King County Zooplankton Monitoring Annual Report 2014

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Project Oversight and Report Preparation

The zooplankton analyses reported herein were conducted in Dr. Julie E. Keister's laboratory at the University of Washington, School of Oceanography. Dr. Keister designed the protocols for the field zooplankton sampling and laboratory analysis. Field sampling was conducted by the King County Department of Natural Resources and Parks, Water and Land Resources Division. Taxonomic analysis was conducted by Amanda Winans, BethElLee Herrmann, and Rachel Wilborn at the University of Washington. This report was prepared by Winans, Herrmann, and Wilborn with oversight by Dr. Keister.

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Report Summary

This report is a summary of the zooplankton analysis conducted as part of King County's Marine Monitoring Program. Data in this report cover the period April-December 2014.

Overview

In 2014, King County initiated zooplankton monitoring as part of their Marine Monitoring Program. Zooplankton samples were collected twice per month, beginning April 9, 2014 at three stations in the Puget Sound Central Basin: Point Jefferson (KSBP01), Point Wells (LSNT01), and East Passage off of Maury Island (NSEX01) (Figure 1). Samples were collected by the King County Environmental Laboratory using two types of net tows: a single ring net was towed vertically to sample zooplankton throughout the full water column; double-ring (bongo) nets were towed obliquely through the upper 30 m of the water column to sample the larger, more motile zooplankton. Samples were taxonomically analyzed at the University of Washington (full detail provided below).

The Central Basin is Puget Sound's most central basin within the Salish Sea. It is a dynamic estuarine ecosystem influenced directly by the Pacific Ocean, several major rivers, and their watersheds. In addition, the Central Basin's proximity to the major metropolitan area of Seattle makes it particularly vulnerable to anthropogenic influences. Effects of global climate change (e.g. ocean acidification, hypoxia, increasing temperatures) are also of concern for Puget Sound (Deppe et al. 2013; Fresh et al. 2011). All of these regional and global factors impact life in the Central Basin, and may threaten the balance of the ecosystem. Zooplankton occupy a key role in aquatic ecosystems—their species composition and abundances can be affected by environmental and anthropogenic influences, which in turn can impact the entire food web. Very little zooplankton data exist from Puget Sound; establishment of baseline data is required to adequately track shifts in the zooplankton and assess the effects that these changes may have on marine life and the economy.

1.0 Methods

1.1. Field Collection

See the Zooplankton Sampling Protocol v.5 (J. Keister, 5 May 2014) provided in the Appendix (F) for full detail.

From April 9, 2014 through December 16, 2014, King County collected 77 samples (49 vertical and 28 oblique) that were processed and analyzed in Dr. Keister's Lab. Three locations in the Central Basin of Puget Sound were sampled: Point Jefferson (KSBP01), Point Wells (LSNT01), and East Passage near Maury Island (NSEX01) (Figure 1; Table 1). Daytime field collections were conducted off the *R/V Liberty.* Two types of net were used: 1) a 60-cm ring net with 200-µm mesh, lifted vertically from ~5 m off the seafloor through the whole water column; 2) 60-cm paired ring (bongo) nets with 335-µm mesh, towed obliquely through the upper 30 m in a double-oblique (down and up) tow. Vertical net tows were conducted at KSBP01 (~282 m depth), LSNT01 (~216 m), and NSEX01 (~180 m); oblique tows (to a depth of 30 m) were conducted at a deep site, LSNT01 (~216 m), and at a shallower (~44 m depth) location to the east of LSNT01—the oblique tow locations are called LSNT01D (for deep) and LSNT01S (for shallow) herein, and the vertical net tow location designated as LSNT01V for clarity. *Sea-Gear* and TSK flow meters were attached to the oblique and vertical ring nets, respectively, in order to quantify the water volume sampled (m^3). Depth sensors (*ReefNet* Sensus Ultra) were attached to the net frames to accurately record tow depths and determine if target depths were achieved. The nets were gently rinsed with seawater and the contents were preserved using $NaHCO_3$-buffered formalin diluted in seawater to achieve a final concentration of 5% formalin. Preserved samples were delivered to the University of Washington for processing and analysis in Dr. Keister's laboratory.

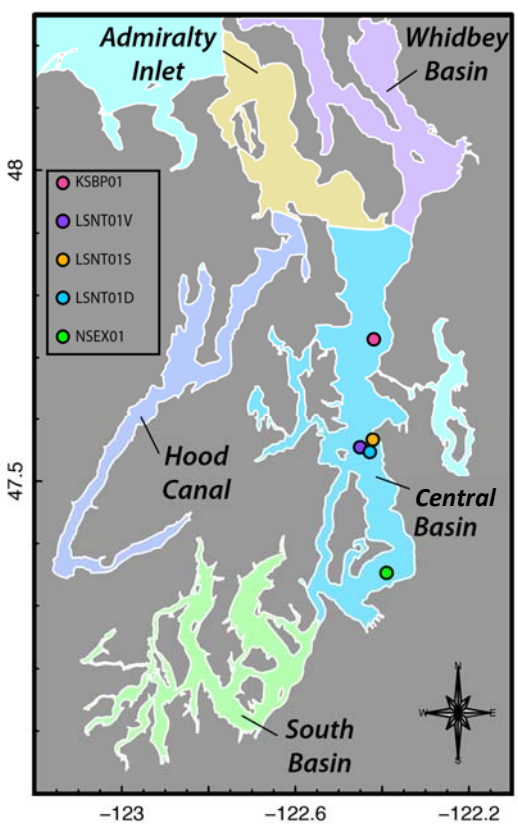

Figure 1. Map of the King County Zooplankton Monitoring Stations

The three vertical plankton tow stations (KSBP01, LSNT01V, NSEX01) and two oblique tow locations (LSNT01D and LSNT01S) are shown.

Table 1. King County Zooplankton Monitoring Station Information

Names and locations of King County's vertical and oblique zooplankton tows. LSNT01V, S, D designate sites within the region of the LSNT01 station where vertical, shallow oblique, and deep oblique tows were conducted. (Detailed station dates and depths given in Appendix A).

Site	Station Code	Target Latitude	Target Longitude	Station Depth (m)	Target Tow Depth (m)
Vertical Tows					
Point Jefferson	KSBP01	47.7440	-122.428	282	200
Fauntleroy Vertical (Point Wells)	LSNT01V	47.5333	-122.433	216	200
Maury Island (East Passage)	NSEX01	47.3586	-122.387	180	170
Oblique Tows					
Fauntleroy Shallow (Point Wells)	LSNT01S	47.543	-122.401	44	30
Fauntleroy Deep (Point Wells)	LSNT01D	47.5333	-122.433	216	30

1.2. Laboratory Processing

The following are detailed descriptions of the protocols used to analyze vertical and oblique zooplankton samples (for Lab Protocol Diagram, see Appendix B).

1.2.1. Vertical Net Analysis Protocol

Overview

The vertical net samples, which were collected with a 200-µm net, were intended to be used as ecosystem indicators. These required a high level of taxonomic and life history identification. Appendix C shows the guidelines for taxonomic levels identified, life history stages differentiated, and which species were measured in the vertical samples. All heterotrophic organisms were identified to at least a broad taxonomic group or (rarely) labeled as unknown. When organisms were measured, up to 30 individuals of each taxon were measured per sample.

In summary - first, the entire sample was briefly examined and the rare, larger organisms were removed for analysis. When abundances were very high, samples were split with a Folsom plankton splitter before picking out the large organisms. Two small aliquots were then taken from the whole sample (or split) for full analysis. Finally, a larger aliquot was taken to quantify mid-size taxa not adequately subsampled by smaller aliquots. All organisms in all subsamples were enumerated, identified, differentiated by life history stage (for certain taxa), and measured (for certain taxa- See Appendix C).

Rinsing and preparing samples

The plankton sample was gently filtered onto a 200-µm sieve under the fume hood and gently rinsed with tap water to remove the preservative.

Picking out rare large organisms

The sample was gently rinsed from the sieve into a large, shallow glass dish and set onto a light table. Large and/or rare organisms (> 1 cm or very rare) that would not be captured by a 1-mL Stempel pipette were removed, identified, counted, and measured with an ocular micrometer or ruler.

Samples with high densities of larger organisms (e.g., crab megalopae, shrimp, or krill) were sometimes split before their removal. To do this, the sample was poured into a Folsom plankton splitter and quantitatively split to a manageable concentration (usually 1/2 – 1/8 split). If the sample was split, the split was then used for further aliquots.

Small aliquots

The whole sample or split (excluding the large organisms that had been removed) was sieved and rinsed into a 100-mL graduated cylinder. The sample was allowed to settle for at least 10 minutes; the settled biovolume was recorded, including an estimate of the proportion of phytoplankton and gelatinous material.

The sample was quantitatively transferred to a wide mouth jar or beaker and diluted with tap water to about 5-10 times the settled volume (not including phytoplankton and gelatinous material). The goal was to attain a concentration of ~200-250 organisms mL^{-1}. The total diluted volume was recorded to use in calculating plankton density from the aliquots counted.

The sample was randomly stirred to obtain a uniform distribution (no vortices), then a subsample was taken with a 1-mL Stempel pipette. This was rinsed into a Bogorov counting chamber and examined under a dissecting microscope. All organisms were identified to the required taxonomic level and developmental stage, and measured with an ocular micrometer when necessary. A replicate 1-mL aliquot was then obtained and sorted.

Mid-size aliquot

After the small aliquots were removed, one 10-40-mL aliquot was taken using a 10-mL Stempel pipette to quantify any mid-size organisms that may have been missed in the small aliquots. Only taxa that had been few or absent in the small aliquots were then counted. The aliquot was rinsed into a petri dish and carefully looked through in its entirety underneath the dissecting microscope. Organisms were identified, staged and measured, as needed.

The whole sample was then sieved, rinsed back into the jar, and re-preserved with 5% formalin.

1.2.2. Oblique Net Analysis Protocol

Overview

The oblique bongo samples, which were collected with a 335-µm net, targeted the larger plankton which are strong swimmers (so can avoid the vertical net tows). These organisms include those that are common prey for fish (e.g. decapods, amphipods, euphausiids). Appendix E shows a detailed table of how organisms were identified taxonomically and by life history stage, as well as which taxa were measured in the oblique samples. All organisms were identified to at least a broad taxonomic group or labeled as unknown. When organisms were measured, only up to 30 individuals of each taxon were measured per sample. The exception to this was larval fish, which were all measured.

In summary – first, the entire sample was briefly examined and the rare, larger organisms were removed for analysis. Then the sample was split with a Folsom plankton splitter, and the organisms that were rare and large in that split were removed for analysis. Finally, the split was diluted to a known volume and two 10-mL aliquots were taken. All organisms in these subsamples were enumerated, identified, staged (for certain taxa), and measured (for certain taxa- See Appendix D).

Rinsing and preparing samples

The plankton sample was filtered onto a 335-µm sieve under the fume hood and gently rinsed with tap water to remove the preservative.

Picking out rare large organisms

The sample was gently rinsed from the sieve into a large, shallow glass dish and set onto a light table. Larger organisms (~5 mm or larger, depending on the size distribution in the sample) that were rare (<30) in that particular sample (such as jellyfish, larval fish, shrimp, crab megalopae, etc.) were removed, counted, identified, staged, and measured.

Splitting samples

The sample was then rinsed into a Folsom plankton splitter and split to a manageable concentration (usually 1/2 – 1/8 split). Large organisms (amphipods, etc.) that were rare in this split (< 30) were removed, counted, identified, staged, and measured.

Taking aliquots of splits

The final split (excluding the large organisms that had been removed) was sieved and rinsed into a 100-mL graduated cylinder. From here, the sample was quantitatively transferred to a wide mouth jar or beaker and diluted with tap water to attain a concentration of ~ 20 organisms mL^{-1}. The total diluted volume was recorded to use for calculating plankton density from the aliquots counted.

The sample was randomly stirred to obtain a uniform distribution (no vortices), then a subsample was taken with a 10-mL Stempel pipette. This was rinsed into a Bogorov counting chamber and examined under a dissecting microscope. All organisms were identified to the required taxonomic level and developmental stage, and measured with an ocular micrometer when necessary. Fish larvae were measured and preserved in 70% ethanol. Fish eggs were measured and saved in a 5% buffered formalin solution. A replicate 10-mL aliquot was then drawn and sorted. Finally, the whole sample was re-preserved with 5% buffered formalin.

1.3. Samples Processed

A total of seventy-seven samples were processed from the 2014 sampling effort (Appendix A). These are currently re-preserved and stored indefinitely at the University of Washington. The data reported here will be available as public record through King County.

1.4. Quality Control and Data Analyses

All data were entered into a Microsoft Access database by data analysts. All data were double-checked by the taxonomists, then queried to generate species densities (abundances) using the following equation (Appendix B):

Individuals m^{-3} = (Whole count + (Folsom count x split multiplier) + ((subsample count#1 x volume#1) + (subsample count#2 x volume#2) + (subsample count#3 x volume#3)) ÷ number of subsamples) ÷ Volume of water filtered by net

Species were then aggregated into general taxa for the analyses described below. For instance, amphipods incorporate *Cyphocaris challengeri, Themisto pacifica, Hyperia, Hyperoche,* etc. (Appendix E).

To create the time-series plots shown below, the semi-monthly sampling dates were categorized as falling on either the 1st or the 15th of the month, regardless of actual date sampled (dates may have deviated a few days around the 1st or 15th). Density for a particular taxon was recorded as zero if a sample was collected and processed, but that taxon was not found. If a sample was not conducted and processed for a particular date, those data points were left blank for analyses. For example, LSNT01V was not sampled in the first part of the months of September or November; therefore, those data time points are blank. LSNT01V was

sampled during the second part of the month in November and December, but because no crabs (for example) were found in those samples, those data points are represented as zeros.

Species abundance matrices were created to analyze in Tableau® 9.0 to examine seasonal cycles of dominant taxa (section 2.4) and in PC-ORD™ 5.05 to examine sample groupings using Nonmetric Multidimensional Scaling (NMS) ordinations (section 2.6).

1.5. General Taxa Assessment

Abundances of all taxa from all tows were calculated. Eggs, copepod nauplii, and *Noctiluca* (in oblique samples) were recorded but not included in analyses, unless otherwise noted, because they are temporally and spatially patchy and can be present in very high abundances. Siphonophore gonophores, a reproductive component of the colonial calycophoran *Muggiaea atlantica*, were also removed before calculating densities. While they will be included for future biomass calculations, siphonophore gonophores are not considered individuals and have no perceived predatory/prey interactions (Purcell 1982). Krill (Euphausiidae) were separated based on life stages of marked developmental differences: "Krill – Early Stages" include nauplii, metanauplii, and calyptopes; "Krill – Late Stages" include furcilia, juveniles, and adults.

2.0 Results

More than 123 taxa were identified in the samples. These were aggregated into 34 broader taxa (e.g. copepods, krill, chaetognaths, etc.) for presentation below. Of those general taxa, 30 were collected by the vertical net tows and 28 were collected by the oblique tows.

The following sections briefly describe the dominant: 1) spatio-temporal patterns in total zooplankton abundance, 2) seasonal variations in dominant taxa averaged over all sites, 3) species diversity, and 4) community patterns determined by Nonmetric Multidimensional Scaling (NMS) ordination analysis.

2.1. Total Zooplankton Abundances by Station

At all stations, peak total zooplankton densities (Ind m^{-3}) occurred between June and August, with the exception of LSNT01S, which also had high abundances in April (Figure 2B). Vertical net samples showed higher abundances than oblique net samples, with the northernmost station (KSBP01) having the highest densities in July, of nearly 7,000 Ind m^{-3} (Figure 2A). *Noctiluca* were excluded from these plots of total abundance at each; however, they were highly abundant at NSEX01 and are shown separately below (Figure 3A). Overall, deep and shallow oblique sites at LSNT01 had similar total abundances except in April and August, when densities differed by ~2,000–3,000 Ind m^{-3} (Figure 2B). [Note: The unusual high and low (seesaw) pattern observed in the oblique tow data in June-August may be an artifact of sampling different tidal phases and is under investigation (Figure 2B).]

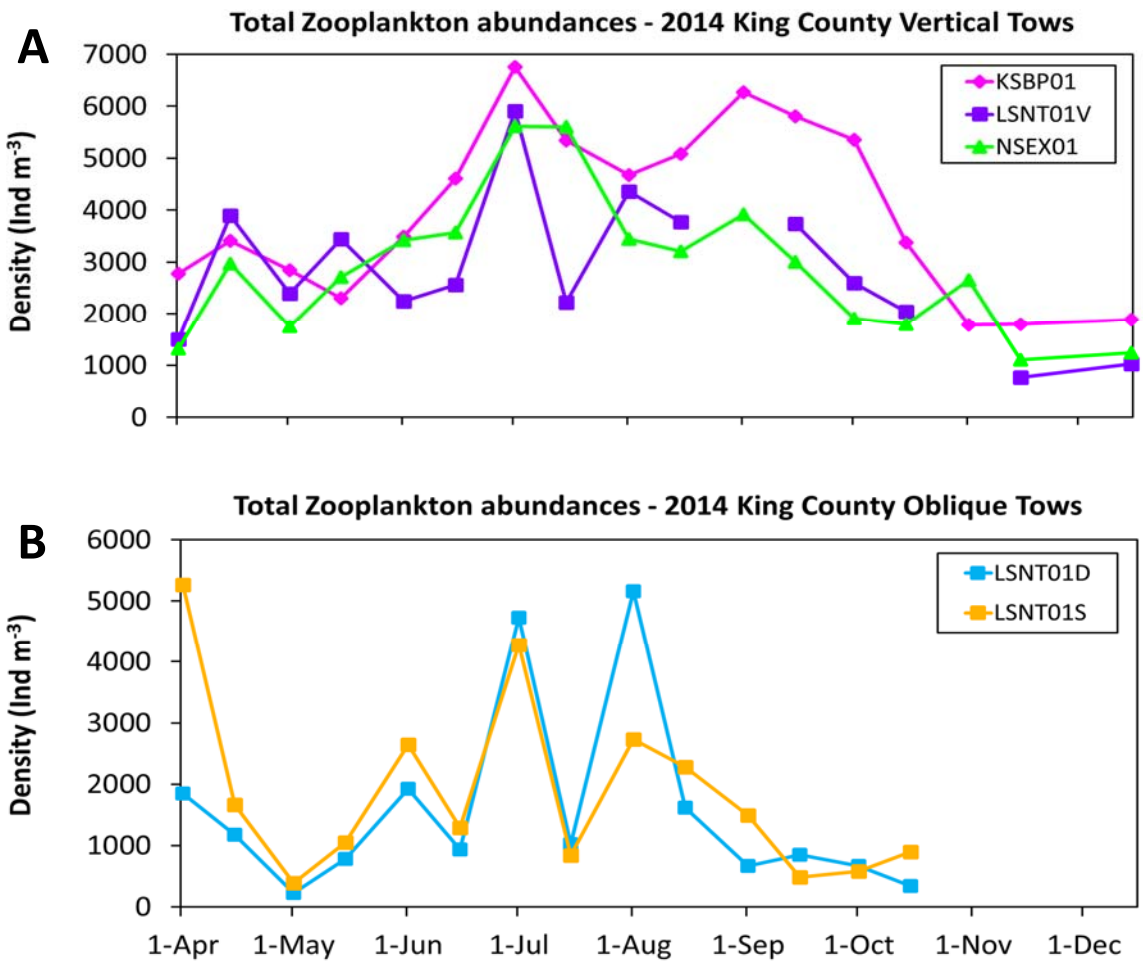

Figure 2. Total Zooplankton Abundances

Total zooplankton abundances (Ind m^{-3}) for A) vertical stations (KSBP01, LSNT01V, and NSEX01) and B) oblique stations (LSNT01D and LSNT01S). Vertical net tows were conducted from April – December, whereas oblique net tows were only conducted from April – October, 2014. (Note: LSNT01V was not sampled in the first half of September or November.) Eggs, *Noctiluca*, siphonophore gonophores, and copepod nauplii were not included here.

2.2. Abundance of Dominant Taxa—Averaged Across All Stations

Vertical net tows generally had higher mean abundances than oblique net tows, and almost 2x the total abundances during the summer months. Copepods and larvaceans were the most abundant taxa across all stations in vertical and oblique tows, with copepods >4x as abundant as larvaceans in the vertical net tows (Figure 3A). Unlike the other most abundant taxa, larvaceans peaked in abundance early in the year, in April (or before sampling began), at about ~1,250 Ind m^{-3} (Figure 3B). *Noctiluca*, which were only quantified in vertical tows, were highly abundant during early summer. Their abundance drastically declined from ~1,700 Ind m^{-3} during early July to $\leq$40 Ind m^{-3} in late July (Figure 3A). Barnacles were ranked 3rd for oblique

tows and 6th for vertical tows; they were among the top taxa at all stations, and showed similar trends to those of larvaceans, but with lower abundances.

Bivalves and gastropods increased during the summer month, although with different peak timing. A similar trend was observed for cladocerans and siphonophores in the oblique net tows. Polychaetes, the 6th top taxa for oblique tows showed a consistent range of 7-12 Ind m^{-3} throughout most of the year, except for a small peak in late April (Figure 3B).

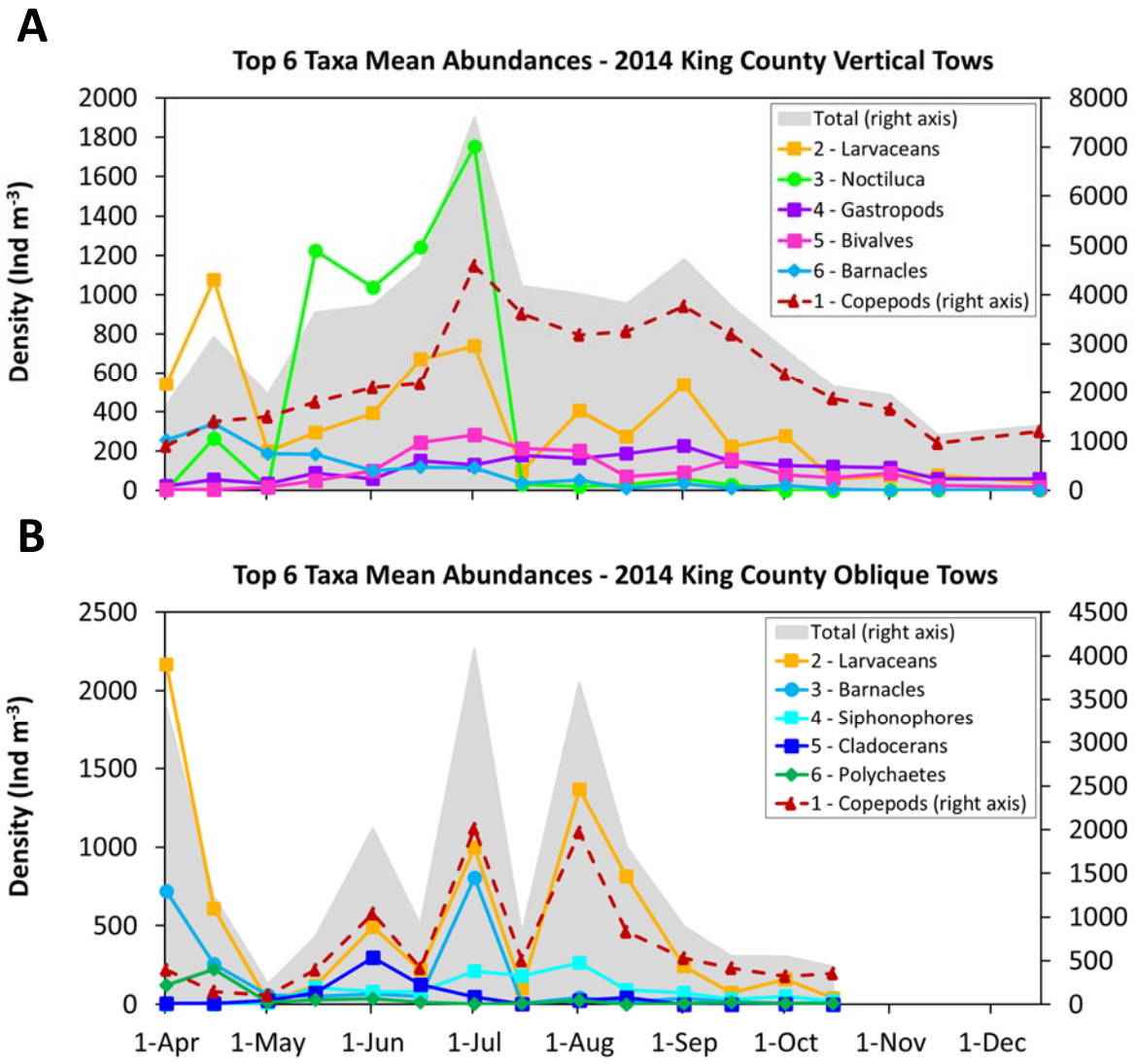

Figure 3. Top 6 Taxa Mean Abundances

Mean abundances of the six most dominant taxa across stations identified from A) vertical net and B) oblique net zooplankton samples. Vertical tows were conducted from April – December, whereas oblique tows were only collected April–October. Taxa shown were ranked (1-6) for dominance. Copepods densities and total abundances are shown on the right axes.

2.3. Abundance of Dominant Taxa—By Station

Vertical Net Tows

Abundances of copepods, gastropods, bivalves, and siphonophores peaked in summer, while *Noctiluca* were most abundant in late spring and diminished through the rest of the year (Figure 4 - panels 1, 3, 4, 5, 9). Bryozoans were least abundant at the time of *Noctiluca* peak abundances, and only increased during the late autumn and early winter (Figure 4 - panels 2, 8). Barnacles were most abundant in early spring and had decreased by summer (Figure 4 - panel 6). Larvaceans were abundant throughout the spring, summer, and early autumn, displaying an almost bi-modal distribution. Early stages of krill (nauplii, metanauplii, and calyptopes) increased briefly in late April and declined rapidly, staying low throughout the rest of the year (Figure 4 - panel 10). Polychaetes showed a strong bi-modal pattern of abundance.

Copepods were most abundant at the northern station, KSBP01, while larvaceans, *Noctiluca*, gastropods, bryozoans, and siphonophores were more abundant at the southern station, NSEX01 (Figure 4 - panels 1, 2, 3, 4, 6, 8, 9). Barnacles, bivalves, polychaetes and early stages of krill were well distributed throughout all of the stations (Figure 4 - panels 5, 7, 10).

Oblique Net Tows

Similar to vertical net tows, copepods and siphonophores were most abundant during the summer months in oblique tows (Figure 5 - panels 1, 4). Chaetognaths, crabs, and cladocerans were also abundant during summer, but only at the deep station (Figure 5 - panels 5, 9, 10). Larvaceans were again bi-modal in their distribution, with peak densities in early spring and late summer. The same trend was observed for cnidarians (Figure 5 - panels 2, 8). Polychaetes, barnacles, and early stages of krill had high abundances in April, but only krill and barnacles peaked again in summer (Figure 5 - panels 3, 6, 7). Polychaetes maintained low densities throughout the rest of the year (Figure 5 - panel 6).

Most taxa were fairly well-distributed between shallow (LSNT01S) and deep (LSNT01D) stations, with some exceptions. Chaetognaths and crabs (Figure 5 - panel 9, 10) were more abundant in the deeper station in July and July- August, respectively. Cladocerans and cnidarians (Figure 5 - panel 5, 8) had a spike in abundance at the shallow station in June, while polychaetes (Figure 5 - panel 6) peaked in April.

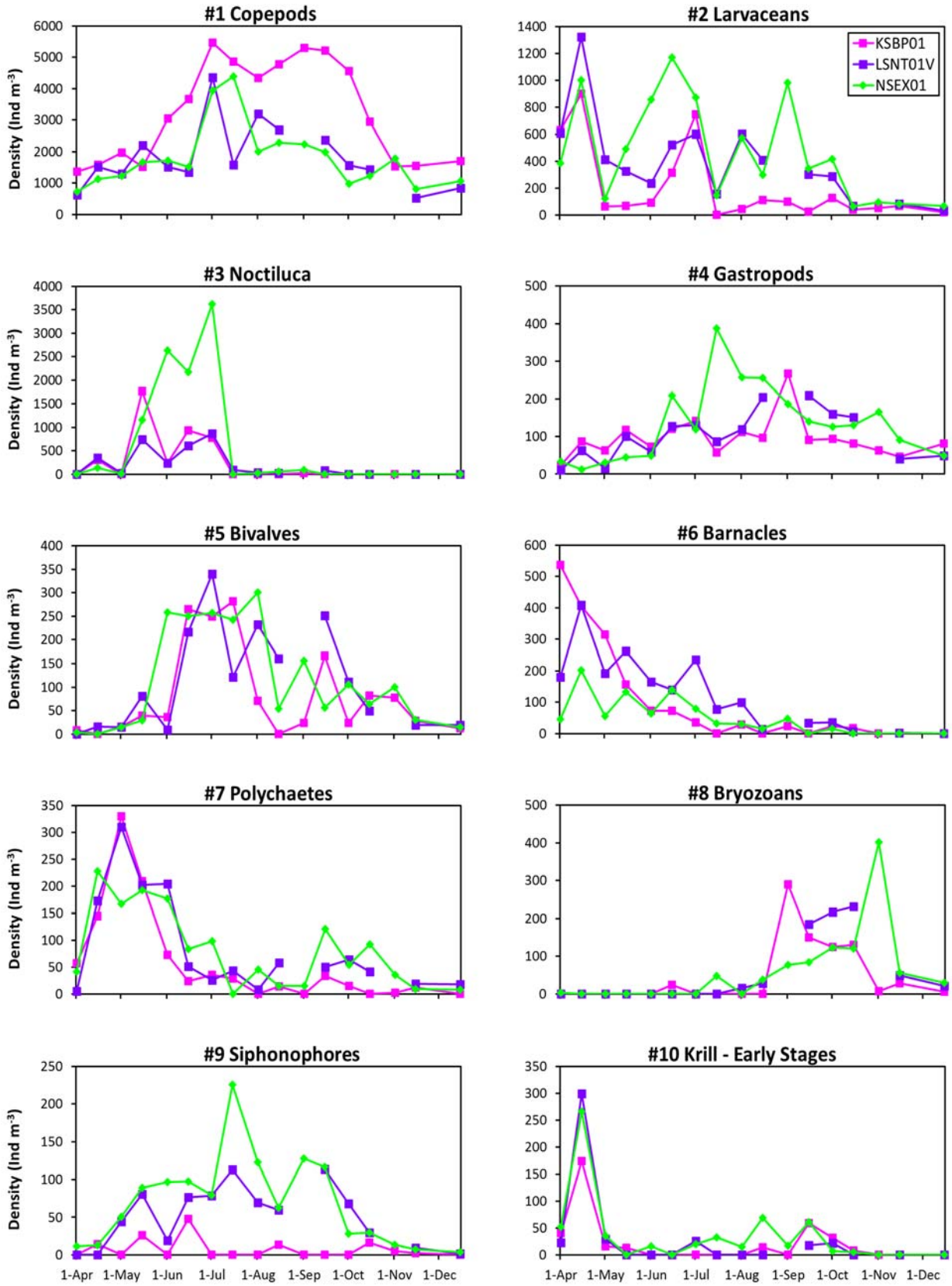

Figure 4. Densities of 10 Most Abundant Vertical Net Taxa by Station

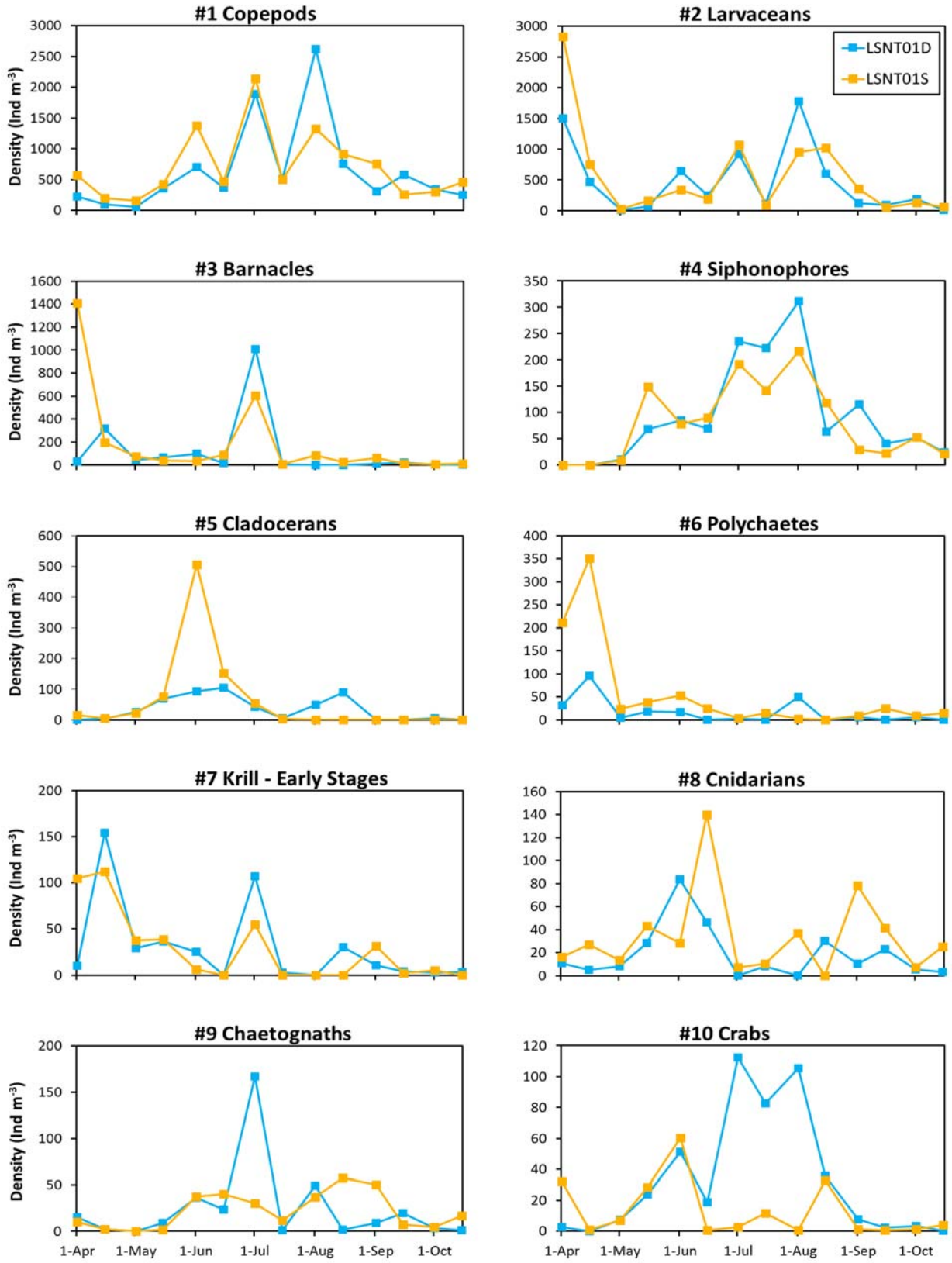

Figure 5. Densities of 10 Most Abundant Oblique Net Taxa by Station

2.4. Taxonomic Composition

Seasonal changes in taxonomic composition resulted in shifts in relative proportions of the dominant taxa over time (Figure 6). Copepods always dominated the zooplankton community except in a few cases where *Noctiluca* were extremely abundant.

Vertical Net Stations

The zooplankton community was notably different in April compared to other months (Figure 6). After copepods (red), larvaceans (orange) were the second most dominant taxon, with barnacles (blue) following. By May, a shift had occurred and *Noctiluca* began to dominate, maintaining a strong presence through July. By August, the large *Noctiluca* blooms had finished for the year. Bryozoan cyphonauts (yellow) appeared in higher proportions in October and November. In April, May, and June, the community was relatively diverse compared to later in the year when copepods vastly dominated (Figure 6).

Oblique Net Stations

The zooplankton community sampled by oblique net tows showed less dramatic seasonal shifts. April was again an anomalous month; whereas copepods numerically dominated the community at the deep and shallow stations in most months, larvaceans dominated in April (Figure 7). The proportion of larvaceans remained high throughout the year. May and July showed higher diversity, with the appearance of cladocerans, siphonophores, krill, and some crabs. Siphonophores maintained a presence throughout the rest of the year, with an increase in June. August, September and October showed little change in the community, although crabs slightly increased in September (Figure 7).

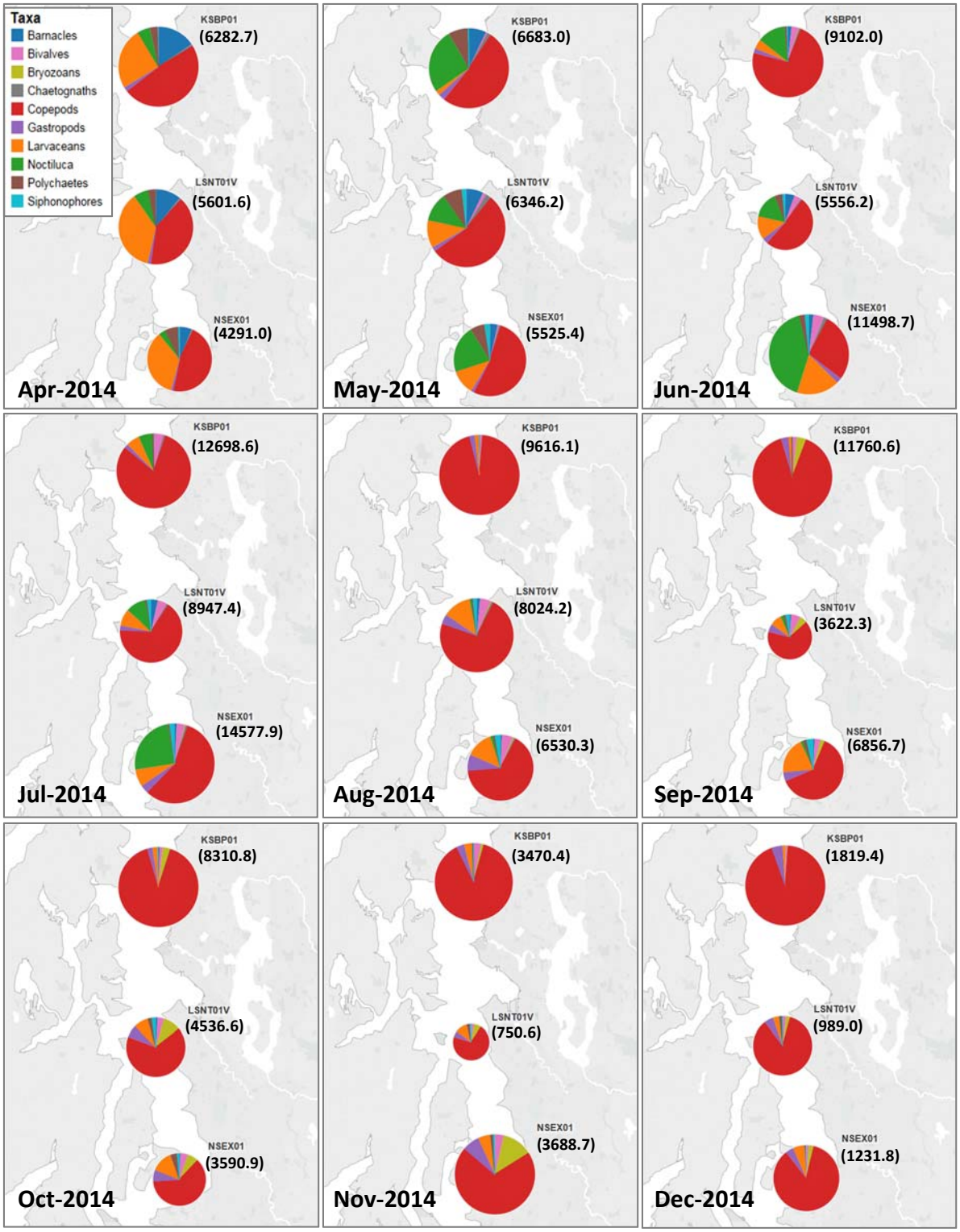

Figure 6. Top 10 Vertical Net Taxa – Monthly Abundances (in parentheses)

Monthly variation in the proportion of dominant taxa from vertical net stations, April–December 2014. Only the top ten most abundant taxa are represented. Pie charts are sized by total station abundance (Ind m^{-3}), but relative sizes can only be compared among stations within each month.

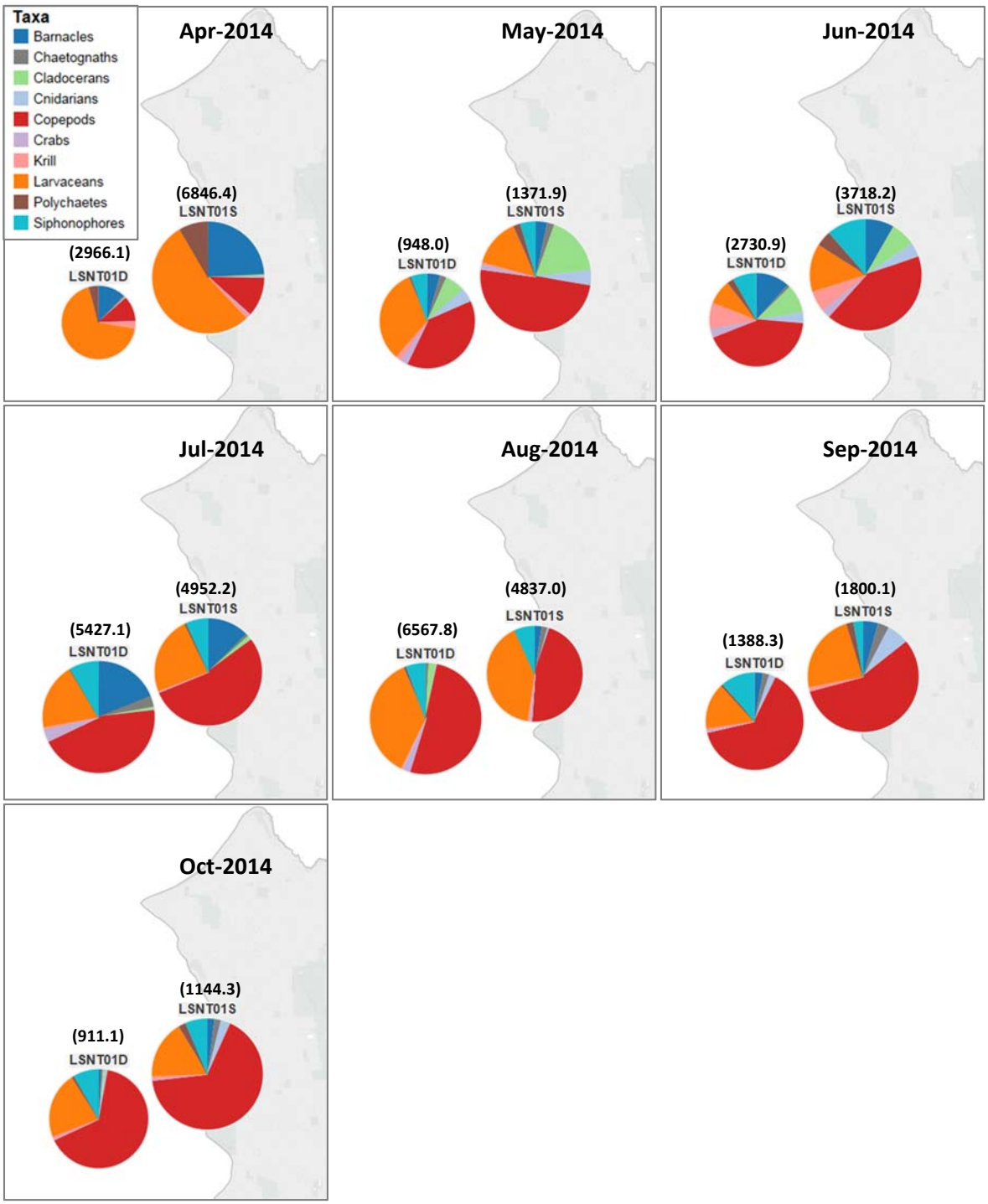

Figure 7. Top 10 Oblique Net Taxa – Monthly Abundances (in parentheses)

Monthly variation in the proportion of dominant taxa from vertical net stations, April–October 2014. Only the top ten taxa are represented. Pie charts are sized by total station abundance (Ind m^{-3}), but relative sizes can only be compared between stations within each month.

2.5. Diversity Indices

Diversity was calculated for each station using:
1) Shannon-Weaver Index:

$$H = \sum_{i=1}^{s} - (P_i * \ln P_i)$$

Where:
H = the Shannon-Weaver Diversity Index
P_i = fraction of the entire population made up of taxa i
S = numbers of taxa encountered
∑ = sum from taxon1 to taxon S

2) Simpson's Index of Diversity (SID):

$$SID = 1 - D$$

Where:

$$D = \sum_{i=1}^{s} P_i^2$$

D = Simpson's Index

3) Richness (S) = numbers of taxa encountered
4) Evenness (J) = H/ln S

The densities of the most specific taxonomic groupings were summed over the entire year for each station. Eggs, copepod and krill nauplii, *Noctiluca*, and siphonophore gonophores were removed from the analyses. As Shannon-Weaver Index and Simpson's Index of Diversity increase, greater diversity is implied relative to other similarly analyzed stations. For the purpose of our study, diversity cannot be compared between vertical net and oblique net samples because analysis protocols differ. Richness is the number of taxa found at each station. As evenness approaches one, densities become more evenly distributed.

Of the vertical tow sites, LSNT01 and NSEX01 supported a more diverse zooplankton community compared to KSBP01. The shallow and deep LSNT01 sites showed similar diversity across all metrics.

Table 2. Diversity Indices

Tow Type	Station	Shannon-Weaver Index	Simpson's Index of Diversity	Richness	Evenness
Vertical	KSBP01	2.46	0.86	84	0.55
	LSNT01V	2.63	0.88	96	0.58
	NSEX01	2.56	0.86	95	0.56
Oblique	LSNT01S	1.87	0.72	68	0.44
	LSNT01D	1.89	0.73	67	0.45

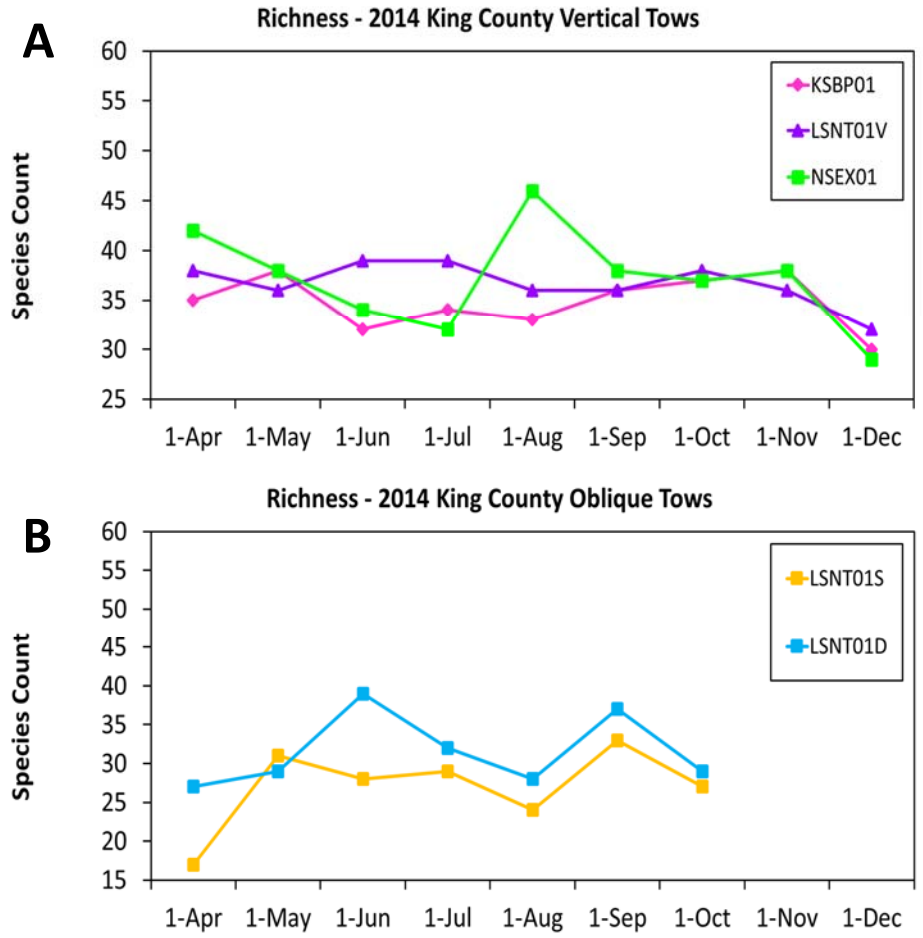

Figure 8. Richness

Richness for all King County stations sampled from April to December 2014.

2.6. NMS Ordinations by Month and Station

Nonmetric Multidimensional Scaling (NMS) ordinations were run using PC-ORD™ 5.05. Species and station matrices were created from general taxa densities (Ind m^{-3}) data (e.g., all copepods were combined, all larvaceans were combined; see Tables 3 and 4 for taxa included in each ordination). Krill were separated into "Krill – Early Stages" (nauplii, metanauplii, and calyptopes) and "Krill – Late Stages" (furcilia, juveniles, and adults). Eggs, nauplii (except for barnacles and krill), unidentified organisms, *Noctiluca* (only for oblique data), and siphonophore gonophores were removed before analyses. Because some taxa were very abundant while others were very rare, data were first normalized using a logarithmic transformation [Log_{10} (Y + 0.001) + 3] and taxa that occurred in <5% of the samples were removed. Ordinations were run on the remaining n=25 taxa for n=49 vertical net samples and n=21 taxa for n=28 oblique net samples using the Sørensen (Bray-Curtis) distance measure.

Following ordination, the sample cloud was freely rotated to load the maximum variance in taxonomic composition along a single axis: for vertical net tows, this major axis was Axis 3; for oblique net tows, it was Axis 2. Distances between points in the ordination indicate the level of dissimilarity between zooplankton communities, where closer points are less dissimilar than points that are farther apart.

A clear seasonal cycle in the zooplankton community aligned with the dominant axis in both the vertical net and oblique net ordinations. Separation of the stations is weak.

2.6.1. Vertical Net Tows

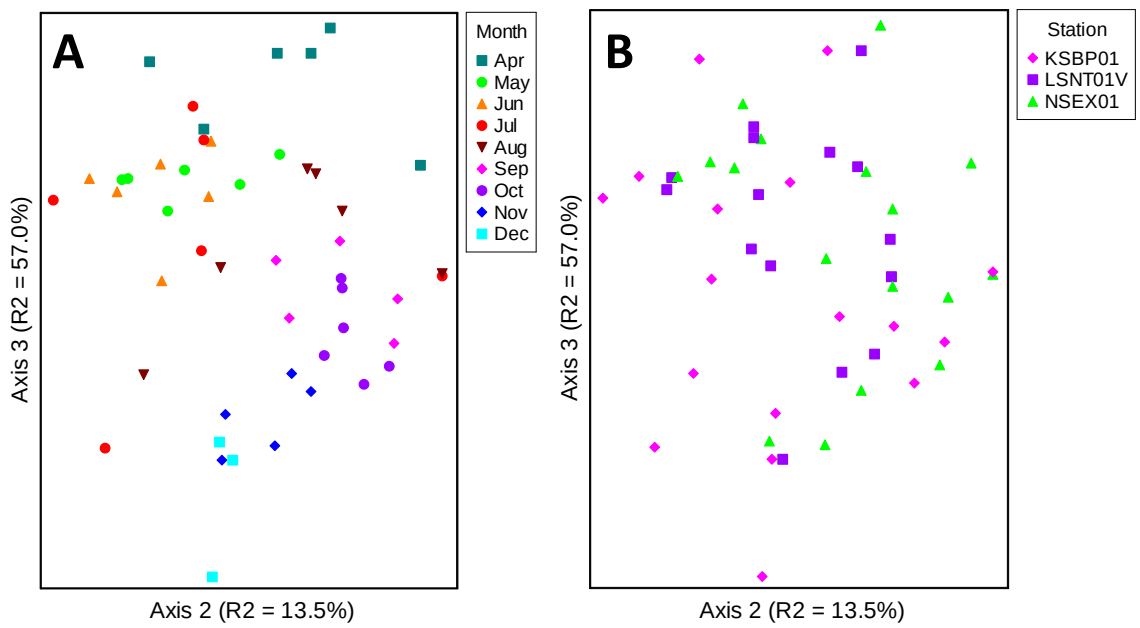

Figure 9. NMS Ordinations – Vertical Tows

Nonmetric Multidimensional Scaling ordinations for 2014 King County Vertical Zooplankton tows, color coded by month (panel A) and station (panel B).

Table 3. Pearson and Kendall Correlations with Ordination Axes – Vertical Net Tows

Correlation coefficients (r) and correlations of determination (R^2) between taxa from vertical net tows and the NMS ordination axes. Taxa strongly correlated with the primary axis (R^2>0.30) are in bold.

CUM R^2 = 0.839	Axis 1 (R^2 = 0.134)		Axis 2 (R^2 = 0.135)		Axis 3 (R^2 = 0.570)	
	r	R^2	r	R^2	r	R^2
Amphipods	-0.217	0.047	0.274	0.075	-0.79	**0.624**
Barnacles	-0.098	0.010	-0.254	0.065	0.788	**0.621**
Bivalves	0.147	0.022	-0.248	0.061	-0.322	0.104
Bryozoans	-0.022	0.001	0.575	0.331	-0.625	**0.39**
Chaetognaths	0.219	0.048	-0.312	0.097	0.654	**0.428**
Cladocerans	0.170	0.029	-0.012	0	0.228	0.052
Cnidarians	0.317	0.101	-0.103	0.011	0.220	0.048
Copepods	-0.245	0.060	-0.093	0.009	-0.046	0.002
Crabs	-0.199	0.040	0.347	0.120	0.403	0.162
Ctenophores	0.261	0.068	0.502	0.252	-0.318	0.101
Cumaceans	0.249	0.062	-0.012	0	0.130	0.017
Echinoderms	-0.358	0.128	0.548	0.300	0.217	0.047
Fish	-0.107	0.012	0.112	0.013	0.225	0.050
Gastropods	0.157	0.025	0.174	0.030	-0.218	0.047
Isopods	-0.279	0.078	0.22	0.049	-0.194	0.038
Krill-Early Stages	-0.444	0.197	0.559	0.312	0.479	0.229
Krill-Late Stages	-0.277	0.077	0.413	0.170	-0.104	0.011
Larvaceans	0.279	0.078	0.110	0.012	0.610	**0.373**
Mysids	0.298	0.089	-0.110	0.012	0.008	0
Noctiluca	0.538	0.289	-0.552	0.305	0.546	0.298
Ostracods	0.027	0.001	-0.176	0.031	-0.256	0.066
Platyhelminthes	0.224	0.050	0.221	0.049	0.244	0.060
Polychaetes	0.409	0.167	-0.171	0.029	0.492	0.242
Shrimp	-0.050	0.002	0.112	0.012	-0.228	0.052
Siphonophores	0.671	0.450	0.220	0.048	0.103	0.011

2.6.2. Oblique Net Tows

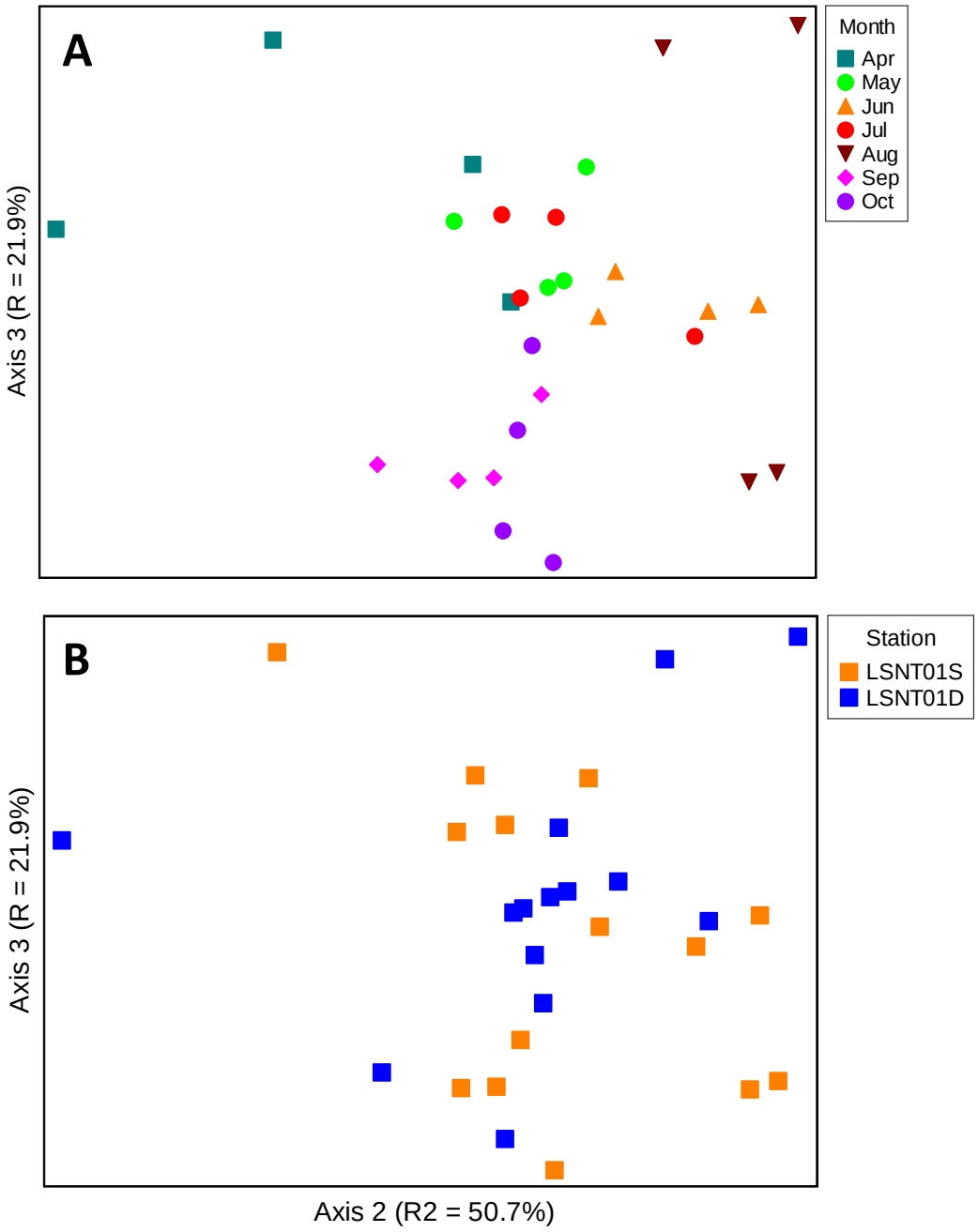

Figure 10. NMS Ordinations – Oblique Tows

Nonmetric Multidimensional Scaling ordinations for 2014 King County oblique zooplankton tows, color-coded by month (panel A) and station (panel B). Move deleted caption text talking about the variance into text section.

Table 4. Pearson and Kendall Correlations with Ordination Axes - Oblique Net Tows

Correlation coefficients (r) and correlations of determination (R^2) between taxa from oblique net tows and the NMS ordination axes. Taxa most strongly correlated with the primary axis (R^2>0.30) are in bold.

CUM R^2 = 0.914	Axis 1 (R^2 = 0.189)		Axis 2 (R^2 = 0.507)		Axis 3 (R^2 = 0.219)	
	r	*R^2*	*r*	*R^2*	*r*	*R^2*
Amphipods	0.151	0.023	0.554	0.307	-0.559	0.312
Barnacles	-0.154	0.024	-0.4	0.16	-0.21	0.044
Bivalves	0.265	0.07	-0.492	0.242	-0.49	0.24
Chaetognaths	0.484	0.234	0.314	0.098	-0.155	0.024
Cladocerans	-0.475	0.225	0.111	0.012	0.763	**0.582**
Cnidarians	-0.17	0.029	-0.14	0.02	-0.142	0.02
Copepods	0.279	0.078	0.531	0.282	0.097	0.009
Crabs	-0.124	0.015	0.608	0.369	0.288	0.083
Ctenophores	-0.418	0.175	0.027	0.001	-0.491	0.241
Echinoderms	0.54	0.292	-0.493	0.243	-0.082	0.007
Fish	0.638	0.406	-0.412	0.17	0.132	0.017
Gastropods	-0.105	0.011	0.356	0.126	-0.604	0.365
Insects	0.488	0.238	-0.138	0.019	-0.42	0.176
Krill-Early Stages	-0.173	0.03	-0.681	0.464	0.331	0.11
Krill-Late Stages	-0.727	**0.528**	0.239	0.057	0.001	0
Larvaceans	0.453	0.205	0.129	0.017	0.437	0.191
Ostracods	-0.251	0.063	-0.32	0.103	0.198	0.039
Phoronids	-0.673	0.453	-0.278	0.077	0.147	0.022
Polychaetes	0.065	0.004	-0.353	0.125	0.346	0.12
Shrimp	0.001	0	0.421	0.177	-0.078	0.006
Siphonophores	-0.197	0.039	0.753	**0.567**	-0.225	0.051

3.0 Future Directions

These 2014 data represent the first year of an on-going zooplankton monitoring program conducted by King County. Field sampling quality for 2015 benefitted from the experience gained over the first year of sampling efforts. The KC captains and crew are now familiar with the specifics of zooplankton collection on their boat, and have a year of experience in sampling and troubleshooting complications that may arise; this should help future sampling run smoothly and consistently. The addition of a depth sensor on the oblique net part way through 2014 has improved the accuracy of tow depths. Because of the similarity in species composition and abundances between the deep and shallow oblique net stations, those sample sites will most likely be revisited for the 2016 sampling year to ensure efficient use of ship resources. In addition to the abundance data summarized herein, calculations for species biomass are currently underway and will be provided as a supplement to this report when completed.

4.0 References

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Appendix A – Station Dates and Depths

Station	Date	Station Depth (m)	Target Tow Depth (m)	Sensor Depth (m)
LSNT01S				
	04/09/2014	112	30	n/a
	04/21/2014	35	30	n/a
	05/06/2014	30	30	n/a
	05/20/2014	32	30	n/a
	06/03/2014	30	30	n/a
	06/17/2014	27	30	12.9
	07/08/2014	40	30	18.5
	07/22/2014	48	30	28.02
	08/05/2014	41	30	12.38
	08/18/2014	39.9	30	22.92
	09/09/2014	39	30	24.18
	09/23/2014	42	30	35.83
	10/07/2014	50	30	n/a
	10/21/2014	50	30	n/a
LSNT01D				
	04/09/2014	218	30	n/a
	04/21/2014	217	30	n/a
	05/06/2014	219	30	n/a
	05/20/2014	218	30	n/a
	06/03/2014	215	30	n/a
	06/17/2014	217	30	19.65
	07/08/2014	215	30	19.5
	07/22/2014	220	30	38.78
	08/05/2014	216	30	13.74
	08/18/2014	218	30	32.31
	09/09/2014	214	30	23.95
	09/23/2014	212	30	30.57
	10/07/2014	215	30	n/a
	10/21/2014	215	30	n/a

Station	Date	Station Depth (m)	Target Tow Depth (m)	Estimated Tow Depth (m)
KSBP01				
	04/09/2014	286	200	200
	04/22/2014	290	200	200
	05/07/2014	284	200	200
	05/19/2014	278	200	200
	06/04/2014	286	200	200
	06/17/2014	292	200	200
	07/07/2014	276	200	200
	07/21/2014	284	200	200
	08/04/2014	284	200	200
	08/18/2014	279	200	200
	09/08/2014	281	200	200
	09/22/2014	284	200	200
	10/06/2014	283	200	200
	10/20/2014	285	200	200
	11/03/2014	284	200	200
	11/17/2014	270	200	200
	12/15/2014	283	200	200
	04/06/2015	275	200	200
LSNT01V				
	04/09/2014	218	200	200
	04/21/2014	217	200	200
	05/06/2014	219	200	200
	05/20/2014	218	200	200
	06/03/2014	215	200	200
	06/17/2014	217	200	200
	07/08/2014	215	200	200
	07/22/2014	220	200	200
	08/05/2014	217	200	200
	08/18/2014	215	200	200
	09/23/2014	215	200	200
	10/07/2014	214	200	200
	10/21/2014	211	200	200
	11/18/2014	217	200	200
	12/16/2014	215	200	200
NSEX01				
	04/09/2014	182	170	170
	04/21/2014	180	165	165
	05/06/2014	181	170	170
	05/20/2014	181	170	170

Station	Date	Station Depth (m)	Target Tow Depth (m)	Estimated Tow Depth (m)
NSEX01				
	06/03/2014	182	170	170
	06/17/2014	180	170	170
	07/08/2014	180	170	170
	07/22/2014	180	170	170
	08/05/2014	180	170	170
	08/18/2014	180	170	170
	09/09/2014	177	170	170
	09/23/2014	177	167	167
	10/07/2014	178	170	170
	10/21/2014	179	170	170
	11/04/2014	180	170	170
	11/18/2014	180	170	170
	12/16/2014	181	170	170

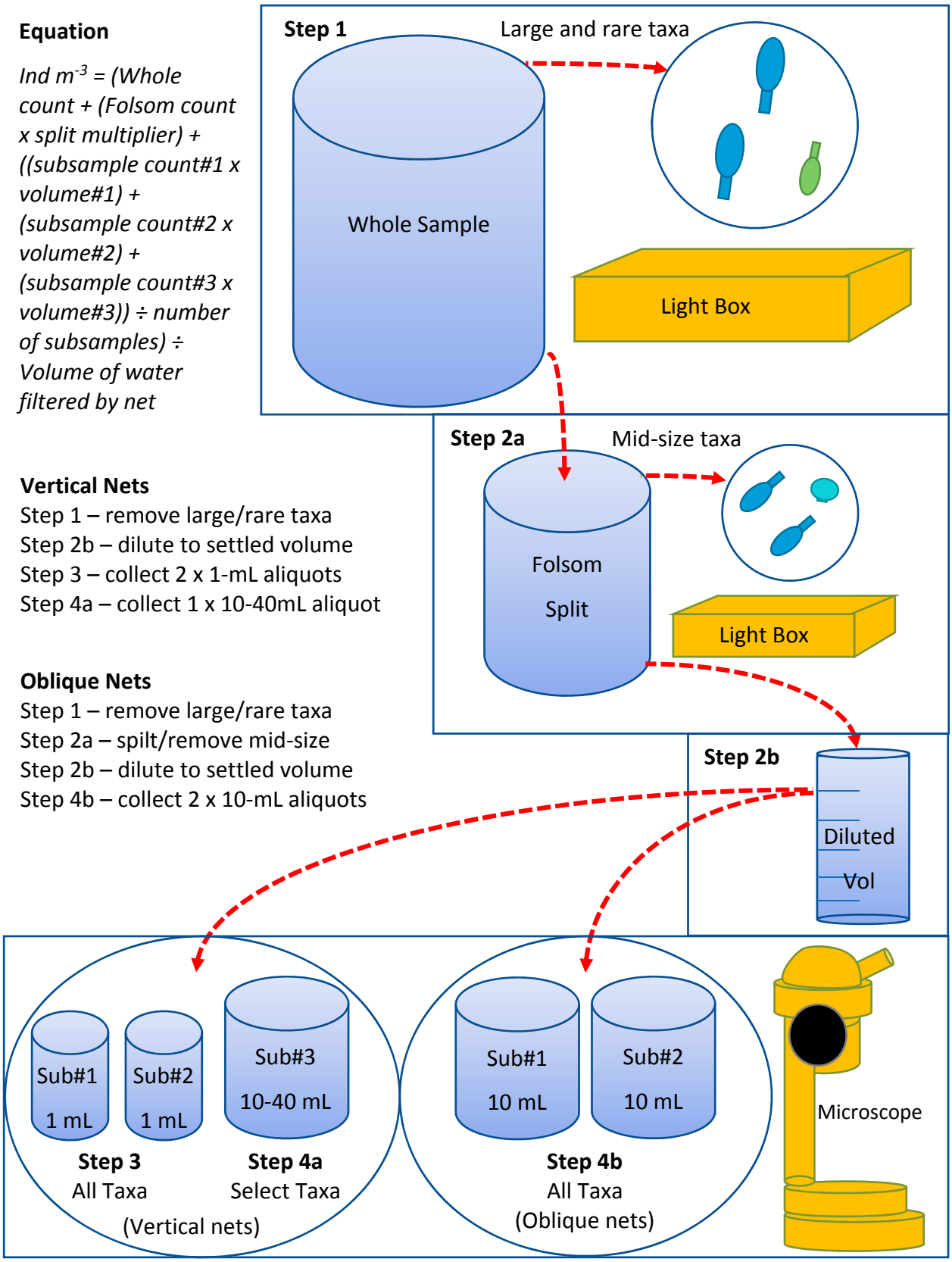
Appendix B – Lab Protocol Diagram

Equation

Ind m^{-3} = (Whole count + (Folsom count x split multiplier) + ((subsample count#1 x volume#1) + (subsample count#2 x volume#2) + (subsample count#3 x volume#3)) ÷ number of subsamples) ÷ Volume of water filtered by net

Vertical Nets
Step 1 – remove large/rare taxa
Step 2b – dilute to settled volume
Step 3 – collect 2 x 1-mL aliquots
Step 4a – collect 1 x 10-40mL aliquot

Oblique Nets
Step 1 – remove large/rare taxa
Step 2a – spilt/remove mid-size
Step 2b – dilute to settled volume
Step 4b – collect 2 x 10-mL aliquots



Appendix C – Vertical Tow Sample Analysis Guidelines

Taxa enumerated, identified, staged (differentiated by life history stage), and measured in vertical net samples. All heterotrophic organisms were counted in these samples. All organisms were speciated unless otherwise noted. For measurements, N = None, TL = Total Length, PL = Prosome Length, CL = Carapace Length, OD = Outer Diameter, H = Height. Table adapted from King County 2015.

Functional Group	Genera	Life Stage	Differentiated Stages/Sex	Measurements Taken	Notes
Copepoda - Calanoida	*Calanus*	Copepodite	1 - 5	PL	
		Adult	Female, Male	PL	
	Metridia	Copepodite	1 - 5	N	
		Adult	Female, Male	N	
Copepoda - Cyclopoida	All	Copepodite	-	N	Identified to genus only
		Adult	Female, Male	N	
Copepoda - other	All	Nauplius	-	N	All nauplii identified as “copepod nauplius”
		Copepodite	-	N	Identified to genus only (to species where possible)
		Adult	Female, Male	N	
Euphausiacea - krill	All	Egg	-	N	
		Nauplius	1 - Metanauplius	N	
		Calyptopis	1 - 3	N	
		Furcilia	1 - 7	TL	
		Juvenile	-	TL	
		Adult	-	TL	
Decapoda - crabs	All	Zoea	1 - 5	CL	
		Megalopa	-	CL	
Decapoda - shrimp	All	Not staged	-	TL	Identified to lowest practical taxonomic level, to species when common
Amphipoda	All	Not staged	-	TL	
Pteropoda	All	Not staged	-	TL	Measured along longest axis.

Functional Group	Genera	Life Stage	Differentiated Stages/Sex	Measurements Taken	Notes
Chaetognatha	All	Not staged	-	TL	Not identified further
Cnidaria - jellies	All	Not staged	-	OD, H	Identified to species when possible. Siphonophora also identified to zooid type (nectophore, eudoxid, bract, or gonophore). When numerous, only representative measurements were taken.
Ctenophora - jellies	All	Not staged	-	OD, TL	Identified to species when possible. When numerous, only representative measurements were taken.
Appendicularia	All	Not staged	-	N	Identified to genus only
Other	-	Not staged	-	N	Identified to lowest practical taxonomic unit, to species when common. Representative picture drawn or organism saved if unknown.

Appendix D – Oblique Net Tow Sample Analysis Guidelines

Taxa enumerated, identified, staged (differentiated by life history stage) and measured in oblique samples. All heterotrophic organisms sampled except the dinoflagellate *Noctiluca* were counted in these samples. For measurements, N = None, TL = Total Length, PL = Prosome Length, CL = Carapace Length, ML = Mantle Length, OD = Outer Diameter. Pteropods and bivalves were measured along the longest axis. Table adapted from King County 2015.

Phylum	Subphylum	Mid-Level Taxonomic Grouping		Species or Genus	Lowest Taxonomic Level	Life Stages Differentiated	Measurements Taken
Arthropoda	Crustacea	Amphipoda	Gammaridea	*Cyphocaris challengeri*	Species	-	TL
				Corophium	Genus	-	TL
				Other	Suborder	-	TL
			Hyperiidea	*Primno macropa*	Species	-	TL
				*Themisto pacifica**	Genus	-	TL
				Hyperoche	Genus	-	TL
				Hyperia	Genus	-	TL
				Other	Suborder	-	TL
			Caprelloidea		Suborder	-	TL
		Copepoda	Calanoida	*Epilabidocera longipedata*	Species	C5 & Adults	PL
				Paraeuchaeta	Species	C5 & Adults	PL
				Neocalanus	Species	C5 & Adults	PL
				Paraeuchaeta	Species	C5 & Adults	PL
				Eucalanus	Species	C5 & Adults	PL
				Calanus	Species	C5 & Adults	PL
			Harpacticoida		Order	-	PL
			Other		Subclass	-	N
		Decapoda (crabs)	Brachyura	Cancridae	*Glebocarcinus oregonensis*	Species	Z1 - megalopa	CL
					Metacarcinus gracilis	Species	Z1 - megalopa	CL
					Metacarcinus magister	Species	Z1 - megalopa	CL
					Romaleon antennarium	Species	Z1 - megalopa	CL

Phylum	Subphylum	Mid-Level Taxonomic Grouping			Species or Genus	Lowest Taxonomic Level	Life Stages Differentiated	Measurements Taken
Arthropoda	Crustacea	Decapoda (crabs)	Brachyura		Other	Family	Z1 - megalopa	CL
				Majidae	*Chionoecetes tanneri*	Species	Z1 - megalopa	CL
					Oregonia gracilis	Species	Z1 - megalopa	CL
					Other	Family	Z1 - megalopa	CL
				Xanthidae	*Lophopanopeus bellus*	Species	Z1 - megalopa	CL
					Other	Family	Z1 - megalopa	CL
				Pinnotheridae	*Fabia subquadrata*	Species	Z1 - megalopa	CL
					Pinnixa	Genus	Z1 - megalopa	CL
					Other	Family	Z1 - megalopa	CL
				Varunidae	*Hemigrapsus oregonensis*	Species	Z1 - megalopa	CL
				Epialtidae	*Pugettia*	Genus	Z1 - megalopa	CL
				Other		Infraorder	Z1 - megalopa	CL
			Anomura	Paguroidea	*Discorsopagurus schmitti*	Species	Z1 - megalopa	CL
					Pagurus hirsutiusculus	Species	Z1 - megalopa	CL
					Pagurus ochotensis	Species	Z1 - megalopa	CL
					Other	Superfamily	Z1 - megalopa	CL
				Porcellanidae	*Petrolisthes cinctipes*	Species	Z1 - megalopa	CL
					Petrolisthes eriomerus	Species	Z1 - megalopa	CL
					Pachycheles	Genus	Z1 - megalopa	CL
					Other	Family	Z1 - megalopa	CL
				Lithodidae	*Lopholithodes*	Genus	Z1 - megalopa	CL
				Galatheoidea		Superfamily	Z1 - megalopa	CL
				Other		Infraorder	Z1 - megalopa	CL

Phylum	Subphylum	Mid-Level Taxonomic Grouping			Species or Genus	Lowest Taxonomic Level	Life Stages Differentiated	Measurements Taken
Arthropoda	Crustacea	Decapoda (Shrimp)	Caridea	Crangonidae		Family	-	TL
				Hippolytidae		Family	-	TL
				Pandalidae		Family	-	TL
				Pasiphaeidae		Family	-	TL
				Alpheidae		Family	-	TL
				Upogebiidae		Family	-	TL
				Other		Infraorder	-	TL
			Axiidae	Callianassidae	*Neotrypaea californiensis*	Species	-	TL
					Other	Family	-	TL
		Cumacea				Order	-	TL
		Mysida		Mysidae	*Orientomysis hwanhaiensis*	Species	-	TL
					Alienacanthomysis macropsis	Species	-	TL
					Archaeomysis grebnitzkii	Species	-	TL
					Pacifacanthomysis nephrophthalma	Species	-	TL
					Exacanthomysis davisi	Species	-	TL
					Neomysis mercedis	Species	-	TL
					Other	Family	-	TL
		Euphausiacea (krill)			*Euphausia pacifica*	Species	N1 - Adult	TL: F1 - Adults
					Thysanoessa longipes	Species	N1 - Adult	TL: F1 - Adults
					Thysanoessa raschii	Species	N1 - Adult	TL: F1 - Adults
					Thysanoessa spinifera	Species	N1 - Adult	TL: F1 - Adults
		Cirripedia (barnacles)				Infraclass	Nauplius - Cyprid	N

Phylum	Subphylum	Mid-Level Taxonomic Grouping		Species or Genus	Lowest Taxonomic Level	Life Stages Differentiated	Measurements Taken
Arthropoda	Crustacea	Cladocera		*Podon*	Genus	-	N
				Evadne	Genus	-	N
		Isopoda			Order	-	TL
		Ostracoda			Class	-	N
		Arachnida (terrestrial spiders)			Class	-	TL
Mollusca	Cephalopoda	Teuthida			Order	-	ML
		Octopoda			Order	-	ML
	Gastropoda	Pteropoda		*Clione limacina**	Genus	-	TL
				*Limacina helicina**	Genus	-	TL
				*Clio pyramidata**	Genus	-	TL
				Other	Polyphyletic	-	TL
		Other			Class	-	N
	Bivalvia				Class	-	TL
	Other				Phylum	-	N
Chordata	Tunicata	Ascidiacea			Class	-	N
		Appendicularia			Class	-	N
	Actinopterygii (ray-finned fishes)				Class	Egg - Juvenile**	OD/TL
Cnidaria	Hydrozoa	Leptomedusae		*Aequorea victoria**	Genus	-	N
		Siphonophorae	Physonectae	*Muggiaea atlantica**	Genus	-	N
				Other	Suborder	-	N
			Calycophorae	*Nanomia bijuga**	Genus	-	N
				Other	Suborder	-	N
		Other			Class	-	N

Phylum Subphylum	Mid-Level Taxonomic Grouping	Species or Genus	Lowest Taxonomic Level	Life Stages Differentiated	Measurements Taken
Cnidaria	Scyphozoa	*Aurelia labiata**	Genus	-	N
		Other	Class	-	OD
Ctenophora		*Pleurobrachia bachei**	Genus	-	N
		Beroe	Genus	-	N
Dinoflagellata		*Noctiluca*	Genus	-	N
Bryozoa			Phylum	-	N
Chaetognatha			Phylum	-	TL
Echinodermata			Phylum	-	N
Nemertea			Phylum	Larva, other	N
Annelida	Polychaeta	*Tomopteris*	Genus	-	TL
		Other	Class	Trochophore	TL
Various	Egg		***	-	OD

* Identified to genus; species is assumed from previous records in the region.
*** Unknown

Appendix E – Vertical Net Sample Species List

Phylum	Subphyllum	Class	Subclass	Infraclass	Order	Infraorder	Family	Lowest Taxonomic Level Identified
Dinoflagellata								*Noctiluca*
Cnidaria		Hydrozoa			Anthomedusae			*Aglantha digitale** *Euphysa* *Leuckartiara* *Proboscidactyla flavicirrata** *Rathkea* *Sarsia tubulosa** Anthomedusa
					Leptomedusae			*Aequorea victoria** *Clytia gregaria** *Mitrocoma cellularia** Leptomedusa
					Siphonophorae			*Muggiaea atlantica* *Nanomia bijuga*
					Unknown			Hydromedusa Hydrozoan polyp
		Scyphozoa						Scyphomedusa
Ctenophora		Tentaculata			Cydippida			*Pleurobrachia bachei*
		Unknown			Unknown			Ctenophore
Platyhelminthes								Flatworm
Nemertea								Nemertean
Nematoda								Nematode

Phylum	Subphyllum	Class	Subclass	Infraclass	Order	Infraorder	Family	Lowest Taxonomic Level Identified
Annelida		Polychaeta						*Tomopteris*
								Polychaete
Arthropoda	Crustacea	Copepoda			Calanoida			*Acartia hudsonica*
								Acartia longiremis
								Aetideus divergens
								Calanus marshallae
								Calanus pacificus
								Candacia columbiae
								Centropages abdominalis
								Clausocalanus
								Eucalanus bungii
								Mesocalanus tenuicornis
								Metridia pacifica
								Microcalanus pusillus
								Neocalanus cristatus
								Paracalanus parvus
								Pseudocalanus mimus
								Pseudocalanus minutus
								Pseudocalanus moultoni
								Pseudocalanus newmani
								Scolecithricella minor
								Tortanus discaudatus
					Cyclopoida			*Corycaeus anglicus*
								Hemicyclops
								Oithona atlantica
								Oithona similis

Phylum	Subphyllum	Class	Subclass	Infraclass	Order	Infraorder	Family	Lowest Taxonomic Level Identified
Arthropoda	Crustacea	Copepoda			Cyclopoida			*Oncaea borealis* *Oncaea subtilis*
					Harpacticoida			Harpacticoid
					Monstrilloida			Monstrilloid
		Ostracoda						Ostracod
						Cladocera		*Evadne* *Podon* Cladoceran
				Cirripedia				Barnacle nauplius Barnacle cyprid
					Mysida			*Alienacanthomysis macropsis* *Pacifacanthomysis nephrophthalma*
					Cumacea			Cumacean
					Amphipoda		Gammaridae	*Cyphocaris challengeri* Gammarid
							Hyperiidae	*Themisto pacifica* *Hyperia* *Hyperoche* Hyperiid
							Caprellidae	Caprellid
					Isopoda			Isopod
					Euphausiacea		Euphausiidae	*Euphausia pacifica* *Thysanoessa raschii* *Thysanoessa*

Phylum	Subphyllum	Class	Subclass	Infraclass	Order	Infraorder	Family	Lowest Taxonomic Level Identified
Arthropoda	Crustacea				Decapoda	Caridea	Alpheidae	Alpheid
							Callianassidae	*Neotrypaea californiensis*
							Crangonidae	Crangonid
							Hippolytidae	Hippolytid
							Pandalidae	Pandalid
							Pasiphaeidae	*Pasiphaea pacifica*
						Anomura	Paguridae	*Pagurus*
							Porcellanidae	*Petrolisthes eriomerus*
								Pachycheles
						Brachyura	Xanthidae	*Lophopanopeus bellus*
							Grapsidae	*Hemigrapsus nudus*
								Hemigrapsus oregonensis
							Pinnotheridae	*Fabia subquadrata*
								Pinnixa littoralis
							Epialtidae	*Pugettia*
							Cancridae	*Glebocarcinus oregonensis*
								Metacarcinus gracilis
								Metacarcinus magister
								Romaleon antennarium
Mollusca		Polyplacophora						Chiton egg
		Gastropoda						Gastropod
				Opisthobranchia				*Gastropteron*
								Limacina helicina
		Bivalvia						Bivalve
		Cephalopoda						Octopus

Phylum	Subphyllum	Class	Subclass	Infraclass	Order	Infraorder	Family	Lowest Taxonomic Level Identified
Phoronida								Phoronid actinotroch
Bryozoa								Bryozoan cyphonaut
Chaetognatha								Chaetognath
Echinodermata								Echinoderm larva
Chordata	Tunicata	Ascidiacea						
		Appendicularia						*Fritillaria, Oikopleura*
	Vertebrata	Actinopterygii						Fish
Unknown								Egg Unknown

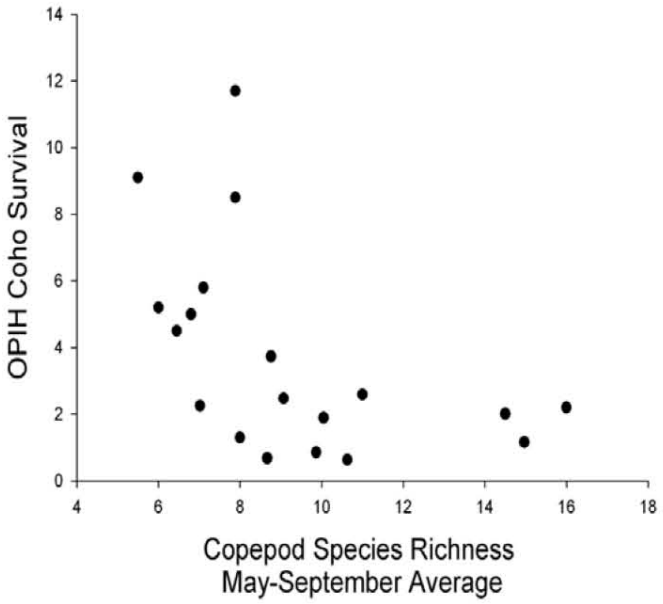
* Identified to genus; species is assumed from previous records in the region.

Appendix F – Zooplankton Sampling Protocol

Last updated by Julie E. Keister, 5 May 2014

These protocols are designed for monitoring zooplankton in Puget Sound for two different objectives: 1) To address how environmental variability affects Puget Sound's ecosystem through changes in zooplankton and 2) To measure how the prey field of salmon and other fish varies spatio-temporally and correlates with survival. The first type of sampling can be used to develop what is referred to in this document as "**Ecosystem Indicators**." The second type provides "**Prey Field Indicators**." Both have been used in other systems to understand how climate variability affects ecosystems and fish survival; indicators developed from both types of sampling have shown strong correlations to fish survival and have helped elucidate the mechanisms by which climate variability affects fish populations.

For example, the "**Ecosystem Indicator**" protocols are based on sampling off Oregon and Washington used by NOAA NWFSC to link climate variability to salmon survival through changes in zooplankton (e.g., [*Keister et al.*, 2011; *Peterson*, 2009; *Peterson and Schwing*, 2003]. The indices developed from this type of sampling strongly correlate with salmon returns and are used in NOAA's "Red-Light, Green-Light" forecasts of salmon returns (see http://www.nwfsc.noaa.gov/research/divisions/fe/estuarine/oeip/ea-copepod-biodiversity.cfm). Another example of use of this type of zooplankton index comes from studies of cod survival in the North Sea ([*Beaugrand and Reid*, 2003; *Beaugrand et al.*, 2003] which revealed that an index of copepod species composition correlates with cod recruitment – larger copepod species dominate during cold climate regimes, which translates to higher growth (and thus survival and recruitment) of cod. These types of indices are powerful components of fish population forecasts. Similar indices can be developed in Puget Sound to add to our understanding of how environmental variability affects fish populations.



Relationship between survival of hatchery-raised coho salmon and copepod species richness off Oregon sampled by vertical net tows. The plot compares data from the summer that the fish entered the ocean. Coho return to their natal streams/hatcheries 18 months after entering the sea. Adapted from Peterson (2009).

The "Prey Field Indicator" protocols are based on sampling that Oregon State University and NOAA NWFSC uses to quantify juvenile salmon prey abundance to understand controls on juvenile salmon survival off Oregon and Washington. As part of the Bonneville Power Administration (BPA) project, prey field sampling off OR and WA has been conducted since 1998. An index of the zooplankton calculated from Bongo net sampling as described below

correlate strongly with salmon growth and survival (C. Morgan, OSU, pers. comm.). The best station depth(s) to sample has not yet been determined and is under discussion and will depend upon initial sampling and analyses. Where capacity allows, sampling stations of several different station depths will help provide the data needed to refine these recommendations.

Monitoring protocols (see Field Methods below for more detail)

Equipment

Ecosystem Indicator sampling protocol: vertical tows

- Bongo or ring net: 50-100 cm diameter (60-cm preferred), 150-212 µm mesh (200 µm preferred), 4:1 or 5:1 filtering ratio (i.e., length:width ratio – longer is better if boat can handle it). Cod end: 3.5-4.5" diameter x 6" length or larger (4.5' x 6" preferred), of same (preferred) or smaller mesh size.
- Flow meter, TSK style preferred. (See section below on flow meters.)
- Daytime sampling
- Vertical tow, sampled at a location that is ideally ~200 m water depth, or at the deepest location in the area.
- Lifted vertically from 5 m off bottom to the surface , deployed and immediately retrieved at 30 m/min. [hand-hauls will almost always be too slow]
- [For the SSMS Monitoring Program: 60-cm diameter ring net, 200 um mesh, 5:1 filtering ratio outfitted with TSK flow meter]

Prey Field Indicator sampling protocol: oblique tows

- 60-cm bongos (preferred) or 1-m diameter ring net, 335 µm mesh.
- Black mesh nets (rather than the standard white).
- Cod end: 4.5" diameter x 12" length, of same (preferred) or smaller mesh size.
- Flow meter required (can be 'torpedo' style from SeaGear Corp or General Oceanics)
- Daytime tows
- Sampled at consistent locations of various water depths, TBD based on location, ideally 3 locations bracketing nearshore to deepest local spot (e.g., 30 m, 50 m, 100 m water depth) trying to sample over constant water depth during the whole tow when conditions allow (tow along a bathymetry contour).
- Towed over upper 30 m where depths are sufficient (net deployed until it is at 30 m depth, then immediately retrieved for a 'double-oblique' tow).
- Towed at 1.5 kts (minimum) to 2 kts, deployed and retrieved with a 30 m/min wire speed, optimally maintaining a 45° wire angle when possible. Adjust amount of line let out to accommodate for actual angle to achieve target depth (see Table below).
- [For the SSMS Monitoring Program: 60-cm diameter black bongo nets, 335 um mesh, outfitted with SeaGear flow meter]

1. Net description – Contact me for recommended vendors if needed

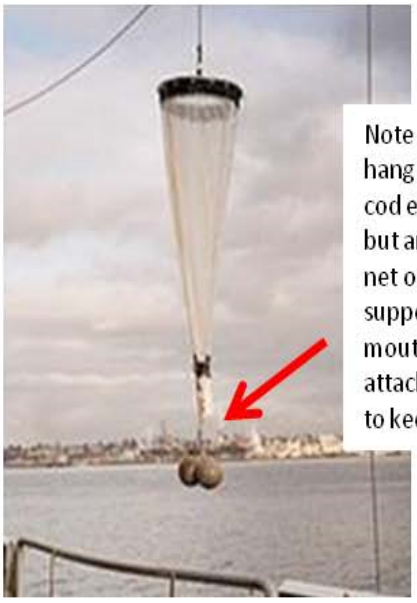
Ring and bongo (double ring) nets are described by their mouth diameter, mesh size, and their filtering ratio. Ring size is given in cm or m; mesh size in micrometers (microns, µm).

The filtration ratio is a description of the length-to-mouth ratio; the larger the filtration ration, the longer the net will be and the less likely the net will clog. We recommend 4:1 or 5:1 – higher is better, but if you work off a small boat, the shorter net is slightly easier to deploy, retrieve, and wash, but the downside is that it clogs more easily which results in a lower quality sample and more time rinsing the net.

The cod end is a removable durable plastic cylinder with holes cut in the sides that are covered with mesh of the appropriate size. The cod end should ideally be the same (or slightly smaller) mesh size as the net. If the mesh size of the cod end and the net disagree, record whichever mesh is larger as that will be the retention size.

Weighting the nets: Some weight added to the net is necessary to make the net sample correctly.

Vertical net with weights attached



Note that the weights hang slightly below the cod end when deployed, but are not pulling on the net or cod end, they are supported from the mouth ring and loosely attached to the cod end to keep it below the net.

Weighting vertical nets is typically done using a 3-string harness made of line. Tie the ends of the 3 lines to the upper net ring (not to the net or cod end itself), equidistant apart. *Make sure the weight lines are long enough to hang ~1 foot below where the cod end will hang when stretched*, tie the bottom ends of the cords to a metal O-ring to attach to the weight. With a small line, tie the cod end to the O-ring with plenty of slack to avoid pulling on the cod end when the weight lines are stretched (~1.5-2 feet of line). This will hold the cod end down near the weight to prevent tangling. *Be careful that the line to the cod end isn't so short that it will stretch the net toward the weight when deployed – that could rip the net. **The net and cod end should never feel the weight.** Attach weights to the O-ring before deployment. [Weighted cod ends are available, but aren't heavy enough to sink the net vertically except when it's very calm.]

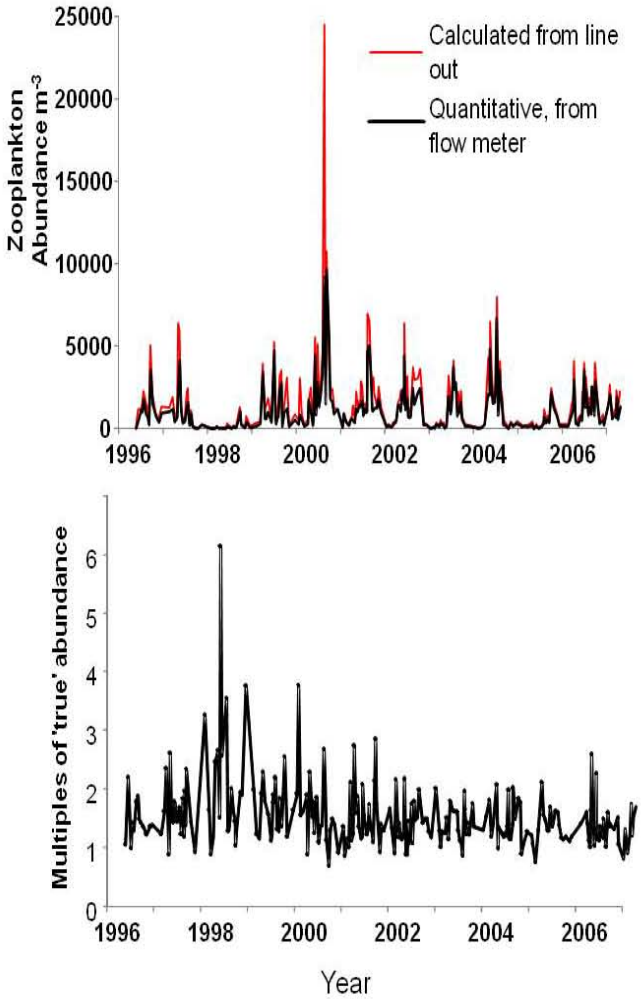
In calm weather with a vertically-lifted net, only enough weight to keep the cod end below the mouth of the net while dropping is needed (maybe 5 lbs). In rough conditions, if there's a strong wind or current, or if undertaking an oblique tow, more weight is needed (20+ lbs). The rougher the seas/current, the more weight that is necessary.

Weighting obliquely-towed ("horizontal") nets is done by attaching a weight to a mid-point on the rings with a short amount of line (e.g., center tow point of the bongo net frame). When lifted by the towing cable, the net opening should be about perpendicular to the deck. This will help the net sample with the mouth opening normal to the water. Rough seas, strong currents, or deeper tows may require more weight to help the net sink to the desired depth. 50+ lbs is not uncommon, but 30-35 lbs is typical.

2. Flow meters

A flow meter is ***absolutely necessary*** to provide quantitative abundance and biomass measures, especially for oblique tows (see plot below). The only exception is where vertical nets are used in shallow, calm waters. If your net always deploys with no net angle (perfectly vertically), then the mouth area x sampling depth can be used to calculate the water volume filtered. If there is any net angle, the net is towing and will sample more water; a flow meter is then required to quantify the volume filtered.

There are many types of flow meters available. However, only a few types are suitable for measuring flow through a vertically-towed net. For vertical tows, the preferred model is a TSK flow meter (http://www.tsk-jp.com/tska/contact.html), which is the only flow meter we've found that is reliably accurate on vertical tows. The problem with most flow meters is that they spin when being deployed (while the net is going down) and retrieved, but not equally in both directions. The TSK style has a 'back-stop' to prevent spinning when going down backwards and a 3-point attachment so they don't flip upside down on depolyment. They are also preferred because they are simple and heavy-duty (which makes for easier maintenance and very rare damage). However, the TSK style requires that the net is retrieved fast enough to depress the backstop and make the propeller spin (or inaccurately low readings will result). They can also be tricky to learn to read and can be costly (>$1000). Other brands are General Oceanics and SeaGear.net – those manufacturers make 'torpedo style' models with back-stops (e.g., SeaGear # MF315), but don't have a good way to mount them in the net mouth that prevents them from flopping over and spinning on deployment.



Without a flow meter, abundances are typically overestimated by ~1.5-2.5 times, occasionally 3-6 times. The overestimate is unpredictable, so can't be corrected for.

Torpedo style flow meters are preferred for oblique tows (e.g., SeaGear # MF315, ~$330, also see General Oceanics). No back-stop is needed for oblique tows.

Field Methods

- **Record** date, time, location, water depth, name of sampler, weather state, etc. on the field sheet.
- **Rig** the nets, attach weights, check equipment for holes, tangles, and loose fittings.
- **Reset** the flow meter to zero (TSK or SeaGear models) or record initial counts (other torpedo models).
- **Deploy** the net at 30 meters/min wire speed to desired depth. When at deepest depth, immediately retrieve the net at 30 m/min.
  For vertical nets, deploy at 30 m/min to 5 m from the bottom, or to a maximum of 200 m in deep water. Record the line angle and, if it's not perfectly vertical, increase the line out to achieve the target depth, calculating total line out to reach target depth from the wire angle (see table below). Retrieve immediately at 30 m/min. Visually check that the flow meter is spinning as it approaches the surface – if not, the retrieval rate may not have been fast enough or the flow meter needs inspection.
  For obliquely-towed nets, deploy to ~30 m depth (or 5-10 m off bottom in shallower water) with the boat moving at ~1.5-2 kts. Steadily let out line at 30 m/min, calculating the amount to let out based on angle (read from table below) to achieve 30 m depth, retrieve immediately at 30 m/min while the vessel is underway, maintaining ~45 degree line angle when possible. If wire angle is regularly >60 degrees, add more weight. For any particular boat, net, and current conditions, the goal is to adjust the total weight of the net (using added weights) needed to get that 45° target angle at 1.5-2 kts ship speed—too little drag or too much weight on the net will cause the net to sample too deep; too much drag or too little weight will keep the net too shallow. This is something you may need to play with at first to optimize. Try not to decrease boat speed to <1.5 kts or strongly swimming organisms will be undersampled – instead, add more weight.
- **Retrieve** the net immediately upon reaching the surface (don't linger just below surface), taking care not to let the flow meter spin in the breeze if windy (note in the log if it does).
- **Rinse** the net downward from the outside using a seawater hose (ideally) or buckets and a hand held sprayer (such as a Spray Doc) to concentrate the sample in the cod end. Be fairly gentle so you don't destroy delicate critters during rinsing. Pay special attention to seams that catch organisms. Once you're satisfied on visual inspection that the plankton are all rinsed into the cod end, unhook it *being careful that it is not full to the top* – if it is, wait for it to drain, or open the cod end over a bucket, so you don't lose any sample, then strain the contents of the bucket through a sieve (or the cod end) to concentrate.
- **Concentrate the organisms in the cod end**, then pour and thoroughly rinse contents into a sample jar, using a funnel if necessary. Use the smallest jar necessary, but do not crowd the sample or it will not preserve well – if the biomass is thick (more than ½ of the jar volume) use a larger jar or split into two jars. Leave enough room for preservative.
  [Note: we've used 700 mL sample jars most often in Puget Sound, but sometimes a larger jar is necessary if ctenophores are dense, or the sample is full of phytoplankton and very slow to drain. Oblique tows may result in larger samples.]
- **Preserve** the sample using neutrally-buffered formalin, adding enough to make the final formalin concentration ~5% (i.e., add 35 ml of buffered formalin to a 700 mL sample jar containing your sample, top off to the threads with seawater to create a 5% formalin solution). It is handy (and safest) to use a dispensette, or a squeeze bottle with a

measured reservoir dispenser (these are great for this: http://www.usplastic.com/catalog/item.aspx?itemid=22892). Always top off the jar to the bottom of the threads with seawater to prevent dehydration. Close tightly and swirl to mix.

All personnel who handle formalin should be familiar with its dangers, protective equipment, and with what to do in case of a spill. Provide absorbent pads in case of spill and an MSDS (http://www.fishersci.com/ecomm/servlet/msdsproxy?productName=F79P4&productDescription=FORMALDEHYDE+ACS+POLY+4L&catNo=F79P-4&vendorId=VN00033897&storeId=10652).

Note: When you purchase formalin, it typically comes unbuffered. You need to add a buffer (we use Borax or baking soda) to bring it to a pH of ~8.2 (surface seawater pH). You can do this by adding the buffer in excess, mixing well, and letting sit for >24 hrs to saturate. The excess will precipitate out, which can get in the way of dispensing, so it's good to buffer in large containers (the original shipping bottles), then dispense into the squeeze dispensers after settling for >24 hrs. [Formalin is the same as 37% formaldehyde.]

- **Label** the jar (We usually write on the jar lid with a Sharpie if it is a matt surface (won't wipe off), or on a label stuck on the side of the jar) with Project, Date, Time, Station name/number, Net mesh size, Net ring size, type of tow (vertical or oblique), depth of tow, and flow meter reading. It is preferable to also make a label for the inside of the jar (in case the outside label gets wiped off, or lids switched accidentally, etc) using waterproof paper and pencil. Label the same things as the lid, plus the lat/long of the station sampled if it is not a consistent location.
  [Note that for the SSMSP Monitoring, it is sufficient to label the jar with: SSMSP, date, station, type of tow (vertical or bongo), flow meter reading) if a field sheet is also filled out.
- Complete the **field sheet** for the station, recording the flow meter reading and wire angle. Note anything unusual about the sampling.

Analysis protocols

The Ecosystem Indicator samples must be analyzed by an expert zooplankton taxonomist. Protocols for analyzing the Prey Field Indicator samples will be provided on request once time series are established.

Acknowledgments

These protocols were written in collaboration with experts in Oregon and British Columbia (W. Peterson (NOAA), C. Morgan (OSU), M. Trudel (DFO)) who have established zooplankton monitoring programs.

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