. Impact: Tranquility Beach, Blue Whale Cove
   b. Other Strata
      i. Tide Height: 0,3,5
2) Analytical Design
   a. Species that are counted
      i. Bray-Curtis Ordination on fourth root transformation.
      ii. Comparison of Treatments using Anosim: Reference found to be different from Impact at **P<0.001**

```
TESTS FOR DIFFERENCES BETWEEN Type GROUPS
(across all Transect groups)
Global Test
Sample statistic (Global R): 0.395
Significance level of sample statistic: 0.1%
Number of permutations: 999 (Random sample from 96621525)
Number of permuted statistics greater than or equal to Global R: 0
```

| | Group Reference | Group Impact | |
|---|---|---|---|
| Species | Av.Abund | Av.Abund | Contrib% |
| Limpets | 2.64 | 1.49 | 23.09 |
| Anemones | 0.51 | 0.5 | 13.21 |
| Strongylocentrotus purpuratus | 0.72 | 0 | 11.66 |
| Mussels | 1.01 | 0.15 | 8.7 |
| Tegula funebralis | 0.47 | 0.18 | 8.48 |
| Sargassum agardhianum | 0.23 | 0.04 | 7.43 |
| Chitons | 0.54 | 0.15 | 7.37 |
| Barnalces | 0.96 | 1.53 | 6.69 |
| Littorina spp | 0.94 | 0.38 | 5.25 |
| | | | |

iii. Decompose **Treatment** effect – test sites separately to determine if **Treatment** effect is driven by one Site. All sites differ at **P=0.001**

*TESTS FOR DIFFERENCES BETWEEN Site GROUPS*
*(across all Transect groups)*
*Global Test*
Sample statistic (Global R): 0.657
Significance level of sample statistic: **0.1%**
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Global R: 0

*Pairwise Tests*

| Groups | R Statistic | Significance Level % | Possible Permutations | Actual Permutations | Number >= Observed |
|---|---|---|---|---|---|
| DH, TB | 0.563 | **0.2** | 1225 | 999 | 1 |
| DH, CB | 0.62 | **0.1** | 1225 | 999 | 0 |
| DH, BWC | 0.446 | **1.1** | 525 | 525 | 6 |
| TB, CB | 0.813 | **0.1** | 6125 | 999 | 0 |
| TB, BWC | 0.83 | **0.2** | 525 | 525 | 1 |
| CB, BWC | 0.798 | **0.2** | 525 | 525 | 1 |

b. Species measured by percent cover.
   i. Bray-Curtis Ordination: no transformation.
   ii. Comparison of Treatments using Anosim: Reference found to be different from Impact at **P<0.039**

*Global Test*
Sample statistic (Global R): 0.051
Significance level of sample statistic: **3.9%**
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Global R: 38

| Species | Group Reference Av.Abund | Group Impact Av.Abund | Contrib% |
|---|---|---|---|
| C_Coralline Algae | 25 | 6.46 | 26.72 |
| Red Turf | 3 | 4.67 | 21.61 |
| C_Phyllospadix | 11.25 | 10 | 13.1 |
| C_Chaetomorpha sp. | 0 | 12.08 | 11.07 |
| Other Browns | 0.38 | 0.46 | 7.4 |
| C_Encrusting Coralline Algae | 6.04 | 1.04 | 7.06 |
| Ralfsiaceae | 0.21 | 0.46 | 6.64 |

   iii. Decompose Treatment effect – test sites to determine if Treatment effect is driven by one Site. All sites differ from each other. P < 0.001

*TESTS FOR DIFFERENCES BETWEEN Site GROUPS*
*(across all Transect groups)*
*Global Test*
Sample statistic (Global R): 0.477
Significance level of sample statistic: 0.1%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Global R: 0

*Pairwise Tests*

| Groups | R Statistic | Significance Level % | Possible Permutations | Actual Permutations | Number >= Observed |
|---|---|---|---|---|---|
| DH, TB | 0.345 | **1** | 42875 | 999 | 9 |
| DH, CB | 0.569 | **0.1** | 42875 | 999 | 0 |
| DH, BWC | 0.356 | **0.9** | 42875 | 999 | 8 |
| TB, CB | 0.682 | **0.1** | 42875 | 999 | 0 |
| TB, BWC | 0.47 | **0.4** | 42875 | 999 | 3 |
| CB, BWC | 0.465 | **0.1** | 42875 | 999 | 0 |

## Sea Ranch

1) Sample Design:
   a. Treatment
      i. Reference: West Control (WC), East Control (EC)
      ii. Impact: West Discharge (WD), East Discharge (ED)
   b. Other Strata
      i. Zone (1,2,3,4)
2) Analytical Design
   a. Species that are counted
      i. Bray-Curtis Ordination on fourth root transformation.
      ii. Comparison of Treatments using Anosim: Reference found to be different from Impact at P<**0.001**

```
TESTS FOR DIFFERENCES BETWEEN Treatment GROUPS
(across all Zone groups)
Global Test
Sample statistic (Global R): 0.238
Significance level of sample statistic: 0.1%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Global R: 0
```

## Comparison of Impact to Reference Sites by zone (followed by species contribution to dissimilarity, if significant)

### Zone 1, P=0.001

```
Sample statistic (Global R): 0.508
Significance level of sample statistic: 0.1%
Number of permutations: 999 (Random sample from 92378)
Number of permuted statistics greater than or equal to Global R: 0
```

| | Group Impact | Group Reference | |
|---|---|---|---|
| Species | Av.Abund | Av.Abund | Contrib% |
| Lottia digitalis | 0.33 | 1.33 | 33.88 |
| Lottia scabra | 0.49 | 1.44 | 31.95 |
| Littorina plena/scutulata | 3.34 | 2.41 | 31.7 |

### Zone 2, P=0.015

```
Sample statistic (Global R): 0.19
Significance level of sample statistic: 1.5%
```

```
Number of permutations: 999 (Random sample from 92378)
Number of permuted statistics greater than or equal to Global R: 14
```

| | Group Impact | Group Reference | |
|---|---|---|---|
| Species | Av.Abund | Av.Abund | Contrib% |
| small limpets | 0.63 | 1.29 | 19.72 |
| Littorina plena/scutulata | 1 | 0.59 | 19.62 |
| Tegula funebralis | 0.61 | 0 | 16.37 |
| Lottia scabra | 0.46 | 1.01 | 15.61 |
| Lottia pelta | 0.33 | 0.32 | 8.42 |
| Nucella ostrina | 0.1 | 0.42 | 7.14 |
| Lottia paradigitalis | 0 | 0.24 | 3.69 |

**Zone 3, P=0.163**

```
Sample statistic (Global R): 0.069
Significance level of sample statistic: 16.3%
Number of permutations: 999 (Random sample from 92378)
Number of permuted statistics greater than or equal to Global R: 162
```

**Zone 4, P=0.321**

```
Sample statistic (Global R): 0.029
Significance level of sample statistic: 32.1%
Number of permutations: 999 (Random sample from 19448)
Number of permuted statistics greater than or equal to Global R: 320
```

iii. Decompose **Treatment** effect – test sites to determine if **Treatment** effect is driven by one Site. Many pairs are different.

```
TESTS FOR DIFFERENCES BETWEEN Site GROUPS
(across all Zone groups)
Global Test
Sample statistic (Global R): 0.327
Significance level of sample statistic: 0.1%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Global R: 0

Pairwise Tests
                 R  Significance      Possible       Actual  Number >=
Groups   Statistic       Level %  Permutations Permutations   Observed
WD, WC        0.57           0.1      18670176          999          0
WD, ED        0.39           0.1     252047376          999          0
WD, EC       0.553           0.1     252047376          999          0
WC, ED       0.242           1.6      18670176          999         15
WC, EC       0.149           8.7      18670176          999         86
ED, EC         0.1           9.3     252047376          999         92
```

(note abundances are expressed in terms of fourth root = $X^{.25}$)

b. Species measured by percent cover.
   i. Bray-Curtis Ordination: no transformation.
   ii. Comparison of Treatments using Anosim: Reference found to be different from Impact at **P<0.006**

*TESTS FOR DIFFERENCES BETWEEN Treatment GROUPS*
*(across all Zone groups)*
*Global Test*
Sample statistic (Global R): 0.103
Significance level of sample statistic: **0.6**%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Global R: 5

**Comparison of Impact to Reference Sites by zone (followed by species contribution to dissimilarity, if significant)**

**Zone 1, P=0.831**
Sample statistic (Global R): -0.057
Significance level of sample statistic: **83.1%**
Number of permutations: 999 (Random sample from 92378)
Number of permuted statistics greater than or equal to Global R: 830

**Zone 2, P=0.042**
Sample statistic (Global R): 0.145
Significance level of sample statistic: **4.2%**
Number of permutations: 999 (Random sample from 92378)
Number of permuted statistics greater than or equal to Global R: 41

| | Group Impact | Group Reference | |
|---|---|---|---|
| Species | Av.Abund | Av.Abund | Contrib% |
| Endocladia muricata | 30.2 | 24.4 | 19.23 |
| Mastocarpus papillatus | 28.6 | 8.4 | 14.65 |
| Gelidium coulteri | 24.7 | 3.8 | 14.51 |
| Cladophora columbiana | 16 | 13.43 | 11.79 |
| encrusting coralline algae | 1.83 | 10.8 | 6.78 |
| Odonthalia floccosa | 0 | 8.63 | 5.42 |
| Petrocelis | 2.6 | 7.33 | 4.85 |
| Halosaccion glandiforme | 6.53 | 2.33 | 4.37 |
| Fucus gardneri | 1.1 | 5.1 | 3.46 |
| Mazzaella flaccida | 0.63 | 5.45 | 3.32 |
| Polysiphonia sp. | 0 | 4.6 | 2.99 |

**Zone 3, P=0.287**
Sample statistic (Global R): 0.014
Significance level of sample statistic: **28.7%**
Number of permutations: 999 (Random sample from 92378)
Number of permuted statistics greater than or equal to Global R: 286

**Zone 4, P=0.002**
Sample statistic (Global R): 0.31

```
Significance level of sample statistic: 0.2%
Number of permutations: 999 (Random sample from 92378)
Number of permuted statistics greater than or equal to Global R: 1
```

| | Group Impact | Group Reference | |
|---|---|---|---|
| Species | Av.Abund | Av.Abund | Contrib% |
| encrusting coralline algae | 60.5 | 19.4 | 26.29 |
| Hedophyllum sessile | 33.8 | 20.8 | 20.47 |
| Odonthalia floccosa | 17.1 | 35.5 | 16.23 |
| Phragmatopoma californica | 0 | 20 | 10.08 |
| erect coralline algae | 9.4 | 12.6 | 6.31 |
| Polysiphonia sp. | 0.93 | 8.1 | 4.55 |
| Petrocelis | 5.2 | 2.7 | 3.58 |
| Endocladia muricata | 0.7 | 4.1 | 3.25 |

iii. Decompose **Treatment** effect – test sites to determine if **Treatment** effect is driven by one Site. All sites differ from each other. **P < 0.001**

```
TESTS FOR DIFFERENCES BETWEEN Site GROUPS
(across all Zone groups)
Global Test
Sample statistic (Global R): 0.285
Significance level of sample statistic: 0.1%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Global R: 0

Pairwise Tests
                R  Significance     Possible       Actual  Number >=
Groups  Statistic       Level %  Permutations  Permutations   Observed
WD, WC      0.503           0.1     252047376          999          0
WD, ED      0.275           0.3     252047376          999          2
WD, EC      0.261           0.2     252047376          999          1
WC, ED      0.274           0.2     252047376          999          1
WC, EC      0.304           0.3     252047376          999          2
ED, EC       0.13           3.7     252047376          999         36
```

## Trinidad

1) Sample Design:
    a. Treatment
        i. Reference: Site 2
        ii. Discharge: Site 1
    b. Other Strata
        i. Region (tide zone): Low, Mid, High (not significantly different, p = 0.35)
        ii. Height above sand: Low, Mid, High
            1. Low vs Mid (p = 0.35)
            2. Low vs High (p = 0.004)
            3. Mid vs High (p = 0.003

2) Analytical Design
    a. Species that are counted
        i. Bray-Curtis Ordination on fourth root transformation.
        ii. Region (tide zone): Low, Mid, High (not significantly different)
        iii. Height above sand: Low, Mid, High
            1. Low vs Mid (p = 0.35)
            2. Low vs High (p = 0.004)
            3. Mid vs High (p = 0.003
        iv. Comparison of Treatments using Anosim: Reference found to be different from Impact at **P<0.002**

```
TESTS FOR DIFFERENCES BETWEEN location GROUPS
(across all Height groups)
Global Test
Sample statistic (Global R): 0.071
Significance level of sample statistic: 0.2%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Global R: 1
```

[redacted]

    b. Species that are sampled by percent cover
        i. Bray-Curtis Ordination on fourth root transformation.
        ii. Region (tide zone): Low, Mid, High (not significantly different, p = 0.65)
        iii. Height above sand: Low, Mid, High
            1. Low vs Mid (p = 0.027)
            2. Low vs High (p = 0.028)
            3. Mid vs High (p = 0.20

iv. Comparison of Treatments using Anosim: Reference found to be different from Impact at **P<0.009**

*TESTS FOR DIFFERENCES BETWEEN Site GROUPS*
*Global Test*
Sample statistic (Global R): 0.063
Significance level of sample statistic: 0.9%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Global R: 8

[redacted]