AQUATIC INVERTEBRATES

OF THE DEVEREUX SLOUGH

2018-19

SB AUDUBON – NCOS – COPR
DEVEREUX SLOUGH MONITORING PROJECT

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I. ABSTRACT

In 2018, the hardscape construction of NCOS (North Campus Open Space), a restored, closed estuary, wetland on the Northern border of COPR (Coal Oil Point Reserve), was completed; thus, approximately doubling its overall size and offering the rather unique opportunity of being able to compare the well-established COPR wetland with the newly constructed, adjoining, NCOS wetland.

Basic water quality and aquatic invertebrate monitoring data collection of both sites were undertaken to help better understand the dynamics of how a newly constructed wetland develops into an established wetland and to establish a baseline for future monitoring.

Aquatic invertebrate sampling protocols were evaluated indicating that sampling in algae gives more than an order-of-magnitude greater abundance and diversity than sampling in open water and that the Filtered Beaker method gives more precise species density information than the Sweep-Net method; when sampling at shallower depths where the Sweep-Net is not fully submerged. Additionally, there are significant issues with how benthic samples are traditionally collected and analyzed.

Four taxa are the more significant contributors to the total taxa observed –Copepods, Ostracods, Cladocera, and Corixidae. Additionally, we found Oligochaete, Chironomids, Nematodes, and Ephydridae in significant abundance.

The type and number of invertebrates collected are evaluated in terms of site, salinity, and location in the sampling column (planktonic or benthic).

Additionally, the effect on other aquatic invertebrates of the use of VectoBac for mosquito abatement was looked at – indicating a minimum, if any, effect on non-Culicidae taxa.

II. INTRODUCTION

The Santa Barbara Audubon Society has undertaken to support the management teams of the North Campus Open Space (NCOS) and Coal Oil Point Reserve (COPR) by developing and implementing a routine water quality and aquatic invertebrate monitoring program based on a Citizen Science approach. Taking a Citizen Science approach makes the program affordable, while simultaneously providing an opportunity for greater student involvement in research, environmental protection, and project management.

The Devereux Slough is an important birding hotspot in the Santa Barbara area (Audubon IBA). Audubon is deeply interested in aiding COPR and NCOS in maintaining it as a 'healthy' habitat for birds. The abundance and diversity of birds at COPR and NCOS is impacted by the abundance and diversity of invertebrates. Many birds feed on invertebrates directly, or indirectly through consumption of something which feeds on invertebrates. Combining water quality with aquatic invertebrate monitoring is an attempt to develop a process to quantitatively evaluate the 'health' of the Slough.

The goals are one, to broaden the factors being monitored over time to create more comprehensive understanding of the interrelated relationship inherent in the ecology of the slough; and perhaps, produce better figures of merit to aid in its adaptive management. And two, to provide a platform for students to get experience in scientific research and project management as they, in time, take leadership roles in the program.

This monitoring also aides in observing the development of the NCOS system by comparing it with the more established COPR system. It is a rather unique situation to have a totally reconstructed landscape come into being on the border of an established one and have the opportunity to track the various plant and animal trajectories as they eventually fully combine into one ecosystem.

Our program consists of UCSB undergraduate volunteers, with majors generally ranging from environmental studies to various branches of biology, some of whom become paid interns who aid in recruiting and training new volunteers; as well ensure protocols and daily procedures run smoothly.

The primary roles of the volunteers are to clean the samples of plant matter and other debris, and to identify, count and record all of the organisms contained in their sample. Then, specific interns check both the discarded ‘debris’, and the counted sample to help ensure data accuracy.

As intermittently open estuaries, such as the Devereux Slough, are not well-studied and COPR and NCOS adjoin the UCSB campus, a rare opportunity is provided for this valuable UCSB student research project.

III. OBJECTIVES

1. Generate data to aid in the management of the NCOS and COPR Estuary-Slough.
2. Generate data in a cost-effective manner; where ‘cost’ also includes the human and infrastructural resources required.
3. Develop a largely self-sustaining undergraduate program to collect and analyze the data. The two-part goal of which is to relieve COPR and NCOS staff from day-to-day management, while simultaneously providing an opportunity for UCSB undergraduates to gain project and data management experience in a scientific context.

IV. SAMPLING PROTOCOLS

Background

The goal of the sampling is to collect a representative sample of the taxa within the habitat being sampled. This is mitigated by practical concerns such as money, time, degree of expertise required, and, perhaps, damage to the site being sampled.

Three different techniques were explored and used: Sweep Net (for planktonic invertebrates and insects), Filtered Beaker (for planktonic invertebrates), and Core (for benthic invertebrates). There are trade-offs involved with each.

Water Column Sampling

Sweep Net – Initially, we began in 2017 with a D-net, 30.5 cm wide and 24 cm high with 1000 um filtering mesh (face area = 640 cm^2), and using five standard sweeps, one-meter long (volume sampled = 320 liters if net is fully submerged). However, in shallower waters, the net is seldom fully submerged; hence the volume sampled is often variable, and difficult to determine. Also, there can be quite some variance, in practice, when trying to make a standard sweep of one-meter length.

Secondly, we found significant taxa down to below 250 um and felt that filtering with a 1000 um mesh did not give an adequate representation of the existing taxa given the resolving power of our dissection microscopes, capabilities of our student volunteers, and the capabilities of dabbling ducks to consume these smaller invertebrates.

Fig. 01 Sweep Net & Filtered Beaker

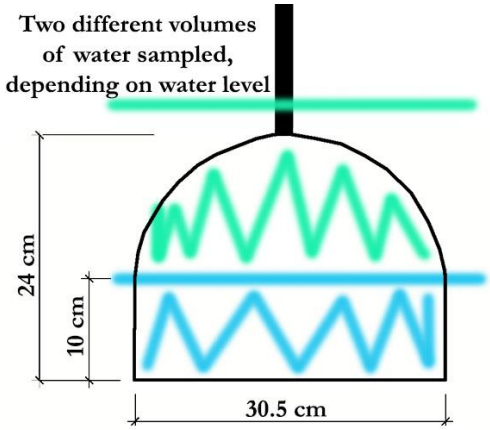


Fig. 02 Water Volume Sampled Problem

Another difficulty with the sweep net protocol is that it is difficult to use within algae – as either one entrains significant algae, which greatly complicates sorting, or, in pushing the algae down, so as not to entrain it, one also pushes the invertebrates down as well. So, as the seasonal algae grew up within our sampling sites, we were forced to choose whether to move into deeper, more algae-free water or find another protocol that would enable us to sample within the algae.

A comparison of results from sampling within the algae, using the Filtered Beaker Method, over sampling just outside of the algae, using either the Sweep Net or Filtered Beaker Method, revealed that there were around 30 times greater numbers of taxa collected within the algae, as well as a greater diversity of taxa, than in sampling outside of the algae.

This led us to abandon the Sweep Net and to use the Filtered Beaker Method for general water column sampling.

Filtered Beaker – The two main advantages of the filtered beaker method are: one, a fairly accurate, repeatable water volume is sampled; allowing one to more accurately compare results from one site or time with another site or time; and two, the smaller sampling cup size allows one push the cup down within algae or other vegetation and take a sample without entraining much, if any vegetation.

By modulating the orientation of the cup, one can sample from any reachable depth. The concern expressed by some that it will tend to miss the faster creatures has not been observed; although we have not done rigorous testing of this hypothesis.

Observing nature, herons (filtered beakers) have no difficulty catching fast fish or gophers; so, with proper technique, we feel that human powered filtered beakers should manage.

Fig. 1 Filtered-Beaker Method – Collecting the Sample

One problem with both the sweep net and filtered beaker is that they are selective for taxa that are free swimming in the water column at the time of sampling. Taxa like Chironomids or Ephydridae, which cling to the vegetation, are less likely to be caught. Including the vegetation in the sample is so far too time consuming to clean, roughly a minimum of 10 times longer; which is presently beyond our resources to deal with.

Benthic Sampling

CORE Protocol – For this study, every three months at each viable site (i.e. covered with water), A CORE sample was taken using a 5 cm diameter, PVC pipe pushed 5 cm into the substrate of the site being sampled. Using a twisting motion, coupled with sliding

one's fingers over the bottom of the pipe, a 5 cm long x 5 cm diameter 'core' sample of the bottom substrate was obtained.

This sample plug has a volume of 0.09 liters (compared to 7.5 liters for the filtered beaker protocol and nominally about 300 liters for the sweep net protocol).

This sample plug was then dissolved between our fingers in a small bucket of previously filtered water. When well mixed, the solution was quickly poured through a 250um filter and the captured results were rinsed into a sample vial using 70% isopropanol.

Generally, benthic samples are taken from mud, or other substrate, at the bottom of the water column. This typically includes the surface of the substrate plus some inches of depth into it. There are a number of issues about how the samples are collected and reported.

One basic issue is that there is typically not a clean demarcation between where the water column ends and the benthic begins – does the benthic include the leaf litter, with its inherent voids that seemingly shelter a plethora of water column invertebrates?

So far, this is included in our CORE samples. However, it is likely that perhaps 90% of taxa recorded are living in the top 5 mm of this 5 cm long plug of substrate.

If true, then the already large taxa densities, reported in our Benthic samples, are even 10 times greater than reported for that 5 mm portion. So, around 150X more taxa density; rather than the around 15X more taxa density that we obtained for the comparable Planktonic sample.

This begs the adoption of another type of protocol that, perhaps, vacuums up the litter at the bottom without entraining significant water column sample. (Perhaps a cap with an array of nozzles that squirt a known amount of filtered water (say one-liter) onto that Benthic-Planktonic layer, while the resultant cloud of material is vacuumed up through a larger central orifice.)

Another issue is that benthic results are typically reported as taxa per unit area; rather than taxa per unit volume; even though the benthic samples are clearly three-dimensional with the depth being quite comparable to the width and height.

It is then stated that one cannot compare benthic results with water column results because the units, $\#/m^2$ for benthic do not match with the units, $\#/m^3$ for water column.

Essentially, first the depth data is thrown away and then it said that the comparison cannot be made because the units no longer match; even though the typical benthic sample is inherently three-dimensional. This does not seem reasonable. In our case, keeping the measurements in units #/Liter allowed us to discover that our Benthic samples contain more than an order of magnitude higher taxa density than found in our Planktonic samples – an interesting discovery.

Normalizing the data to 'Numbers per Liter' allows us to both compare our data for Benthic to Planktonic and to data sampled with different volumes.

A ***third issue is that the volume of the benthic sample is small relative to the volume of the planktonic samples***, resulting in a greater amount of scatter in the number and types of benthic taxa found. The issue is similar to the problem of Rarefication; only instead of # of samples required to give a stable # of species, here it is the Volume of the sample required.

Increasing the Benthic volume sampled has two problems:

1. One, as it is, benthic samples are much more time-consuming to clean and sort than water column samples. Increasing the volume sampled would swamp our volunteer resources.
2. Two, if we sampled that much volume regularly, at a given site, it would likely damage the site due to the much greater disturbance that benthic sampling creates.

A possible solution to this third issue would be to place an array of plates on the bottom at site and just pull up selected plates each time one samples, minimally disturbing the surrounding ones, keeping track of when and which ones were removed so that no one plate would be checked say more than once a year. However, while giving a better measurement of the Benthic-Planktonic interface, this would not be a true Benthic Sample extending into the substrate.

This leads to the larger issue of "How best to use NCOS as a learning laboratory without significantly damaging it or stressing the existing wildlife?"

Water Quality - Invertebrate Water Quality Sampling

For the invertebrate sampling in shallow water, the YSI 2030 probe is held horizontally and 10 cm below the surface of the water. It is waved gently (about 5cm per second velocity), while the DO (Dissolved Oxygen), Conductivity, Temperature, and Barometric readings are taken.

Additionally, using a separate meter, the pH is measured.

The rationale behind just sampling the top 10 cm of the water column is that, most of the invertebrates, being mobile, can travel to this likely more-oxygenated water and

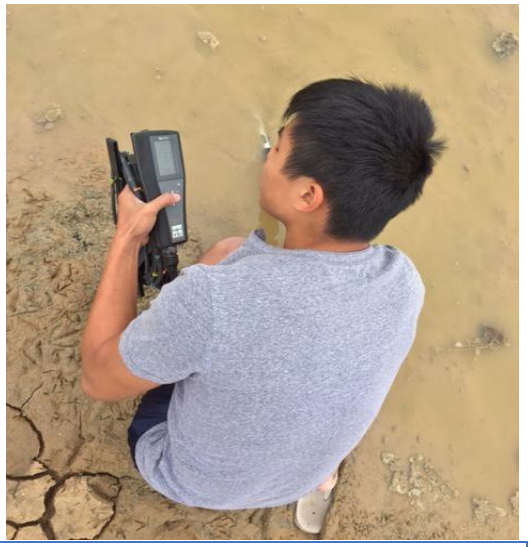


Fig. 4 Invertebrate Water Quality Sampling.

therefore, more habitable region of the water column. From there they can expand to, or contract from, other areas as conditions vary. While this does skew the data, it skews it to a significantly large volume (the top 10 cm of the water), in ready reach of the birds and other consumers of invertebrates.

Before the readings are taken, the DO calibration is checked using the YSI quick-calibration procedure.

Salinity and pH calibration is done every 3 months using standard solutions.

V. SAMPLING LOCATIONS

The Devereux Slough consists of two portions: COPR, a relatively untouched closed estuary for at least the past 40 years, and NCOS, a newly reclaimed portion (2018), having been a golf course for more than 60 years, directly to the North and bordering on COPR.

In 2018, there were a total of twelve sample sites, four in COPR and eight in NCOS. These were intended to be representative of the different microbiomes of each region.

In 2019, these sites were expanded to include the Pier, NPB1, and NPB2. Additionally vernal pools, VOW1, NVP10, VWCB, VCC7, VDSW, VM3, VM7, and VMM were added for that year. For this report, only the data for the sites corresponding to the 2018 data are used. The additional vernal pool sites will be reported in a separate report focused on all of the vernal pools. The sites NPB1, NPB2, and the Pier will be included in this report in the future.

Description of Sites:

COPR

1. MO1 – Mouth of the Slough – saline to hyper-saline, shallow, sandy bottom.
2. CUL1 – Culvert exit on Slough Road – Part of main body of Slough water during wet portion of year – separate small hypersaline pond during dry portion of year. Appears to have water year-round. Clay bottom with shallow organic layer.
3. VBR1 – South (COPR) side of Venoco Bridge – clay bottom, about 0.6 to 1.2 m deep during year. Channel edged with pickle-weed. Top layer of water can be relatively fresh-to-brackish during rainy season, saline to hypersaline at bottom.
4. DSP – Dune Swale Pond – Seasonal, shallow, brackish-water pond with cattails along edge. Clay and organic sediment bottom.

NCOS

1. NVBR – North (NCOS) side of Venoco Bridge – Scraped-bare earth, clay bottom. Brackish near surface during and just after rainy season. Sampling site is about 30 meters across the road from VBR1 in COPR.
2. NEC – Slough-side of East Bridge – scraped-bare earth, clay bottom. Fresh-to-saline water depending on time of year.
3. NMC – Main Channel (during rainy season, sampled with kayak). Scraped-bare earth, clay bottom. Brackish-to-Saline depending on season.
4. NPB – Slough-side of Phelps Bridge – Entrance of Phelps Creek into Slough. Scraped-bare earth, clay bottom with some medium boulders. Fresh-to saline depending on rain.
5. NWP – West Pond – Scraped-bare earth, clay bottom. Fresh water pond.
6. NDC – Devereux Creek – Narrowed after filling in 1960s, narrow setting, clay bottom with some organic material at top. Fresh water.
7. NVP2 – Vernal Pool #2 – Scraped-bare earth, clay bottom. Seasonal fresh water pool. Formed in late 2018.
8. NVP4 - Vernal Pool #4 – Scraped-bare earth, clay bottom. Seasonal fresh water pool.

Fig. 5 2019 COPR and NCOS Sampling Sites

VI. SORTING PROTOCOL

Sorting is done to separate the invertebrates from the algae and general detritus, dividing the invertebrates into taxa while counting.

The process of sorting requires, at minimum, a dissection microscope, tweezers, pipet, petri dish(es), 70% isopropanol (or equivalent), a small, sample-vial, and a larger, 'debris' vial.

The volunteer takes a previously collected sample from the "To be Sorted" box, a larger, 'debris' vial for the detritus, and a small sample vial, containing 70% denatured ethanol, for the invertebrate specimens. Both the sample vial and the 'debris' vial are then labeled with the site name, date, and sampling technique used to collect the sample (e.g., CUL1, 20 MAR 19, CORE)

A portion of the sample is poured into a petri dish and looked at under the microscope. An iterative process then begins of either: separating the 'debris', using tweezers or pipette, into the 'debris' vial; or separating the taxa, while identifying, counting and recording them, pipetting or tweezering them into the sample-vial.

When complete, the debris-vial and sample-vials, along with a form containing the invertebrate-counts, are placed in the "To-Be-Checked" box.

A designated checker then reviews the vials and form to verify the accuracy. If acceptable, the debris-vial is discarded and the sample vial is stored in the designated cabinet. The data is then recorded in the log book – to be later uploaded into the database. There can be many months between sampling the specimens, analyzing them, and entering them into the database.

Meanwhile, the water quality data is entered into its own section of the database soon after being collected.

Once the specimen data is entered, it is then merged with the corresponding water quality data.

VII. INTRODUCTION TO RESULTS

While we began collecting data in 2017, we don't include it here because our sampling protocols evolving and were not directly relatable. As well, with our program attracting more volunteers, we realized near the beginning of 2018 that some of our volunteers were not only mis-identifying some of the taxa, but were not even seeing some of them, and were discarding them with the debris.

At that point we implemented the protocol of saving the debris and having an experienced person check both the debris and the sample before logging the data. This boosted the accuracies of identification and counting and, as well, allowed for directed feedback to aid in the volunteer's learning process.

Thus, we have limited this report to the calendar years 2018 and 2019. Covid-19 curtailed the program in 2020 and we are still (2021) recovering from that impact.

VIII. RESULTS

Taxa Abundance

To create some ad hoc baseline against which to compare the results for the individual sites, the results of all the 2018 sites were averaged by individual taxa for both 2018 and 2019. The most common taxa are displayed in order of their abundance, for both the filtered beaker (planktonic) method and the CORE (benthic) sampling protocols.

Most Common Taxa Using Filtered Beaker Protocol

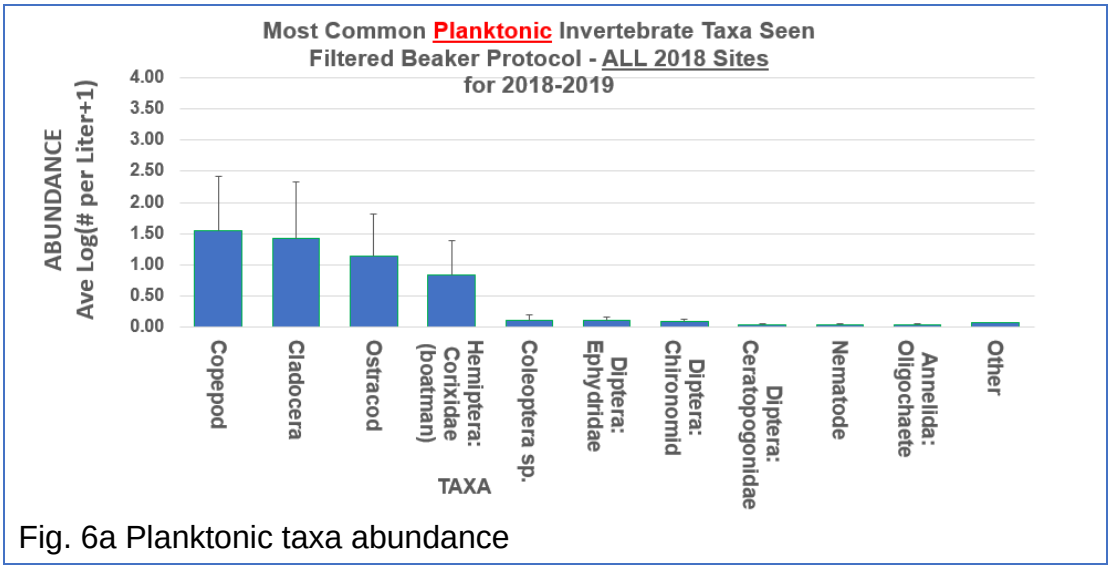


Fig. 6a Planktonic taxa abundance

Most Common Taxa Using CORE Protocol

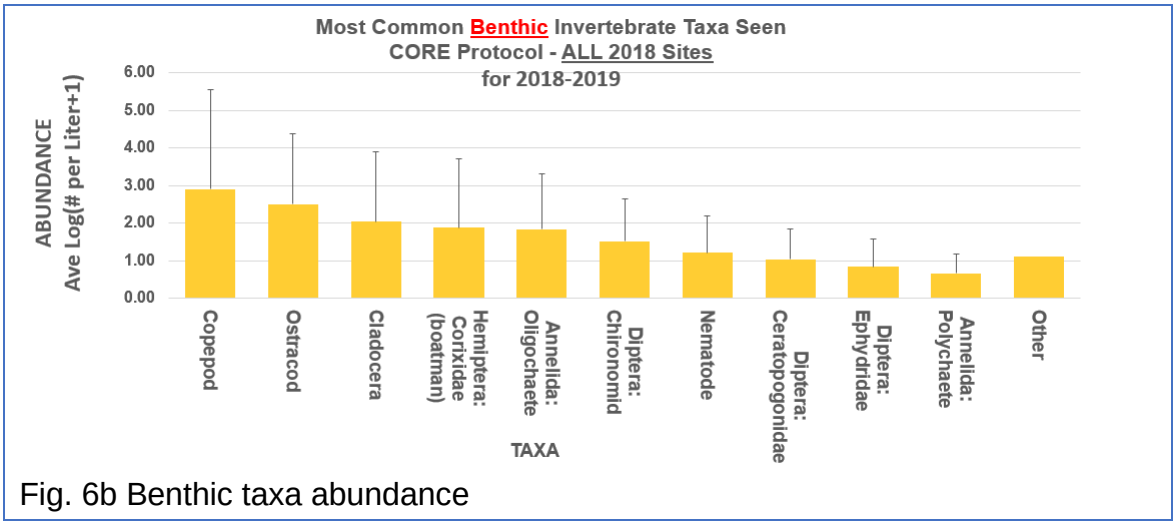


Fig. 6b Benthic taxa abundance

Planktonic v Benthic Sampling Protocols v Taxa Abundance Distribution

The charts in Figs. 6a & 6b display the general trends found in both the 2018 and 2019 data.

- Four major taxa are found: Copepod, Ostracod, Cladocera, and Corixidae; however, with the Benthic, there is a gradual decline with abundance of other, significant taxa.
- Planktonic – There is a rather abrupt fall-off in abundance after the first four taxa.
- Benthic – much greater abundance (concentration) (generally greater than 15X and as much as 950X) and more taxa of significant abundance than with the planktonic. Considering that most of the nominally Planktonic taxa found in the Benthic samples likely exist in the top 5mm of the 5cm CORE plug of substrate, this greater abundance (concentration) may be as much as 150X greater (or more) for the Benthic samples.

In looking at the results shown in Figs. 6a and 6b, one notices both a higher concentration and larger distribution of taxa. While one would expect that taxa such as the Annelida would be more prevalent in the Benthic than the Planktonic; both the larger concentrations of the Copepod-Ostracod-Cladocera-Corixidae and the Chironomid-Ceratopogonidae-Ephydridae groups would not be expected to be such dwellers in the mud.

As we see significant numbers of the Copepod-Ostracod-Cladocera-Corixidae group in both the Planktonic and Benthic samples, the differences in concentration (roughly 15X greater in the Benthic) could be attributable to lifestyle (they prefer the possibly greater protection and food available amongst the bottom litter).

However, while we see roughly the same relative rankings for the Chironomid-Ceratopogonidae-Ephydridae group in both the Planktonic and Benthic samples, that we see roughly 23-to-124X more in the Benthic is perhaps due to their propensity to cling to vegetation and thus be harder to gather with either the sweep net or filtered beaker methods.

This leads to the question about ‘degree of accuracy’ v ‘amount of resources or expense required’ – a question to keep in mind as the costs of monitoring continue to decrease. While individual entries vary, these general patterns remain with most of the sites. The full results are in the appendix.

In the future, it would be worthwhile to explore the actual distribution of the taxa within the CORE plug. This illustrates the importance of the points brought up in the Sampling Protocol discussion – keeping the result units in numbers per Liter or m3, normalizing the data, seeing how the taxa are distributed vertically, and perhaps discovering a sampling protocol that truly only looks at the surface of the benthic.

Taxa Abundance by Site

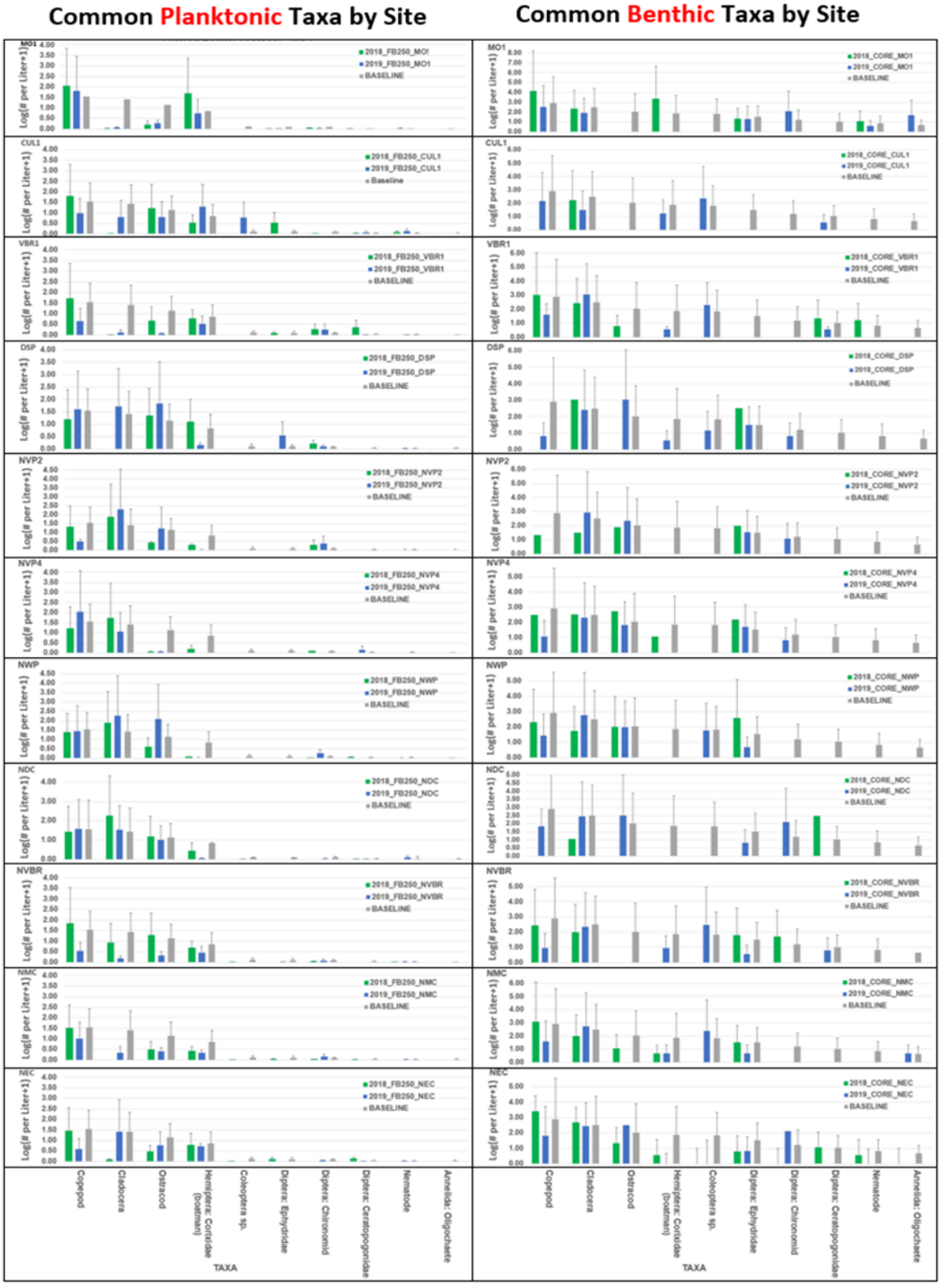


Fig. 7 All Abundance v Site Data

Taxa Abundance by Salinity

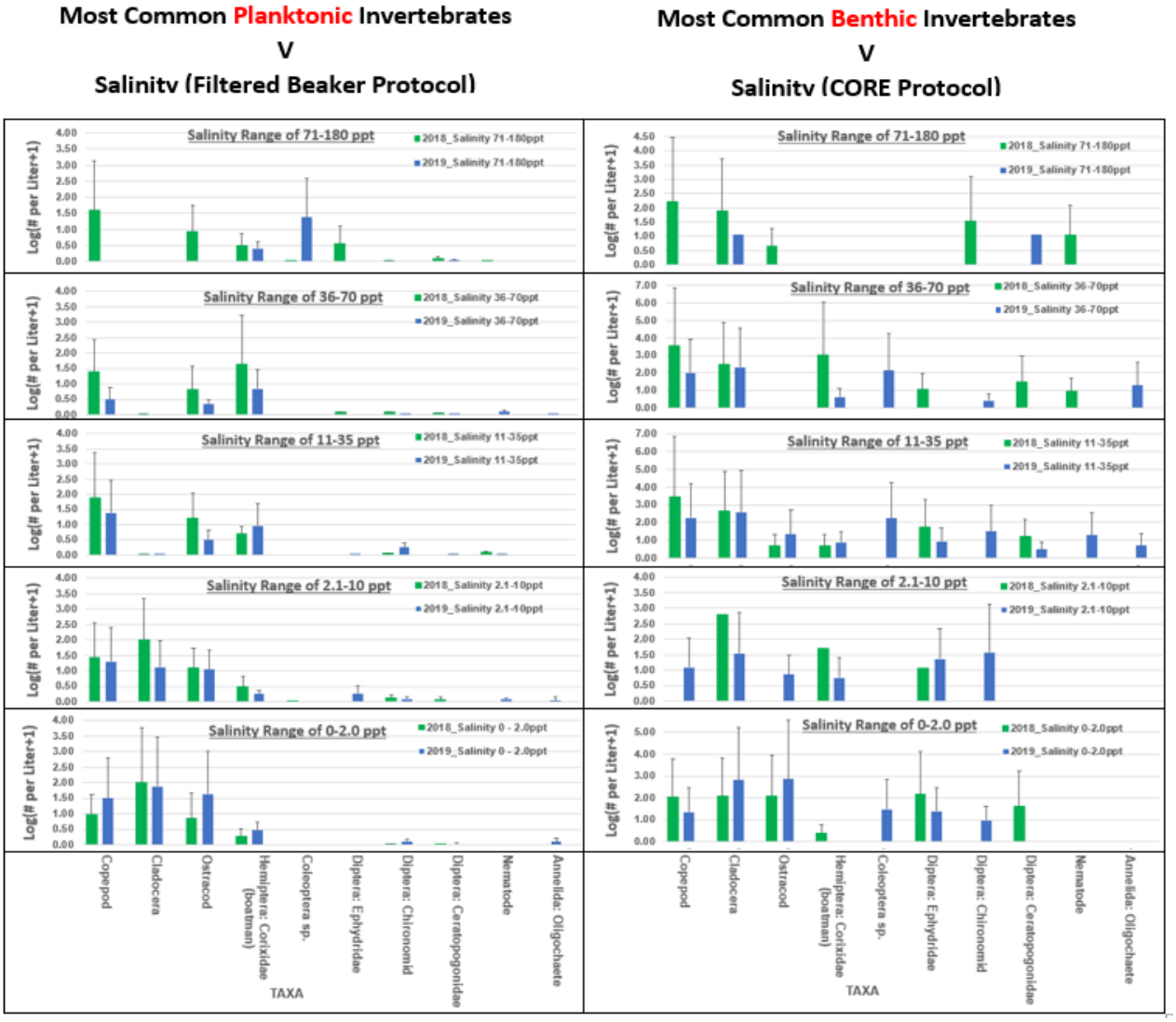


Fig. 8 Planktonic and Benthic Taxa Distribution v Salinity

With the Planktonic samples, the degree of salinity has a pronounced effect on some taxa, particularly Cladocera; with the trend being 'Less abundance with greater salinity'; however, there seem to be some exceptions.

However, with the Benthic samples, generally, there seems to be little correspondence between abundance and salinity, or even that the greater abundance occurs with sea water salinity and slightly higher and less abundance with brackish or hyper-saline conditions. (Note: freshwater is nominally 0 to 2 ppt salinity and seawater is nominally 35ppt.)

It should be again noted that, at present, the Benthic sample size is probably too small in volume to give consistent results; especially given that many of the invertebrates tend to 'clump' together, resulting in what is likely a rather "hit or miss" result.

In any case, more clarity could come with a couple of more years of data.

The more meta-results, shown in Fig. 7 are still puzzling in that some of the same taxa are represented in both groups, planktonic and benthic, with the benthic versions having one or two orders-of-magnitude higher densities. ***Copepods and Cladocera do not seem to be well equipped for burrowing into the mud and if they are just residual catch from the water column, why are their true densities consistently so much greater than the water column itself?***

This could be because of the way the Benthic is commonly sampled, collecting what is arguably the bottom of the planktonic with the actual ground matrix; where the bottom of the planktonic is where many of the planktonic taxa prefer to exist.

It may also be that some of planktonic taxa also go dormant in higher levels of salinity. This could be checked by collecting live samples (not put in alcohol) and seeing if they are moving around or not.

This study is a broad-brush investigation into first order relationships; which could present opportunities for more detailed investigations. These are some interesting research topics. It would, perhaps, be good to clean up some of the difficulties with the benthic protocol (discussed above in 'Protocols') and get another few years of data before spending a lot of time trying to minutely analyze this.

Taxa Abundance by Sampling Protocol

Filtered Beaker v Sweep Net

250um v 500um filter

Sampling in Algae v Open Water

A number of questions arose during the first year of sampling (2017) regarding protocols and how specific protocols might be influencing, or skewing, the data. For example, how will the results be different using the sweep-net v the filtered beaker protocol? Will using a 500um mesh give significantly different results from using a 250um mesh? Will sampling outside algae patches give different results than sampling within algae patches. In June 2018 an attempt to get some handle on these issues was made by conducting the following matrix of test to more '*accurately indicate*':

1. The relationship of the results, if any, between the Sweep-Net and the Filtered-Beaker methods.
2. The degree of difference between the results of samples taken within the algae to samples taken outside of the algae.
3. How the results differ between using a 500um mesh and a 250um mesh filter.
4. How taking the same type of sample 2 meters (and 10 minutes) apart would affect the results.

5. How taking the same set of samples 30 meters (and 90 minutes) apart would affect the results.

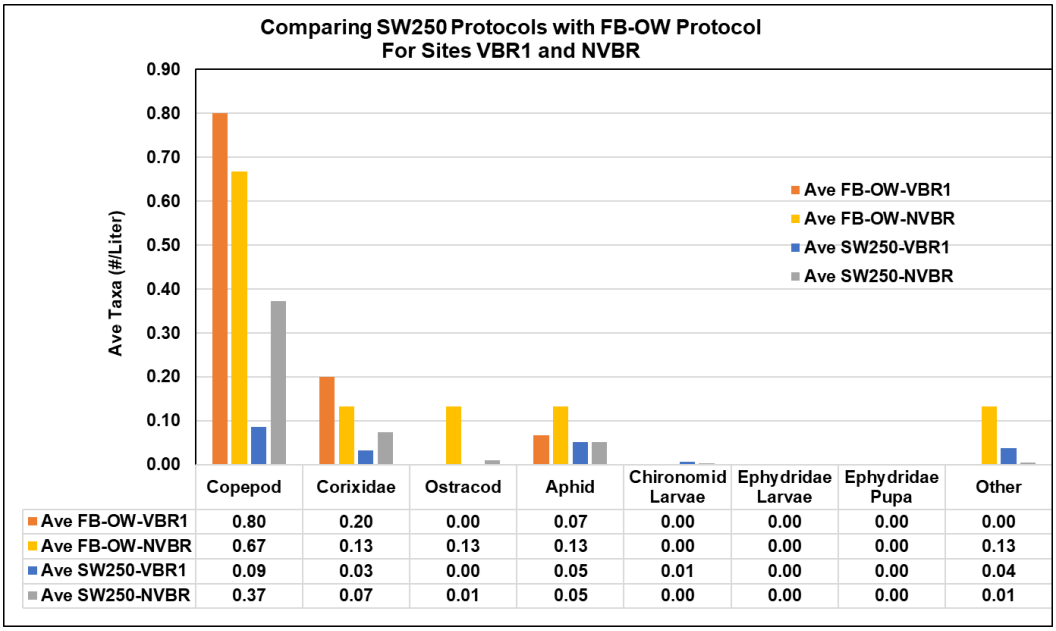
The Protocol Matrix Test - 2018

A total of sixteen samples were taken, eight on the COPR (South) side of the Venoco Bridge and eight on the NCOS (North) side – the waterway being connected below the bridge. The COPR side representing the established wetland and the NCOS side representing the newly created wetland.

The eight samples from each side were broken down as follows:

1. SW500-1 - 500 um Sweep Net in Open Water
2. SW500-2 - 500 um Sweep Net in Open Water 2 meters from SW500-1
3. SW250-1 - 250 um Sweep Net in Open Water – 2 meters from other SW's
4. SW250-2 - 250 um Sweep Net in Open Water – 2 meters from other SW's
5. FB250-OW1 - 250 um Filtered Beaker in Open Water – 2 meters from other samples
6. FB250-OW2 - 250 um Filtered Beaker in Open Water – 2 meters from other samples
7. FB250-Algae1 - 250 um Filtered Beaker in the Algae
8. FB250-Algae2 - 250 um Filtered Beaker in the Algae 2 meters away

The results in Figure 10a show the ***Filtered Beaker protocol obtaining roughly two to four times the taxa/liter as with the sweep net***. The larger taxa density obtained using the filtered beaker protocol is possibly due to the fact that the filtered beaker samples were taken in the top 40cm of the water column; while the sweep net samples were taken throughout the full 100 cm depth of the water column (the sweep net pole being longer than my arm). At the Venoco Bridge, the water near the bottom tends to be anoxic; thus, possibly having a lower density of taxa.

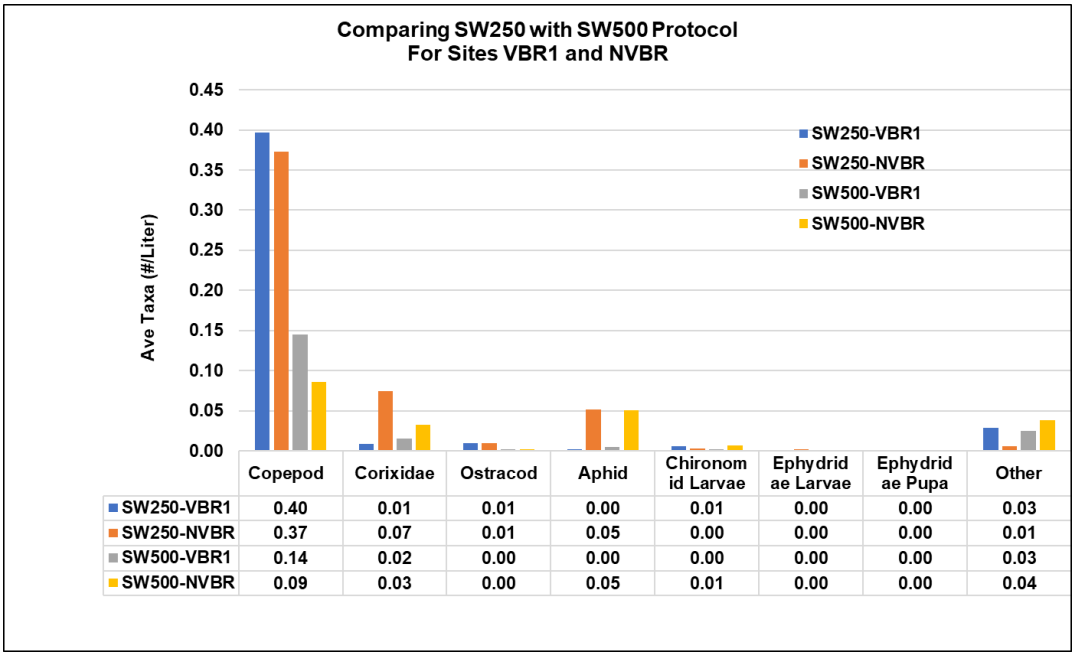


	Copepod	Corixidae	Ostracod	Aphid	Chironomid Larvae	Ephydridae Larvae	Ephydridae Pupa	Other
Ave FB-OW-VBR1	0.80	0.20	0.00	0.07	0.00	0.00	0.00	0.00
Ave FB-OW-NVBR	0.67	0.13	0.13	0.13	0.00	0.00	0.00	0.13
Ave SW250-VBR1	0.09	0.03	0.00	0.05	0.01	0.00	0.00	0.04
Ave SW250-NVBR	0.37	0.07	0.01	0.05	0.00	0.00	0.00	0.01

Fig. 10a Comparing Sweep Net to Filtered Beaker results

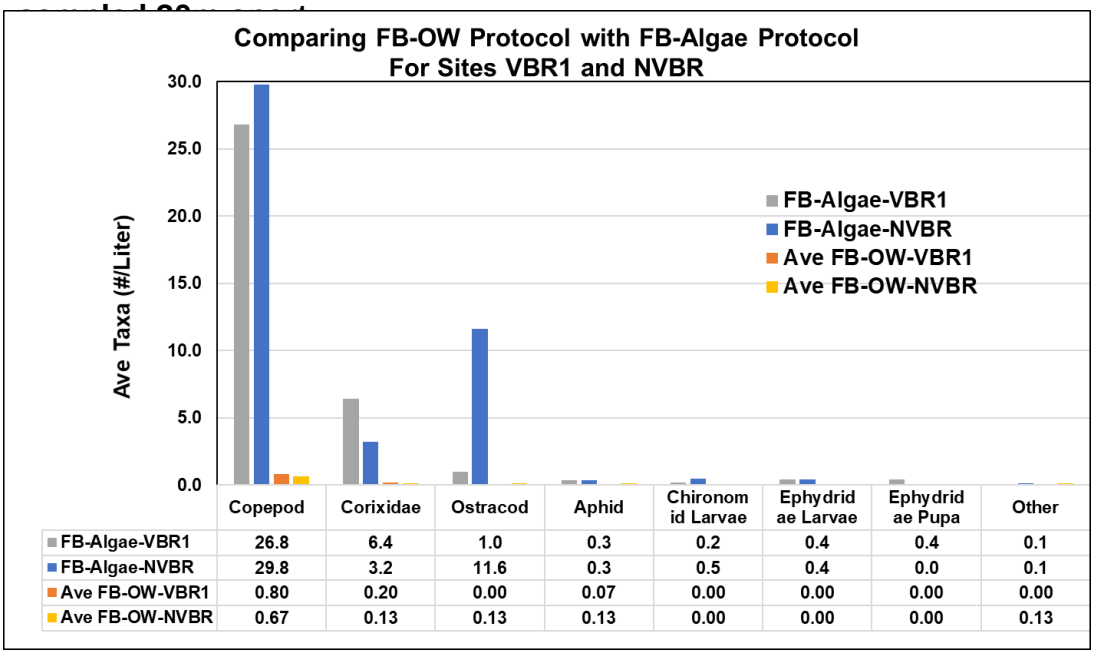
Figure 10b shows the major difference between the 500um mesh and 250um mesh results are with the taxa that span that difference in range (Copepods and Ostracods); with approximately ***4x more Copepods and 6x more Ostracods being collected with the 250um mesh***. The numbers of larger invertebrates are largely unaffected by this difference in mesh size – as would be expected.

The difference in the results of sampling 30m apart was significant; but somewhat random. (With larger sample sizes, the NCOS side of the Bridge does show greater abundance/liter.) It tends to support our ad hoc observations that the invertebrates tend to exist clumps, contributing to significant scatter in the data.



	Copepod	Corixidae	Ostracod	Aphid	Chironomid Larvae	Ephydridae Larvae	Ephydridae Pupa	Other
SW250-VBR1	0.40	0.01	0.01	0.00	0.01	0.00	0.00	0.03
SW250-NVBR	0.37	0.07	0.01	0.05	0.00	0.00	0.00	0.01
SW500-VBR1	0.14	0.02	0.00	0.00	0.00	0.00	0.00	0.03
SW500-NVBR	0.09	0.03	0.00	0.05	0.01	0.00	0.00	0.04

Fig. 10b Comparing 250um to 500um mesh results and results



	Copepod	Corixidae	Ostracod	Aphid	Chironomid Larvae	Ephydridae Larvae	Ephydridae Pupa	Other
FB-Algae-VBR1	26.8	6.4	1.0	0.3	0.2	0.4	0.4	0.1
FB-Algae-NVBR	29.8	3.2	11.6	0.3	0.5	0.4	0.0	0.1
Ave FB-OW-VBR1	0.80	0.20	0.00	0.07	0.00	0.00	0.00	0.00
Ave FB-OW-NVBR	0.67	0.13	0.13	0.13	0.00	0.00	0.00	0.13

Fig. 10c Comparing Open Water Sampling to Within Algae Sampling Results

Figure 10c illustrates the crux of the issue - of how choice of sampling protocol affects the results. Sampling the water within the algae (but excluding the algae) results in both

a ***more diverse*** sample as well as ***around 30x more abundance*** (on the basis of this limited sample-size-test).

Consequently, due to the greater accuracy of determining the sample volume, the possibility of easily increasing the sample volume to be comparable to the sweep net protocol, and the ability to work close-in with vegetation, we plan to focus on the filtered beaker protocol in the future.

Planktonic v Benthic Sampling Protocols v Taxa Abundance Distribution

In looking at the results shown in Figs. 6a and 6b, one notices both a higher concentration and larger distribution of taxa. While one would expect that taxa such as the Annelida would be more prevalent in the Benthic than the Planktonic; both the larger concentrations of the Copepod-Ostracod-Cladocera-Corixidae and the Chironomid-Ceratopogonidae-Ephydridae groups would not be expected to be such dwellers in the mud.

As we see significant numbers of the Copepod-Ostracod-Cladocera-Corixidae group in both the Planktonic and Benthic samples, the differences in concentration (roughly 15X greater in the Benthic) could be attributable to lifestyle (they prefer the possibly greater protection and food available amongst the bottom litter).

However, while we see roughly the same relative rankings for the Chironomid-Ceratopogonidae-Ephydridae group in both the Planktonic and Benthic samples, that we see roughly 23-to-124X more in the Benthic is perhaps due to their propensity to cling to vegetation and thus be harder to gather with either the sweep net or filtered beaker methods.

A point of reference is that with the Oligochaete and Polychaete group, they are 957X and 167X more likely to be in the Benthic group – as one might expect, being in the Annelida.

This leads to the question about ‘degree of accuracy’ v ‘amount of resources or expense required’ – a question to keep in mind as the costs of monitoring continue to decrease.

IX DISCUSION OF RESULTS

Figures of Merit

Complex systems such as an automobile, a large corporation, the world economy, or an ecosystem have 'Figures of Merit' to help people, who do not have all the specific knowledge to all the detailed information available to evaluate such a system.

For an automobile, one has miles/gallon (city and highway), 0-60mph, braking distance, turning radius, etc. For a corporation, there is Price/Earnings, Price/Revenue, Price/Book, Short Ratio, etc. For the economy there is GDP per capita, Balance of Trade, Debt as a function of GDP, percent literacy, etc. These figures, while neither stand-alone or particularly precise, when taken together, paint a picture that is often much more helpful than not in evaluating the system – hence their near ubiquitous use.

For the general ecosystems, it is recognized that greater Abundance and Diversity equate to a more stable, therefore 'healthier' system.

The Shannon-Wiener attempts to measure the diversity of taxa in a standard manner. The higher the number, the greater the diversity. Along with the Shannon Index is the Evenness index; or a measure of how uniform the various taxa are in number – is there one dominant species or many taxa, similar in number? Again, the larger the number, the more uniform the distribution.

In the future, it would be interesting to work out other Figures of Merit that would allow us track the variables that are most influencing Abundance and Diversity in the slough.

Applying the Shannon-Wiener Index to our data, we get the following:

Shannon-Weiner Index Comparisons	# of Sites Sampled - 2018	# of Species - 2018	# of Individuals/Liter - 2018	Shannon Index - 2018	Evenness - 2018	# of Sites Sampled - 2019	# of Species - 2019	# of Individuals/Liter - 2019	Shannon Index - 2019	Evenness - 2019
All Sites - Planktonic	12	16	93	**1.19**	0.41	12	25	68	**1.37**	0.43
All Sites - Benthic (CORE)	12	10	2,392	**0.90**	0.39	12	18	773	**1.60**	0.55
COPR- Planktonic	4	13	99	**0.93**	0.34	4	24	65	**1.46**	0.46
NCOS - Planktonic	8	13	90	**1.06**	0.41	8	22	71	**1.19**	0.38
COPR - Benthic (CORE)	4	7	5,018	**0.68**	0.31	4	14	961	**1.57**	0.56
NCOS - Benthic (CORE)	8	10	1,371	**1.01**	0.44	8	16	642	**1.55**	0.54

Fig. 11 Shannon-Wiener Diversity Index and Evenness

Shannon-Wiener is useful for comparing various results from various ecosystems; but perhaps a more intuitive representation of the data for COPR and NCOS would be to just list the # of Species each year (and their site distribution), and their abundance in # / Liter / sample?

Some other comparisons of interest:

Interesting Comparisons	# of Species - 2018	# of Species - 2019		# of Individuals/Liter - 2018	# of Individuals/Liter - 2019
All Sites - Planktonic	16	25		93	68
All Sites - Benthic (CORE)	10	18		2,392	773
COPR- Planktonic	13	24		99	65
NCOS - Planktonic	13	22		90	71
COPR - Benthic (CORE)	7	14		5,018	961
NCOS - Benthic (CORE)	10	16		1,371	642

Fig. 12 Other Interesting Comparisons

Figure 12 shows a rather strange set of relationships:

1. In all cases, the number of different taxa increased from 2018 to 2019
2. In all cases, the abundance of taxa (# of individuals/Liter) decreased from 2018 to 2019

To some degree, the increase number of distinct taxa could, in part, be explained by a better trained volunteer group being able to do the identification more discerningly; but the increase is roughly the double, and is made up largely of easily identifiable taxa, so, I think there is a more organic reason(s). Perhaps the extra year gave the taxa a better foothold in NCOS and that bounty then flowed into COPR. However, this begs the question as to why they did not already exist in COPR – the established ecosystem?

That this increase in taxa was correspondingly accompanied by a decrease in abundance is puzzling. A better trained volunteer corps would likely find more numbers, not less. And the greatest drops occurred in COPR which is the more established and is downstream from NCOS; so, would have more water.

A few more years of data would be useful for a better understanding of the dynamics.

Necessity for Species-Level Identification

Figure 11a shows that there is a **total of 18 planktonic taxa and 10 benthic taxa, of significant quantity, *reported***. This is rather conservative. For example, we certainly have two and probably more than three Copepod species; as well, at least two or three Ostracod species, and at least two Cladocera species. There are a number of issues here:

- Some of our undergraduate volunteers are challenged to distinguish between a Copepod and debris at first, much less, which kind of Copepod; so, going to the species level with Copepod is not readily possible at this time.
- Given that the major goal of this research is to begin to quantify the 'health' of this ecosystem, does it significantly matter whether it is this Copepod or that Copepod (or this Ostracod or that Ostracod) – given their relative ecological niches? In other words, would the resources required for the additional accuracy be justified by the benefit obtained? At this point, we feel the answer is "no". If the choice is between 80% accuracy and no data (because it is too difficult to get, and say 95% accuracy, then, at this point, we think that 80% accuracy or better, is acceptable.
- However, when using the Shannon-Wiener Diversity Index, that we have at least 23 different planktonic taxa, rather than 18 could be significant.
- Additionally, that with Copepods, we are at the level of 'Subclass'; with Ostracod, 'Class'; and with Chironomid, 'Family'; etc. The question becomes, "For the results to be truly meaningful, do we need to do our comparisons at, say, the 'Class' level? This begs a larger question, "Are these classifications particularly relevant to the ecological niche of the particular creature or are they mostly useful for assigning a name to a particular creature?" My feeling is that, due to a lack of 'Complete Knowledge' there is an unavoidable ambiguity here. Practically, we simply need a way to assign the best name that we can to a particular creature and work out, generally, what roles that creature plays in the ecosystem. In other words, does a dabbling duck really care much if it is eating a Cladocera or a copepod, much less which kind of copepod? And, if it did, could it actually separate them out when eating?

Taxa Abundance by Sampling Protocol

Combining the results shown in Fig. 7 with those in Fig. 8 raises larger procedural questions. *"If the densities of taxa are greater for benthic samples than for planktonic samples, shouldn't we be sampling the benthic more frequently?"*

This comes down to *"How to optimize the given monitoring resources to collect the most useful sets of data?"*

And that comes down to: *"Given what we now know, what is the ranking of most useful data?"*

Initially, it took say three-to-five times more effort to process benthic samples compared to aquatic samples due to the amount of debris entrained in these samples; hence, not knowing the relative specimen densities at that time, we decided to sample the easier-to-process planktonic samples more frequently.

Taking a step back, given an increase in size and efficiency of our volunteer workforce, is it better to sample the invertebrates more precisely or expand our efforts to include also sampling the algae and looking at 'who is eating what' using DNA identification techniques?

However, as noted in the Protocol Section, benthic sampling would be rather destructive to the site if done more frequently, especially if the volume of the sample were to be increased by sampling multiple places at each site, each time. Feedback on these questions is very welcome. We see benthic sampling as a work in progress.

Culicidae v Vector Control

The question arose whether the substance that Santa Barbara Mosquito & Vector Management was applying in the Slough would be adversely affecting the larger invertebrate population.

In researching the substance, VectoBac, the literature claims that it is a bacterium highly specific to mosquito larvae (Culicidae). In our data, we only saw small incidences of Culicidae:
Devereux Creek, Planktonic, 04 June and 30 Dec 2018,
Dune Swale Pond, Planktonic, 06 Feb and 24 Sept 2019
Northwest Pond, Planktonic, 27 June and 01 Aug 2019).
Meanwhile, two closely related Diptera, Order taxa, Chironomid and Ceratopogonidae registered 2X and 6X overall at various sites.

X. CONCLUSION

The results reported here are an indication of the Slough environment; but at least a couple of more years of data, perhaps one or two non-drought years, and some fine tuning or testing of sampling protocols would give more depth and consistency to the data.

From the data so far, the Slough and its associated ponds and vernal pools contain a fairly small set of invertebrate inhabitants. While we will need more time to determine what NCOS's steady-state environment will be like, COPR's portion of the Slough has some relatively extreme conditions with regard to Salinity, Temperature, and Dissolved Oxygen.

Given that a great deal more of the nominally Planktonic invertebrates seem to live in the nominally Benthic, perhaps many of these species go dormant when conditions become too extreme (as opposed to laying 'resting eggs' as with the Cladocera – we are counting taxa, not eggs.)

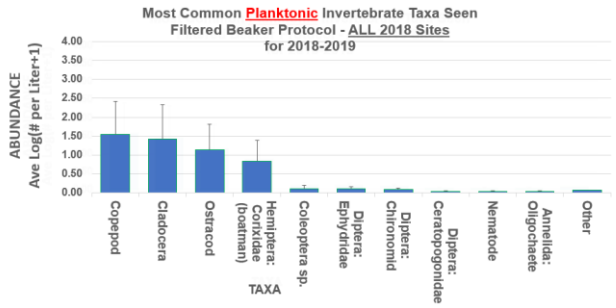
Also, with more data, we will be better able to separate out the more freshwater/brackish ponds from the more saline/hyper-saline Slough. This, and the possibility that the NCOS portion of the Slough is less harsh than the COPR portion, could help to understand the dynamics involved.

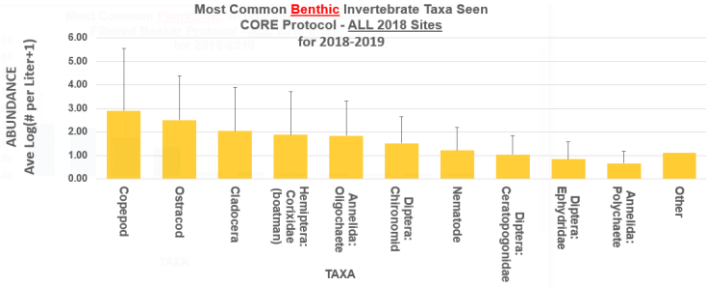
The take-aways:

1. NCOS, in its first two years of existence, has a generally equivalent diversity, compared to COPR, as measured by the Shannon-Wiener Index.
2. Overall, the diversity of taxa increased from 2018 to 2019 for both COPR and NCOS; with both COPR and NCOS being equivalent.
3. Overall, the abundance of taxa decreased significantly from 2018 to 2019, with COPR-Benthic being the more dramatic (roughly a 5X decrease).
4. Only four Planktonic taxa appear in any great, relative, abundance: Copepod, Corixidae, Ostracod, and Cladocera.
5. There are eight Benthic taxa of significant abundance: the four Planktonic plus Oligochaete, Chironomid, Nematode, and Ceratopogonidae.
6. The Benthic substrate has much higher concentration of taxa than the Planktonic, including the four main Planktonic taxa.
7. The Benthic data likely suffers from small sample sizes (rarefication issues) and ambiguity of how to include (or not) the interface with the Planktonic.
8. The Benthic-Planktonic interface needs to be studied and rationalized as it is by far the most important in terms of abundance.
9. While not mentioned in this report, the role of the streams washing fresh invertebrates into the slough can be further studied using the sites NPB1 and NPB2 (upstream from NPB). We discovered a large number of Amphipods there in 2019. Perhaps, when the golf course existed, and Devereux Creek extended to the Venoco Bridge, Amphipods were being injected into COPR in significant numbers – thus solving the mystery of how Darcie Goodman found so many.

APPENDICES

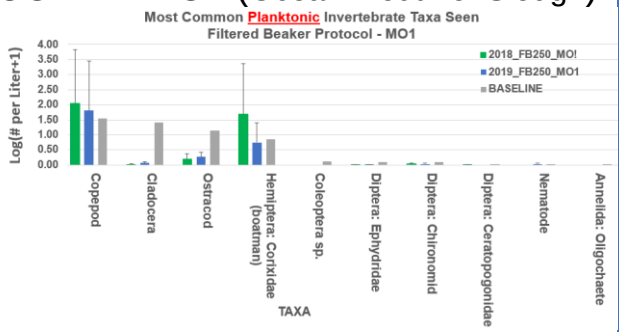
1. Taxa Abundance by Site - COPR

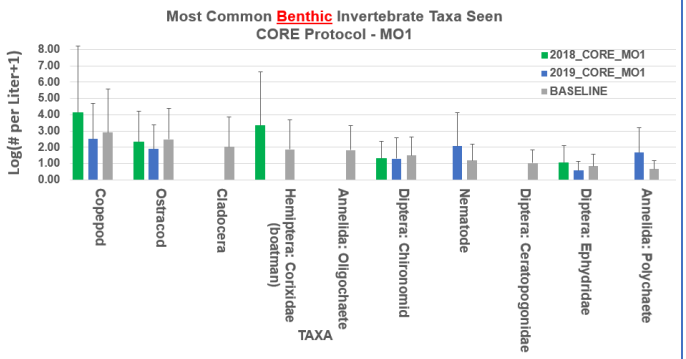




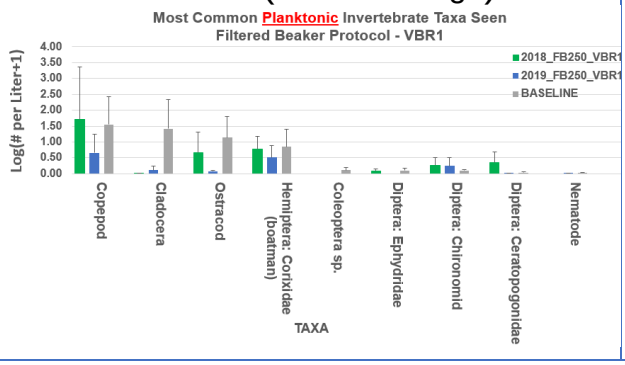
All 2018 Sites (COPR + NCOS) for 2018 and 2019 – Baseline

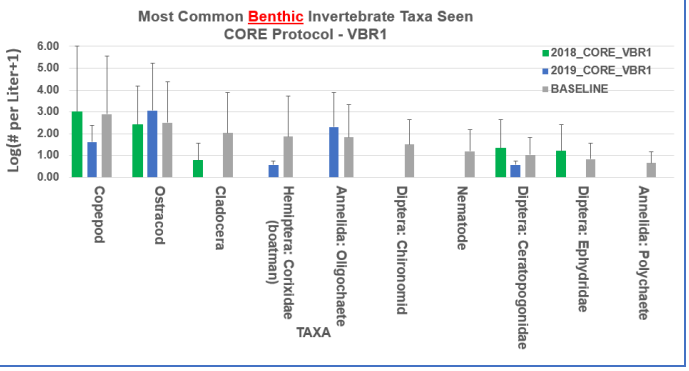
COPR – MO1 (Ocean Mouth of Slough)



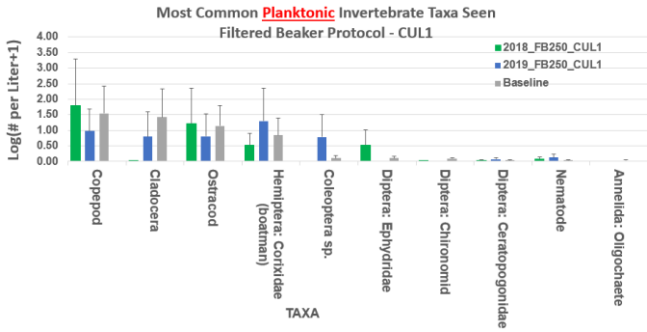


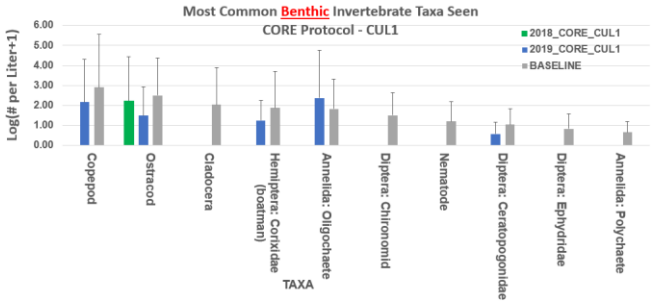
COPR – VBR1 (Venoco Bridge)



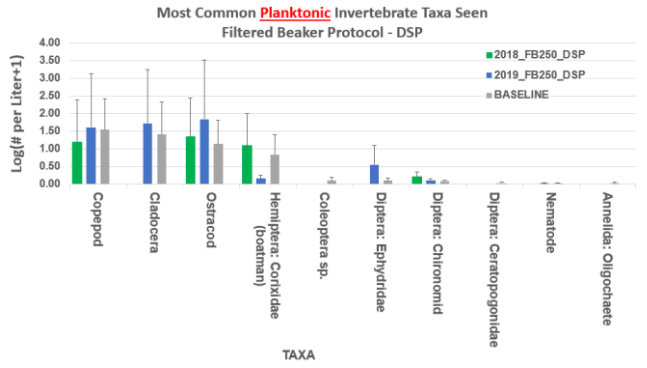


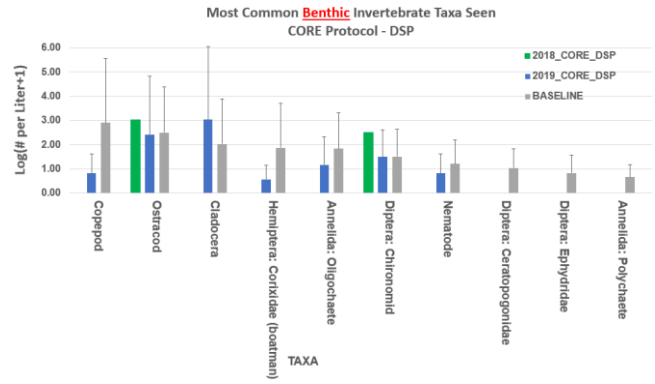
COPR – CUL1 (Culvert)





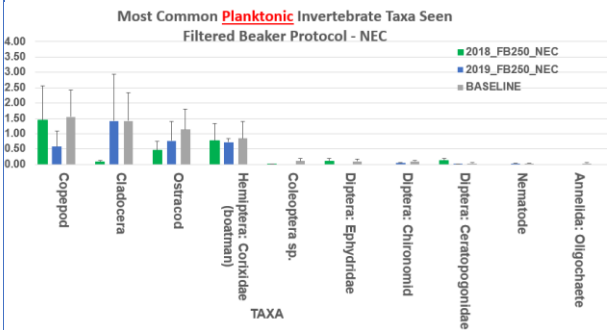
COPR – DSP (Dune Swale Pond)

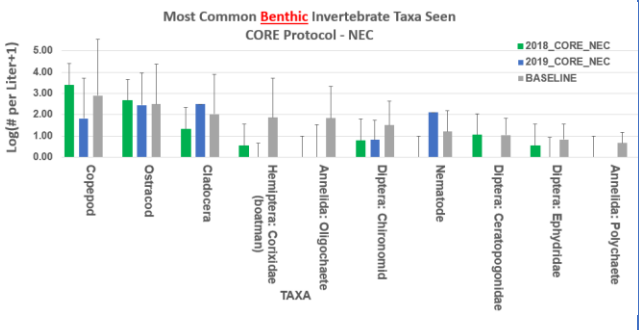




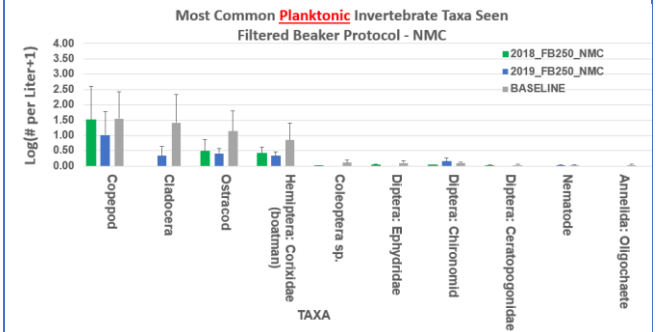
Taxa Abundance by Site - NCOS

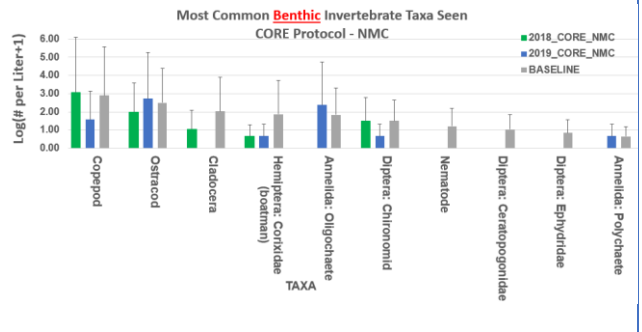
NCOS – NEC (East Channel)



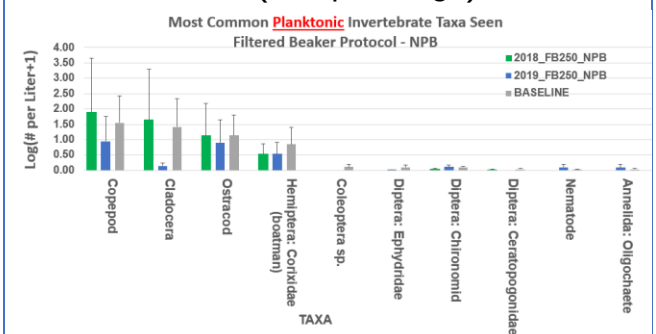


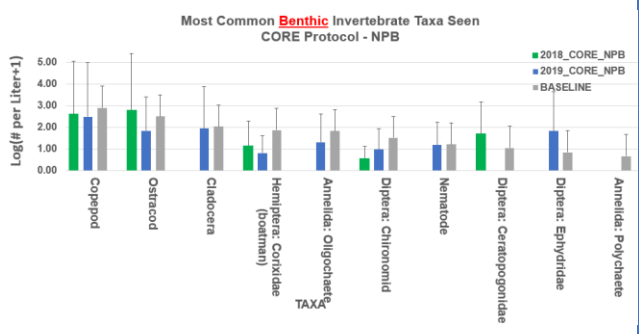
NCOS – NMC (Main Channel)



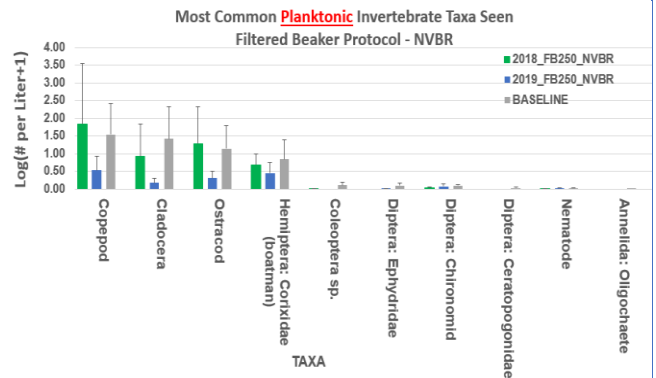


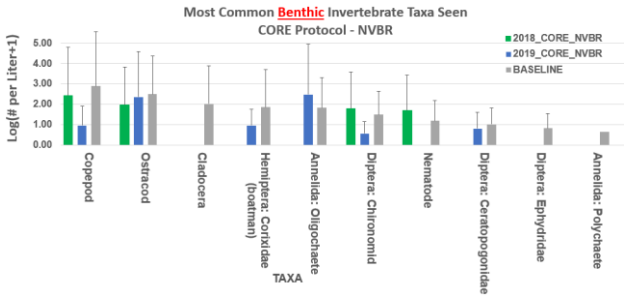
NCOS – NPB (Phelps Bridge)



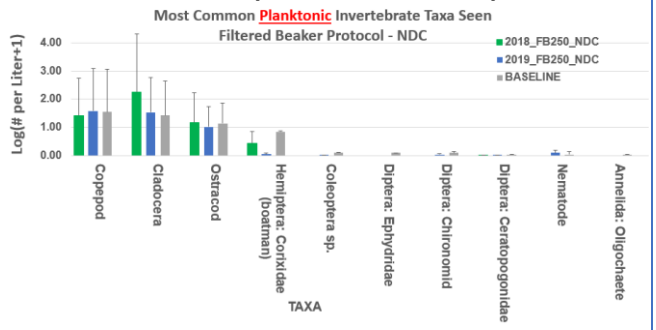


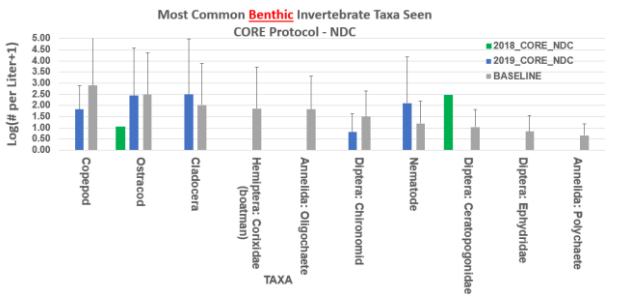
NCOS – NVBR (Venoco Bridge)



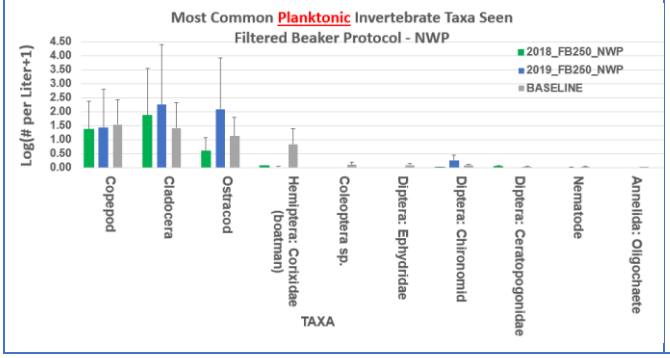


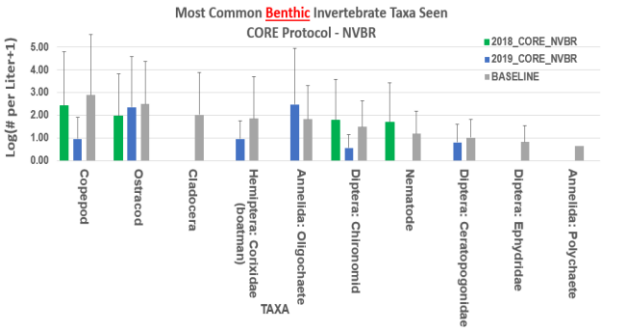
NCOS – NDC (Devereux Creek)



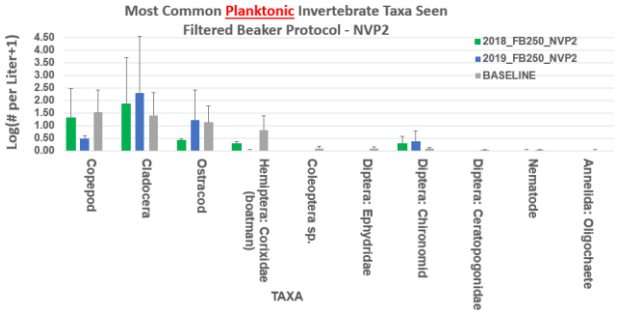


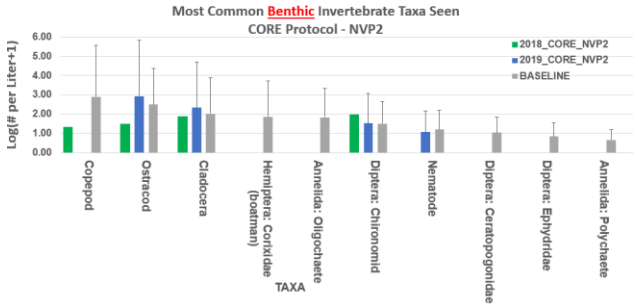
NCOS – NWP (West Pond)



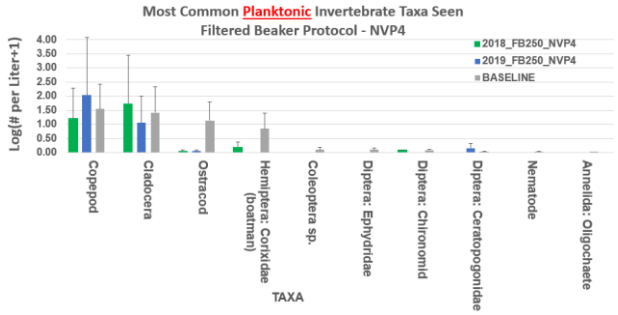


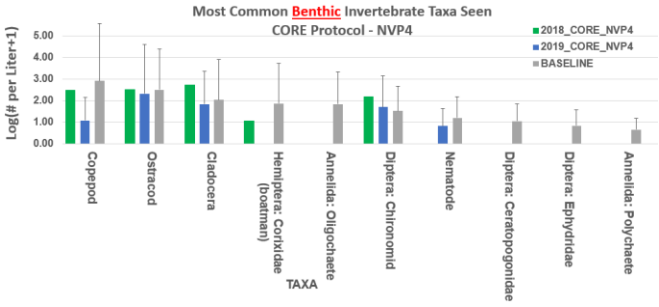
NCOS – NVP2 (Vernal Pool 2)





NCOS – NVP4 (Vernal Pool 4)

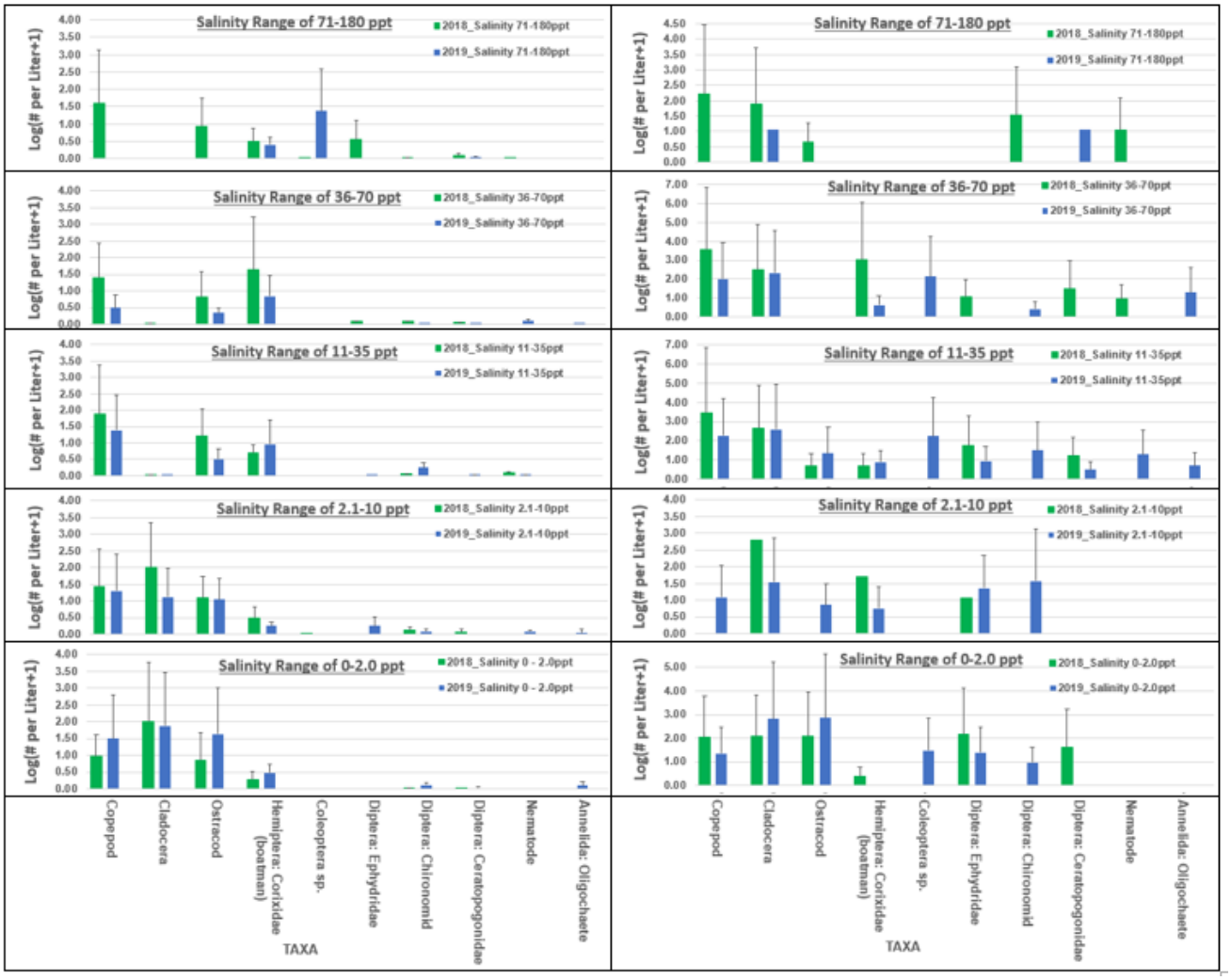




2. Taxa Abundance by Salinity

Most Common Planktonic Invertebrates
V
Salinitv (Filtered Beaker Protocol)

Most Common Benthic Invertebrates
V
Salinitv (CORE Protocol)



3. Average Abundance v Salinity

The following graphs represent the total number of invertebrates divided by number of samples taken in each salinity grouping. Notice the difference in vertical scales.