Salish Sea Marine Survival Project: Zooplankton Monitoring Program 2014-2015 Final Report

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Project Oversight and Report Preparation

The 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 King County Department of Natural Resources and Parks – Water and Land Resources Division, Kwiáht, the Nisqually Indian Tribe, NOAA, the Port Gamble S'Klallam Tribe, the Tulalip Tribe, Lummi Nation, the WA Department of Fish and Wildlife, and the WA Department of Ecology. Taxonomic analysis was conducted by Amanda Winans, BethElLee Herrmann, Rachel Wilborn, and Olga Kalata at the University of Washington.

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From Long Live the Kings, we thank Michael Schmidt and Iris Kemp. From King County, we thank Kimberle Stark, Amelia Kolb, Wendy Eash-Loucks, Christopher Barnes, the King County Environmental Laboratory field scientists, and the captain and crew of the *R/V Liberty*. From the Tulalip Tribe, Mike Crewson, Max Lundquist, and Todd Zackey. From Lummi Nation, Evelyn Brown and Mike MacKay. From Kwiáht, Russel Barsh and Madrona Murphy. From the Nisqually Indian Tribe, Jed Moore, Chris Ellings, Emiliano Perez, and Walker Duval. From NOAA, Correigh Greene, Jason Hall, and Dan Lomax. From the Port Gamble S'Klallam Tribe, Hans Daubenberger and Juliana Sullivan. From the Department of Fish and Wildlife, Mark Millard. From the WA Department of Ecology, Nate Schwarck, Jay Dimond, Julia Bos, and Christopher Krembs. We would like to thank all of the many field crew members who assisted with these collections over the years.

We would also like to thank our collaborators Moira Galbraith and Kelly Young from Fisheries and Oceans Canada Institute of Ocean Sciences for their expert guidance in species identification and Cheryl Morgan from Oregon State University for assistance in designing sampling and analysis protocols.

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Overview

This report is the zooplankton analysis for the Salish Sea Marine Survival Zooplankton Monitoring Project (SSMSP) covering the first two years of the program, 2014-2015 and describe spatial and temporal variations using abundances, biomass, and NMS Ordinations. In addition to this report, we analyzed an existing zooplankton time series from the 2003-2015 Joint Effort to Monitor the Straits (JEMS) currently run by the WA Dept. of Ecology.

The Salish Sea Marine Survival Project has been collecting and analyzing zooplankton since 2014 as part of a marine monitoring program to the explore relationships between zooplankton, climate, and juvenile salmon survival. Zooplankton samples were collected twice per month, March through October, by eight groups at fourteen stations throughout Puget Sound (Figure 1) using two types of net tows: a single ring net towed vertically to sample zooplankton throughout the full water column; a double-ring (bongo) net towed obliquely through the upper 30 m of the water column to sample the larger, more motile zooplankton.

The geographic scope of this project includes seven major regions of the Salish Sea: Bellingham Bay and the San Juan Archipelago to the north of Puget Sound; Admiralty Inlet, Whidbey Basin, Central Basin, South Sound, and Hood Canal within Puget Sound. The region is a dynamic estuarine ecosystem directly influenced by the Pacific Ocean, as well as several major rivers and their watersheds. The study includes regions of densely populated metropolitans including Seattle, Everett, and Tacoma, which makes it particularly susceptible to anthropogenic influences. Additionally, the effects of global climate change (e.g. ocean acidification, hypoxia, increasing temperatures) are of great concern (Deppe et al. 2013; Fresh et al. 2011). Since zooplankton can be affected by environmental and anthropogenic influences, they are key indicators in the marine ecosystem, and shifts in their species composition and abundances can greatly impact the marine food web. Due to the paucity of zooplankton data from Puget Sound and surrounding regions, an established baseline is required to assess future shifts in zooplankton communities and predict the effects these changes may have on the ecosystem.

Summary of Samples

In 2014, six groups participated in the SSMSP zooplankton sampling: King County (KC), Kwiáht (KWT), the Nisqually Indian Tribe (NIT), NOAA, the Port Gamble S'Klallam Tribe (PGST), and the Tulalip Tribe (TUL). A total of 216 bongos samples and 142 vertical net samples were collected in 2014, and are currently archived at UW (Table 1). In 2015, the Lummi Nation (LUM) and WDFW began sampling. Currently, 325 bongo and 184 vertical samples collected in 2015 are archived at the UW.

Since the inception of the project, we initiated and improved the zooplankton monitoring program including development and refinement of field and analysis protocols, training and oversight of field crews. In 2014, several field sampling challenges and constraints were identified which led to equipment and protocol improvements which substantially improved the sampling consistency among groups, particularly for the oblique bongo sampling. As a result, the number of high-quality samples that were collected increased from 92% of the total in 2014 to 98% in 2015.

Table 1. Summary of SSMSP samples collected and analyzed.

Summary of 2014-2015 SSMSP samples collected and 2011-2015 JEMS samples. Low Quality samples were not processed due to various field equipment failures or lack of recorded flow volume. Unfunded samples are those collected by sampling groups, but not funded through SSMSP (mostly winter samples and UW 2015 samples), and therefore not processed. Processed – Not Analyzed samples are those not included in the following analyses due to flow meter or station-name inconsistencies.

2014 SSMSP Samples	KC	KWT	LUM	NIT	NOAA	PGST	TUL	Sub Totals
Bongo Totals	**29**	**38**	**0**	**73**	**20**	**6**	**50**	**216**
Processed & Data analyzed	28	38	0	73	18	0	49	206
Low Quality	1	0	0	0	2	6	1	10
Vertical Totals	**50**	**19**	**0**	**25**	**10**	**18**	**20**	**142**
Processed & Data analyzed	49	18	0	25	10	18	3	123
Low Quality	1	1	0	0	0	0	17	19
2014 Totals	**79**	**57**	**0**	**98**	**30**	**24**	**70**	**358**

2015 SSMSP Samples	KC	KWT	LUM	NIT	NOAA	PGST	TUL	Sub Totals
Bongo Totals	**34**	**32**	**42**	**68**	**24**	**48**	**77**	**325**
Processed & Data analyzed	34	31	31	60	17	45	72	294
Low Quality	0	0	2	5	1	0	2	10
Processed - Not analyzed	0	1	9	3	6	3	3	24
Vertical Totals	**64**	**18**	**14**	**23**	**11**	**28**	**26**	**184**
Processed & Data analyzed	64	18	14	22	11	28	26	183
Processed - Not analyzed	0	0	0	1	0	0	0	1
2015 Totals	**98**	**50**	**56**	**91**	**35**	**76**	**103**	**509**

Methods

1.1 SSMSP Sampling and Analysis

See the Zooplankton Sampling Protocol v.7 (J. Keister, 22 October 2015) provided in the Appendix for full detail.

From March 2014 through December 2015, fourteen locations throughout the Salish Sea and Puget Sound were sampled: Eliza Island (ELI) in Bellingham Bay; Cowlitz (COW) and Watmough Bay (WAT) at the north and south of the San Juan Islands; Hope Island (HOPE) and North Saratoga Passage (SARA) at the north end of Whidbey basin; Camano Head (CAM) and Mukilteo (MUK) at the south end of Whidbey basin; Admiralty Inlet (ADI) and Thorndyke Bay (TDB) before and after the mouth of Hood Canal; Point Jefferson (KSBP01), Point Wells (LSNT01), and East Passage near Maury Island (NSEX01) in Central Puget Sound; and South Ketron (SKET) and Dana Passage (DANA) in South Sound (Figure 1, Table 2). Field collections were conducted by King County (KC), Kwiáht (KWT), Lummi Nation (LUM), Nisqually Indian Tribe (NIT), NOAA, Port Gamble S'Klallam Tribe (PGST), and Tulalip Tribe (TUL). All collections were made during daytime. 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. *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). A depth sensor (*ReefNet* Sensus Ultra) was attached to the bongo net frame to accurately record tow depths and determine if target depths and good tow profiles 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.

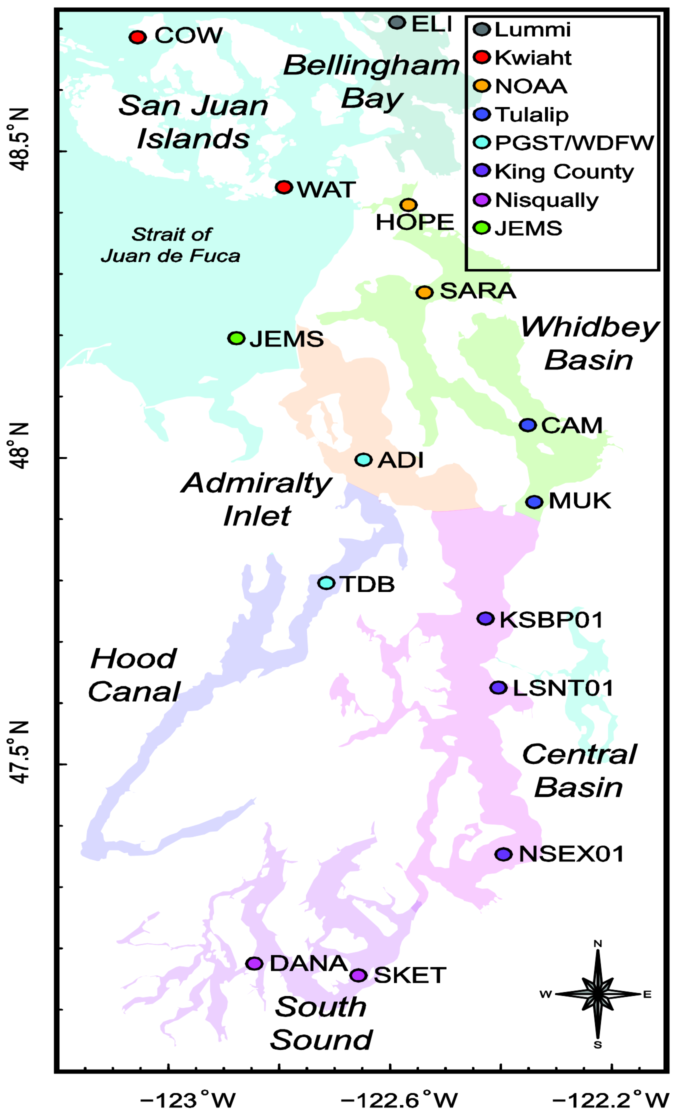

Figure 1. Map of the SSMSP and JEMS Monitoring stations.

Table 2. SSMSP Zooplankton Monitoring station information

Station names, locations, and depths of SSMSP vertical and oblique zooplankton tows. Station Code suffixes designate sites within the region of the Group where vertical and oblique shallow, mid, and deep station tows were conducted (e.g. ELIV, ELIS, ELID are vertical, bongo-shallow, and bongo-deep, respectively).

Site	Station Code	Group	Target Latitude	Target Longitude	Station Depth (m)	Target Tow Depth (m)
Vertical Tows						
Eliza Island	ELIV	LUM	48.64	-122.57	114	109
Cowlitz	COW3V	KWT	48.68	-123.04	66	61
Cowlitz	COW3V2	KWT	48.67	-123.05	75	70
Watmough Bay	WAT3V	KWT	48.44	-122.79	50	45
North Hope Island	HOPEV	NOAA	48.41	-122.58	37	32
North Saratoga Passage	SARAV	NOAA	48.26	-122.54	73	68
Camano Head	CAMV	TUL	48.06	-122.39	186	181
Admiralty Inlet	ADIV	PGST	48.00	-122.64	122	117
Mukilteo	MUKV	TUL	47.97	-122.32	200	200
Thorndyke Bay	TDBV	PGST	47.78	-122.73	116	111
Point Jefferson	KSBP01V	KC	47.74	-122.43	277	200
Fauntleroy (Point Wells)	LSNT01V	KC	47.53	-122.43	212	200
Maury Island (East Passage)	NSEX01V	KC	47.36	-122.39	180	170
Dana Passage	DANAV	NIT	47.18	-122.83	52	47
South Ketron/Solo Point	SKETV	NIT	47.15	-122.66	150	120
Oblique Tows						
Cowlitz	COW1S	KWT	48.68	-123.04	30	25
Cowlitz	COW2D	KWT	48.68	-123.04	64	30
Cowlitz	COW2D2	KWT	48.67	-123.05	72	30
Eliza Island	ELIS	LUM	48.64	-122.59	31	26
Eliza Island	ELIM	LUM	48.64	-122.59	54	30
Eliza Island	ELID	LUM	48.63	-122.59	87	30
Watmough Bay	WAT2D	KWT	48.44	-122.80	50	30
Watmough Bay	WAT1S	KWT	48.43	-122.80	29	24
North Hope Island	HOPED	NOAA	48.41	-122.57	38	30
North Hope Island	HOPES	NOAA	48.40	-122.56	18	13
North Saratoga Passage	SARAS	NOAA	48.27	-122.55	23	18
North Saratoga Passage	SARAD	NOAA	48.26	-122.54	73	30
Camano Head	CAMD	TUL	48.07	-122.40	100	30
Camano Head	CAMM	TUL	48.07	-122.39	50	30

Camano Head	CAMS	TUL	48.06	-122.39	28	23
Admiralty Inlet	ADID	PGST	48.00	-122.64	124	30
Admiralty Inlet	ADIM	PGST	48.00	-122.67	63	30
Admiralty Inlet	ADIS	PGST	47.99	-122.69	35	30
Mukilteo	MUKD	TUL	47.96	-122.29	101	30
Mukilteo	MUKM	TUL	47.96	-122.29	50	30
Mukilteo	MUKS	TUL	47.96	-122.29	28	23
Thorndyke Bay	TDBS	PGST	47.80	-122.73	31	26
Thorndyke Bay	TDBM	PGST	47.79	-122.73	51	30
Thorndyke Bay	TDBD	PGST	47.78	-122.73	115	30
Fauntleroy (Point Wells)	PWBONGOS	KC	47.54	-122.40	40	30
Fauntleroy (Point Wells)	LSNT01S	KC	47.54	-122.40	55	30
Fauntleroy (Point Wells)	LSNT01D	KC	47.53	-122.43	212	30
Dana Passage	DANAD	NIT	47.18	-122.83	43	30
Dana Passage	DANAM	NIT	47.18	-122.84	31	26
Dana Passage	DANAS	NIT	47.18	-122.84	23	18
South Ketron/Solo Point	SKETD	NIT	47.14	-122.64	40	30
South Ketron/Solo Point	SKETD2	NIT	47.14	-122.65	102	30
South Ketron/Solo Point	SKETM	NIT	47.14	-122.64	35	30
South Ketron/Solo Point	SKETS	NIT	47.14	-122.64	28	23

In the laboratory, all organisms were identified and quantified from subsamples. Speciation in oblique tow samples focused on taxa which are key prey items for juvenile salmon and forage fishes (i.e., crab larvae, amphipods, large copepods, euphausiids, polychaetes, other decapods, chaetognaths, pteropods). Vertical net tow samples were analyzed to a higher taxonomic level by highly-trained taxonomists to capture the relatively subtle shifts in species dominance that occurs in response to environmental variations. Organisms were measured for conversion from abundances to biomass. Abundances (Ind./m^3) and biomass (mg C/m^3) were calculated.

A juvenile salmon Prey Field Index (PFI) was calculated from the oblique tow biomass data following methods provided by C. Morgan (Oregon State University). The PFI includes only taxa that are in juvenile salmon diets, primarily crustaceans >2.5 mm body length, ichthyoplankton, some polychaetes, pteropods, and insects. Results shown herein are preliminary as these methods are currently being refined for application to Puget Sound.

Results

2.1 SSMSP Zooplankton Time Series

Vertical Tows

Peak total zooplankton abundances were higher at most stations in 2015 than in 2014 (Figure 2). At many stations, the increase was driven by small copepods while larvaceans decreased significantly and many other taxa decreased slightly. Strong seasonality was observed in both years, with consistently high values in the summer (July - August). South Sound stations (Dana Passage and S. Ketron Island) peaked earlier than other regions, then declined in summer. Peak biomass was higher overall in 2015 than 2014. Admiralty Inlet and Camano Head had some of the highest biomass values in 2015, which differed from the pattern in abundances. Biomass values were highest in June through August in both years.

Oblique Tows

Abundances and biomass of zooplankton collected in oblique tows were much more variable on bi-weekly to monthly time scales than in vertical net collections (Figure 3). Strong seasonality was still apparent. Abundances peaked at most stations between April and August in 2014; in 2015, peaks occurred in March and September at some stations, apparently broadening the season of high zooplankton abundance. Some stations, such as Saratoga Passage, Hope Island, and Dana Passage, had peak abundances in 2015 that were almost double those measured in 2014. One of the more southern stations, Point Wells, had a higher peak abundance in 2014.

Biomass (middle panel, Figure 3) was generally high in the later summer months (July - September) in both years. Some of the highest peaks were in the spring (April - May) 2015. Preliminary data exploration of The Prey Field Index (PFI) - composed of taxa that are important components of juvenile salmon diets (bottom panel of Figure 3) – shows peak biomass in the spring of both years, starting in May in 2014 and slightly earlier (April) in 2015. Prey Field Index biomass was consistently high earlier in the season (June - July) in 2014 than in 2015 (July - August).

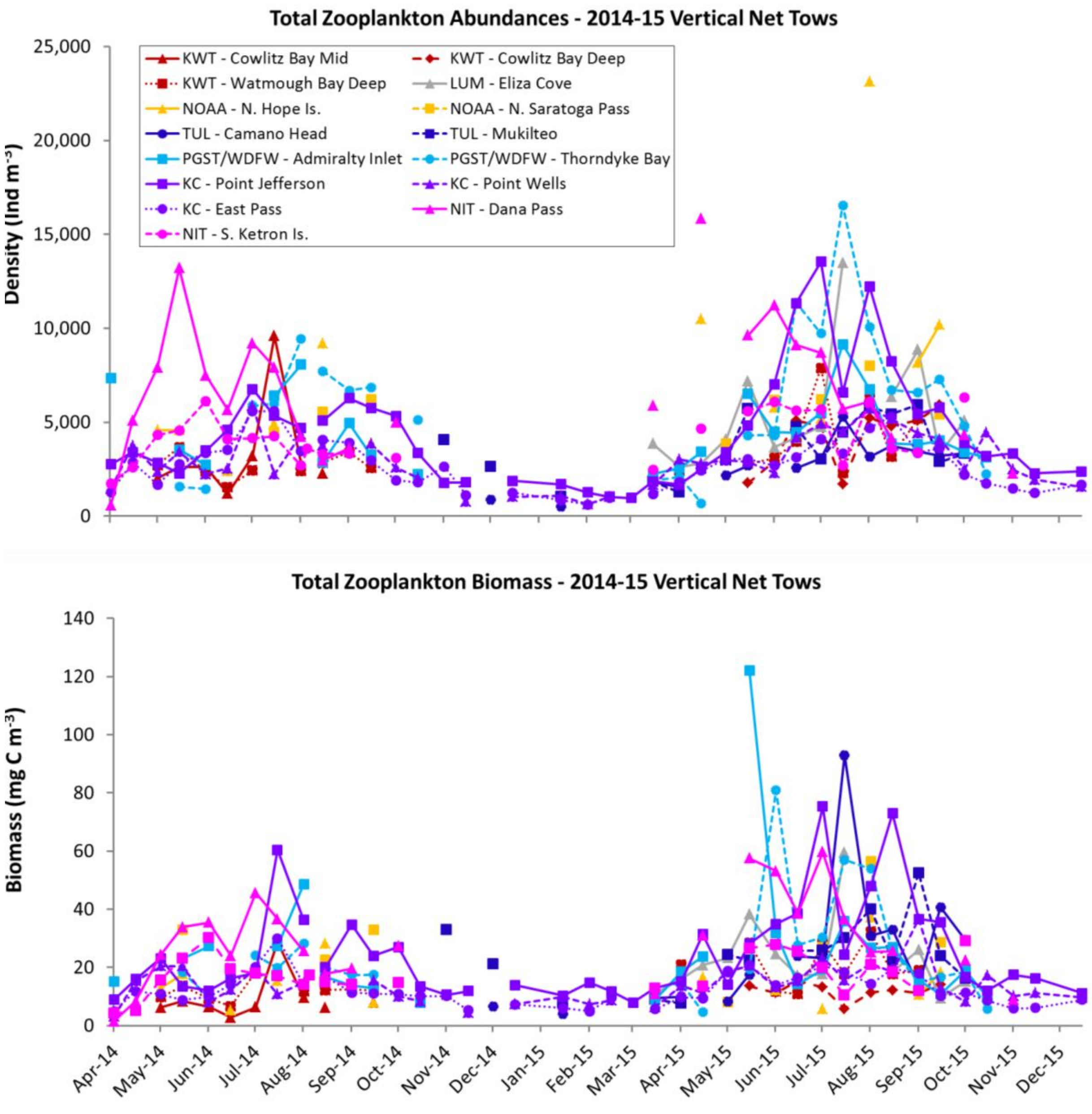

Figure 2. Total Zooplankton Abundances and Biomass from Vertical Net Tows, 2014-2015

Total zooplankton A) abundances (Ind m^{-3}) and B) biomass by station. Samples were only collected on dates with data points. Eggs, *Noctiluca*, and copepod and krill nauplii were not included in totals. Siphonophore gonophores were not included in abundances.

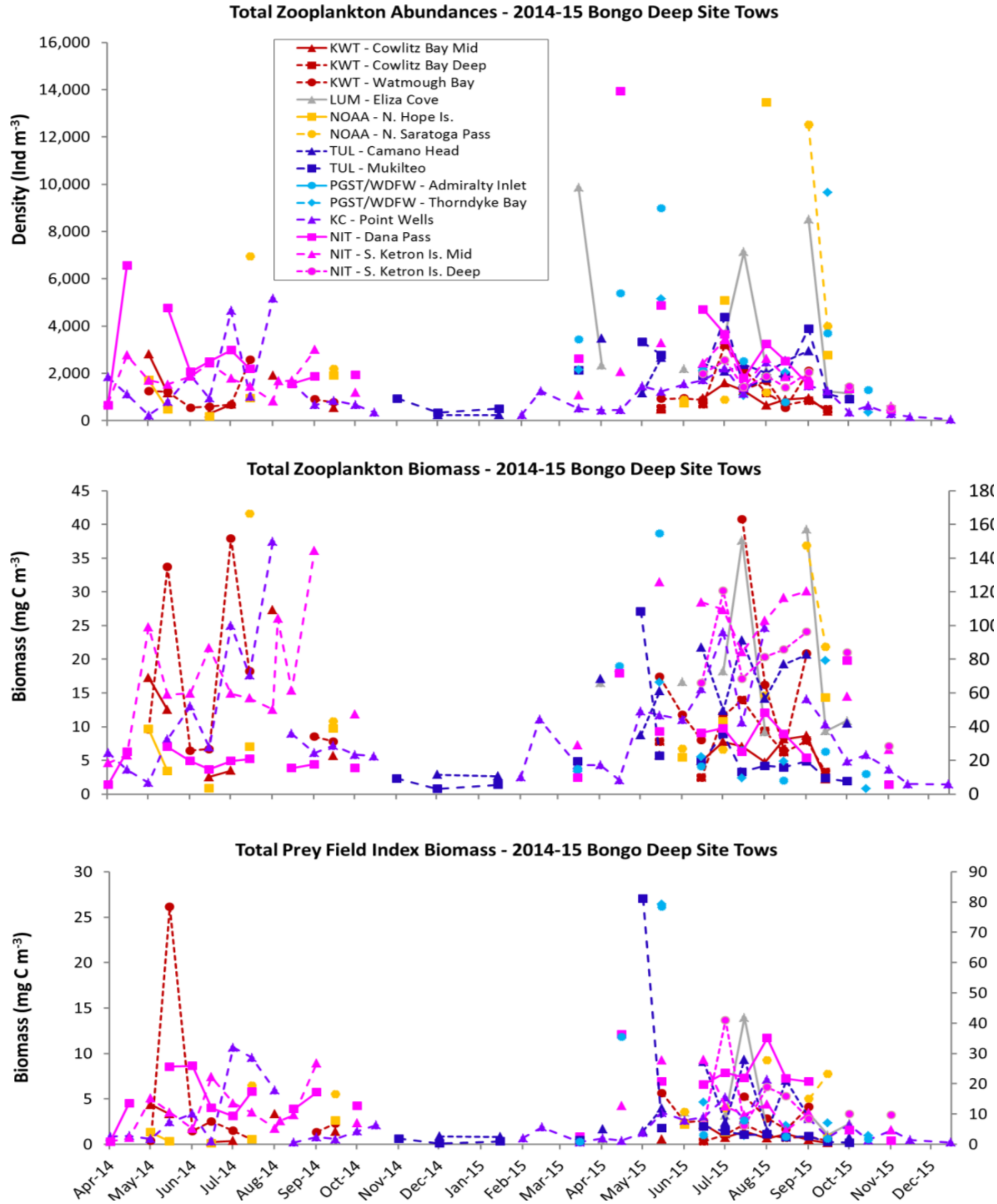

Figure 3. Total Zooplankton Abundance and Biomass from Oblique Net Tows, 2014-2015

(Upper panel) Total zooplankton abundances (Ind m^{-3}). (Middle panel) Total zooplankton biomass (Mukilteo, Admiralty Inlet, Thorndyke Bay and Dana on right axis). (Bottom panel) Biomass of the Prey Field (Mukilteo and Admiralty Inlet on right axis). Samples were only collected on dates with data points. Eggs, *Noctiluca*, copepod and krill nauplii were not included in totals. Siphonophore gonophores were not included in abundances.

2.2 Prey Field Community Composition

The Prey Field Index (PFI) showed high variability in time and space (Figures 4, 5). Decapod biomass (crabs and shrimp) vastly dominated the prey field biomass in both years, during April – October. Hyperiids and large copepods were most noticeable in the winter and early spring times, especially in 2015, whereas higher ichthyoplankton biomass was detected in March 2015 and dropped off shortly after. “Other” taxa were more dominant in 2014 compared with 2015. Insects, euphausids, and pteropods were largely undetected compared to other taxa.

Overall PFI biomass increased throughout spring to summer, with a dramatic increase in Admiralty Inlet in April 2015. A minor spatial variation occurred in 2015 where PFC biomass increased from north to south latitudes through April to September – a pattern not as distinguishable in 2014. Increases for 2014 started later in the year and maintained higher numbers in the south. Note: there are little sampling data for the mid latitudes (Admiralty Inlet and Central Puget Sound) in 2014.

The following figures (4-5) show data collected from oblique tows collected from the deepest water stations from each region in 2014-2015. The “Other” category incorporates the following taxa: amphipods (other than hyperiids), barnacles, cephalopods, cumacea, isopods, mysids, and polychaetes. Plots were created in Tableau® 10.2.

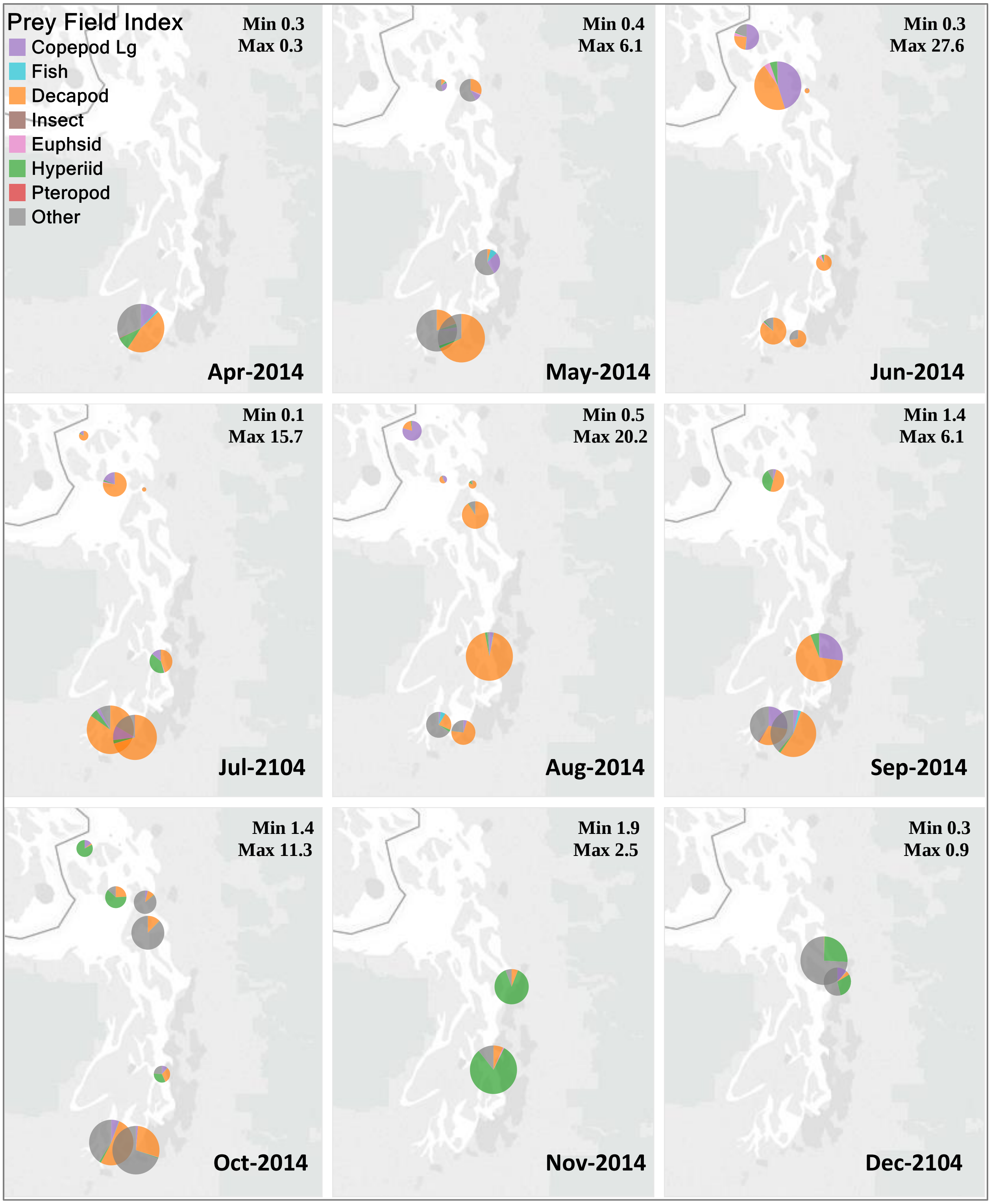

Figure 4. 2014 Prey Field Taxa – Total Monthly Biomass

Monthly variation in the proportions of Prey Field Taxa at deep oblique tow stations, April–December 2014. Pie charts are sized by total station biomass (mg C m^{-3}), but relative sizes can only be compared among stations within each month. Monthly Max and Min biomass are given.

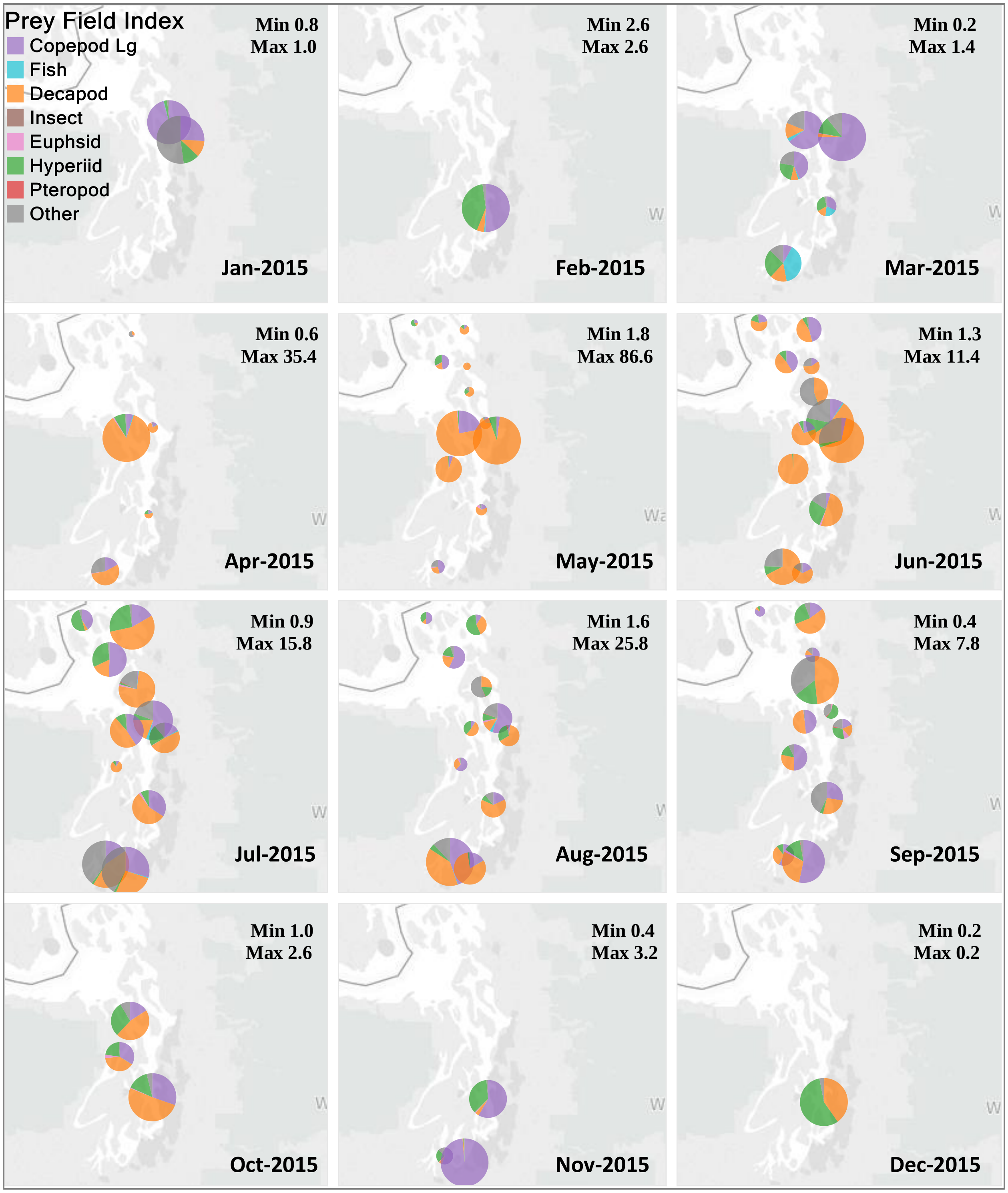

Figure 5. 2015 Prey Field Tax – Total Monthly Biomass

Monthly variation in the proportions of Prey Field Taxa at deep oblique tow stations, January–December 2015. Pie charts are sized by total station biomass (mg C m^{-3}), but relative sizes can only be compared among stations within each month. Monthly Max and Min biomass are given.

2.3 NMS Ordinations of Vertical Net Tows

Zooplankton community relationships over time and space were explored using Nonmetric Multidimensional Scaling (NMS) ordination. An ordination was run on the vertical net tow data using PC-ORD™ 5.05. Species x Sample matrices were created from species density (Ind m^{-3}) data. Because some species were very abundant while others were very rare, data were first normalized using a logarithmic transformation [$\text{Log}_{10}(Y + 0.001) + 3$] and species that occurred in less than 5% of samples were dropped from analysis. The ordination was run on the remaining n=177 species for n=306 vertical tow 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 Axis 1. Distances between points in the ordination indicate the level of dissimilarity between zooplankton communities—closer points are less dissimilar than points that are farther apart.

The ordination revealed differences in zooplankton community structure among basins, and showed a general latitudinal shift in communities along the dominant axis (Axis 3) from the San Juans to South Sound: the San Juan, Bellingham Bay, and Admiralty Inlet regions separated from the Main Basin, Whidbey Basin, and South Sound. Hood Canal was an exception – it fell among the more southern basins although the sampling location is in the northern portion of Hood Canal. The pattern among basins was observed in both years, but greater similarity, indicated by tighter clustering, occurred in 2015.

A seasonal cycle in the zooplankton community was observed in the ordination, with the dominant axis (Axis 3) capturing a seasonal shift from winter (Jan-Feb and Nov-Dec) to late spring-summer (March-Sept) whereas Axis 2 captured a difference between the first half of the year (Jan-May) and end of the year (Sept-Dec). The strongest pattern that was apparent is a shift along Axis 2 from 2014 (open symbols) to 2015 (filled symbols) and tighter clustering of all samples in 2015 compared to 2014, indicating lower spatial and temporal variance in the community structure in 2015.

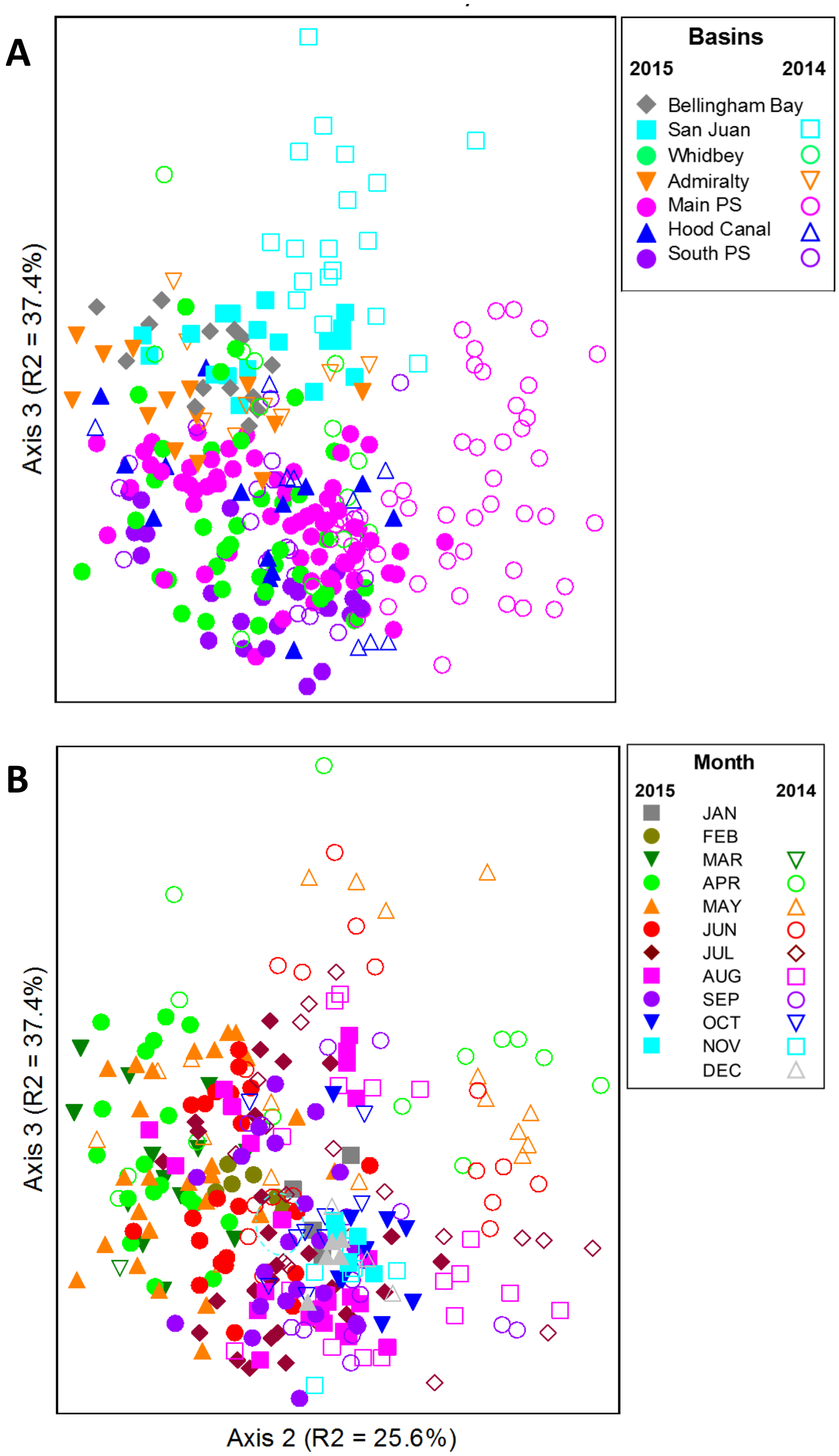

Figure 6. NMS Ordinations – Vertical Tows.

Nonmetric Multidimensional Scaling ordinations of 2014-2015 vertical zooplankton tows, symbol and color coded by basin (panel A) and month (panel B) of sampling.

Table 3. Pearson and Kendall Correlations with ordination axes – Vertical Tows

Correlation coefficients (r) and correlations of determination (R^2) between taxa from vertical net tows and the NMS ordination axes. Only species strongly correlated with one or more axis ($R^2 \geq$ 0.25) are included and those with $R^2 \geq 0.4$ are in bold.

CUM R^2 = 0.734	Axis 2 (R^2 = 0.256)		Axis 3 (R^2 = 0.374)	
	r	R^2	*r*	R^2
Aartia	0.249	0.062	0.532	0.283
Acartia longeremis	0.095	0.009	0.692	**0.479**
Aetideus divergens	-0.011	0	-0.576	0.331
Glebocarcinus oregonensis	-0.501	0.251	0.061	0.004
Epilabidocera amphitrites	-0.283	0.08	0.578	0.334
Larvacea	0.661	**0.436**	0.168	0.028
Miocrocalanus spp.	-0.007	0	-0.524	0.274
Muggiaea atlantica	0.179	0.032	-0.614	0.377
Oikopleura sp.	-0.683	**0.467**	-0.162	0.026
Pseudocalanus moultoni	-0.147	0.022	0.636	**0.404**

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Appendix – Zooplankton sampling protocols

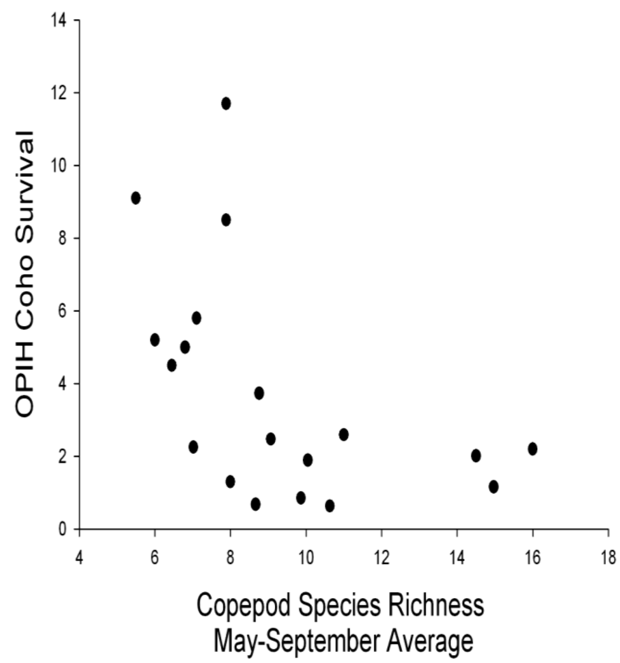
SSMSP Zooplankton Sampling Protocol

Julie E. Keister

(Last updated by Julie E. Keister and Amanda K. Winans, 22 October 2015)
For the latest update, contact jkeister@uw.edu

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***

- Ring net: 60 cm diameter, 200 µm mesh, 4:1 or 5:1 filtering ratio (i.e., length: width ratio – longer is better if boat can handle it). Cod end: 4.5" diameter x 6" length or larger (4.5' x 6" preferred), of same (preferred) or smaller mesh size.
- Flow meter, TSK style. (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 (but to a maximum of 200 m tow depth) to the surface, deployed and immediately retrieved at 30 m/min. [hand-hauls will almost always be too slow]

Prey Field Indicator ***sampling protocol: oblique tows***

- 60-cm bongos, 335 µm mesh.
- Black mesh nets.
- Cod end: 4.5" diameter x 12" length, of same mesh size.
- Flow meter required ('torpedo' style from SeaGear)
- Sensus Ultra depth/temperature sensor attached to the inside of the net ring.
- Daytime tows
- Sample at consistent locations of various water depths: ideally 3 locations bracketing nearshore to deepest local (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).

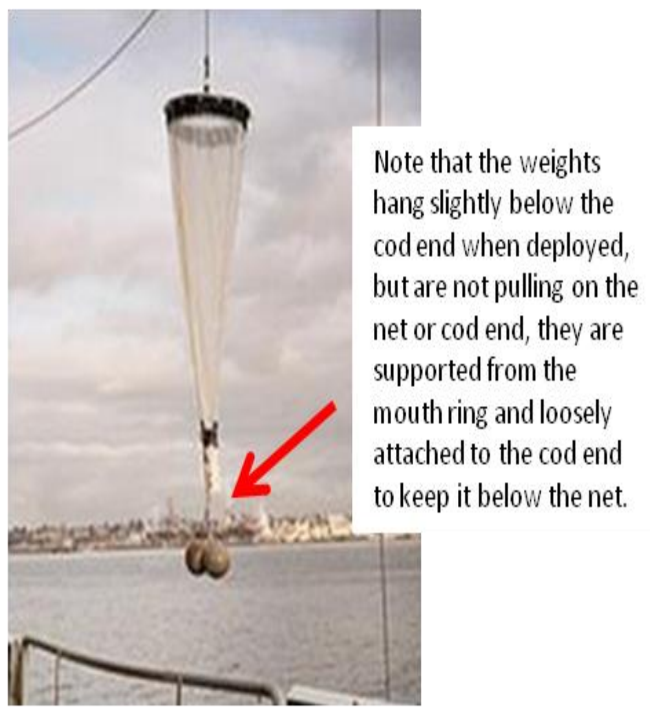
- Towed at 1.5 kts (minimum) to 2 kts, deployed and retrieved with a 30 m/min wire speed, optimally maintaining a 45º wire angle when possible. Adjust amount of line let out to accommodate for actual angle to achieve target depth (see Wire Angle table below).

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.

Vertical net with weights



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

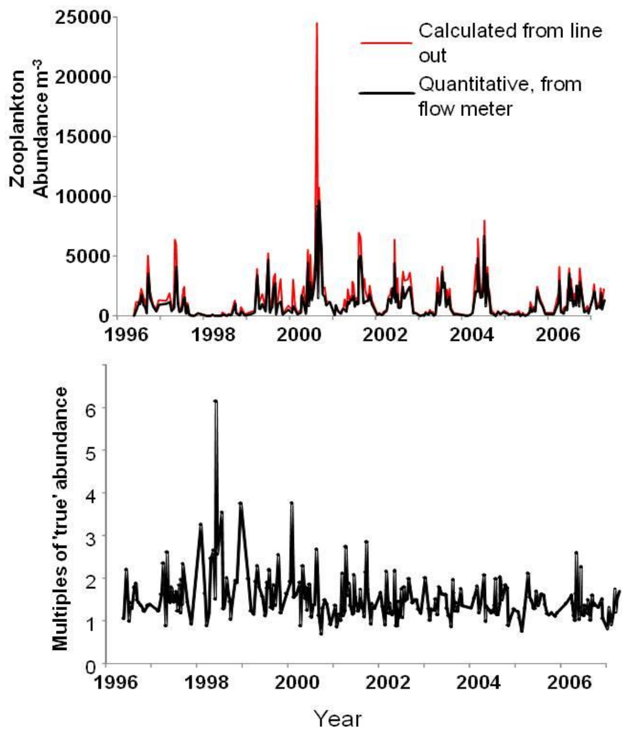
Weighting vertical nets is typically done using a 3-string harness made of line. Tie the ends of the 3 lines to the upper net ring (not to the net or cod end itself), equidistant apart. *Make sure the weight lines are long enough to hang ~1 foot below where the cod end will hang when stretched*, tie 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.



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.

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 deployment. 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.

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 samplers, weather state, winds, currents, etc. on your field sheets.
- **Rig** the nets, attach weights, check equipment for holes, tangles, and loose fittings.
- **Attach Sensus Ultra** depth sensor inside bongo frame (see ReefNet instructions, provided).
- **Reset** the flow meter to zero (TSK or SeaGear models) or record initial counts.
- **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 deeper 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. Recast when in doubt.

  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 <u>immediately</u> at 30 m/min while the vessel is underway, maintaining ~45 degree line angle when possible. [Note: At a 45 degree angle and 30 m/min wire speed, a 30-m depth tow would take 3 minutes *in the water*.] 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 45º 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. If the net hits the bottom, please re-do the tow. Rinse the net out well and redeploy. <u>Use depths recorded with the Sensus Ultra to adjust future tows to achieve 30 m depth.</u>
- **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). Check the flow meter reading and record it on your data sheet.
- **Record** engine RPMs and length of tow time for bongo (oblique) tows; wire angle and any issues for both net tows.

- **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. Start with a gentle rinse so you don't destroy delicate critters. Pay special attention to seams that catch organisms. When you think you've got everything off that you can with a moderate-pressure rinse, then do a higher pressure rinse to get off any leftover algae or other substances that would otherwise stay stuck on the net. 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. Make sure to use a sieve that is the same mesh size or smaller than the mesh size of the net.
- **Concentrate the organisms in the cod end or sieve of the correct mesh size** (200 µm vertical net, 335 µm bongo net), then pour and thoroughly rinse contents into a sample jar with a squirt bottle, using a funnel if necessary. **For bongo (oblique) tows, only save the sample from one codend, preferably the one with the flow meter.** Do not discard the other codend until the first is preserved, in case there's an accidental spill. 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 or multiple jars are necessary if ctenophores are dense, or the sample is full of phytoplankton and very slow to drain. Oblique tows may result in larger samples.]

  If larger jellyfish are caught, rinse the plankton off of them into the sample, ID the jellyfish (see provided ID guide), measure and record the bell diameter, and toss them back. You may also do a "field split" of the sample if it is very large. Do this by continuously mixing the sample well in a large container (e.g., bucket) while distributing equal volumes into two containers, continuing until full sample has been split in half. Repeat again if necessary, preferably saving at least ¼ of the full sample. Preserve and record on the jar lid and data sheets the split that was saved (i.e. ¼ split).

- **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). ***Work outside or in a hood and wear gloves while using formalin*** (Nitrile are recommended). Make sure the reservoir cap is on (but loosened) when squeezing the bottle to avoid spray.

  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 preferably 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 >48 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 (e.g., the original shipping bottles), then dispense into squeeze dispensers after settling for >48 hrs. [Formalin is the same as 37% formaldehyde.]

- **Top off the jar** to the bottom of the threads with seawater to prevent dehydration. Close tightly and swirl to mix.
- **Label** the jar (We usually write on the jar lid with a Sharpie if it is a matt surface (won't wipe off) with**: SSMSP, Group, date, time, station, type of tow (vertical or bongo), mesh size, depth of tow, and flow meter reading** (See attached example). 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.
- Complete the **field sheet** for the station, recording the flow meter reading, wire angle and coordinates. Note anything unusual or any issues with the sampling or equipment, especially for the bongo tows.
- After collection, **Rinse all equipment in fresh water.** Rinse all flow meters well. Rinse nets down with fresh water, including the rings and codend. Power-washing the net (being careful of flow meters) may help dislodge phytoplankton buildup.
- **Check equipment periodically.** Look over entire net carefully to check for holes. Check the flow meters to see if they seem to be spinning at their normal rate. See equipment maintenance guide for further information.
- **Store equipment carefully.** Store vertical net with flow meter down, so that the metal won't rub on the net and wear holes into it. Do not step on the nets. Try not to allow stiff folds/creases to form in the nets.

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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