Marine Zooplankton Monitoring Program
Sampling and Analysis Plan

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King County

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Julie Keister (University of Washington) and Christopher Barnes (King County Environmental Laboratory) provided technical information on field sampling methodology. General sampling protocols were designed by Julie Keister in collaboration with the following experts, who have established zooplankton monitoring programs in Oregon and British Columbia: William Peterson (Northwest Fisheries Science Center), Cheryl Morgan (Oregon State University), and Marc Trudel (Department of Fisheries and Oceans, Canada).

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EXECUTIVE SUMMARY

King County's (KC) marine water quality monitoring program collects data on the physical, chemical, and biological characteristics of marine waters within the boundaries of King County. These data are used to improve our understanding of ecosystem structure and function and for assessing the impact of human activities, such as wastewater treatment plant and combined sewer overflow discharges. The zooplankton component of this larger program, beginning in spring 2014, will collect information on zooplankton community composition, abundance, and biomass.

This technical document provides the plan for implementing the marine zooplankton monitoring program, which is standard practice for monitoring programs. This document follows a standardized format and includes background information, program objectives, sampling design, sample collection and analysis protocols, and data management procedures for this program.

King County's zooplankton monitoring program is a partnership with Julie Keister at the University of Washington (UW) and will contribute to the Salish Sea Marine Survival Project headed by Long Live the Kings. A grant from SSMSP will fund a portion of the program for two sampling years (2014–2015). After SSMSP funding ends, KC will continue zooplankton sampling in collaboration with UW.

Zooplankton are organisms that, due to their small size and/or weak swimming ability, are carried along with the flow of ocean and estuarine currents. They are an important component of the marine food web. Zooplankton are consumers of the tiny plant-like phytoplankton that form the base of the marine food web, and in turn, are important prey for juvenile salmon and small fish. Due to their ubiquity, diversity, and key role in the ecosystem, zooplankton community composition is a useful indicator of ecosystem and food web function. Zooplankton community composition and abundance are also likely to be good predictors of salmon survival and return in Puget Sound, where poor early marine diet has been linked to reductions in juvenile salmon size/growth rate and survival.

Zooplankton sampling using two types of nets, which capture different portions of the zooplankton community, will occur on routine ambient marine sampling cruises on the King County Environmental Laboratory's *R/V Liberty*. These cruises occur twice monthly from February through November and once monthly in December and January (22 sampling events per year). Three sampling locations (see map on page 10) will provide broad spatial coverage in County offshore waters. These locations are also sampled for community composition and abundance of phytoplankton as well as routine water quality parameters such as salinity, nutrients, chlorophyll, and dissolved oxygen. A total of five zooplankton samples will be collected during each sampling event (5 samples × 22 events = 110 samples per year). Samples will undergo detailed analysis by expert taxonomists in J. Keister's group at UW. Zooplankton will be identified to species where possible, measured for biomass estimates, and counted for abundance estimates.

Data will be used by program partners to develop biologically relevant indicators, which KC will use to explore ecosystem and food web dynamics in the Central Basin of Puget Sound. Data will be interpreted in the context of anthropogenic inputs, weather, estuarine circulation, phytoplankton, water quality, and historical zooplankton datasets. Analysis will be reported in summaries on the web each year and in water quality reports every five years. A database to archive phytoplankton and zooplankton data will begin development in 2015 and will include a web portal for public data viewing and download.

1.0 INTRODUCTION

King County's (KC) marine water quality monitoring program collects data on physical, chemical, and biological parameters in marine waters of central Puget Sound within the boundaries of King County. These data are used to improve our understanding of ecosystem structure and function and for assessing the impact of local and larger-scale human activities. In particular, KC conducts monitoring to assess the impact of the County's treated wastewater and combined sewer overflow (CSO) discharges on the marine environment. This monitoring effort involves collecting environmental data from sites near discharge locations (outfall pipes) as well as data from "ambient" sites outside the immediate vicinity of known discharges. KC's marine monitoring program also contributes to the Puget Sound Ecosystem Monitoring Program (PSEMP), an interagency effort tasked with monitoring the health of the Puget Sound environment on a regional basis.

This sampling and analysis plan (SAP) presents the extension of KC's marine ambient monitoring program to include regular sampling and taxonomic analysis of zooplankton in the Central Basin of Puget Sound. This addition, beginning in 2014, is a partnership with Julie Keister at the University of Washington (UW) and will contribute to the Salish Sea Marine Survival Project (SSMSP) headed by Long Live the Kings (LLTK). A grant from SSMSP will fund a portion of the program for two sampling years (2014–2015). After SSMSP funding ends, KC will continue zooplankton sampling in collaboration with UW.

The current lack of zooplankton abundance and community composition data in the Puget Sound has been identified by PSEMP and SSMSP as a substantial gap in long-term monitoring in Puget Sound (PSEMP, 2014; SSMSP, 2013). KC's zooplankton program is part of a regional effort to increase monitoring of important biological parameters, and resulting data will be made available to many users in multiple capacities. This document includes background information, program objectives, sampling design, sample collection and analysis protocols, and data management procedures for this program. Details of other KC marine monitoring activities and general field and laboratory procedures can be found in separate program SAPs or in the general Marine Monitoring SAP (King County, *in prep*.).

2.0 BACKGROUND INFORMATION

2.1 Zooplankton Ecology

Marine plankton are organisms that, due to their small size and/or weak swimming ability, are carried along with the flow of ocean and estuarine currents. Zooplankton are mainly heterotrophic, meaning they must ingest other organisms as their energy and carbon source. They are between approximately 20 µm and several centimeters or more in size. Within this diverse functional group, the target organisms for this program's sampling effort are known more specifically as the mesozooplankton, which are organisms larger than 200 µm. Mesozooplankton are a diverse group of holoplanktonic (remaining in the water column for their entire lifecycle) and meroplanktonic (existing in the water column only in the larval stage) animals. The taxonomic groups represented in the mesozooplankton include: crustaceans, euphausiids, cnidarians, ctenophores, mollusks, amphipods, fish, and many others. In all marine waters, copepods (subphylum Crustacea) are usually dominant in terms of numbers and biomass, but euphausiids, amphipods, gelatinous zooplankton (phylums Cnidaria and Ctenophora), and larval benthic crustaceans can also make up a large proportion of the mesozooplankton. The smaller fraction of the zooplankton, the microzooplankton (20–200 µm), consists of single-celled heterotrophic and mixotrophic (which can both photosynthesize and ingest other organisms) protists as well as the larvae of copepods and some other organisms. These tiny and delicate organisms cannot be effectively sampled with nets and are not considered further in this program.

In coastal and estuarine food webs, mesozooplankton occupy an important position. Mesozooplankton consume large phytoplankton such as diatoms. However, the smaller microzooplankton are frequently the primary consumers of phytoplankton, as well as of heterotrophic bacteria (Calbet and Landry, 2004). Nitrogen-dense microzooplankton are in turn often the preferred and primary prey of many mesozooplankton, including copepods (e.g., Fessenden and Cowles, 1994). Thus, the omnivorous mesozooplankton are an important link transferring carbon and energy from the entire microplanktonic assemblage (phytoplankton, heterotrophic protists, and bacteria) to higher trophic levels.

As important prey of juvenile salmonids and forage fishes, mesozooplankton species composition and abundance have direct consequences for fish populations (Trudel et al., 2002). To what degree variability in the mesozooplankton assemblage is a control on survival and growth of higher trophic-level organisms is critical information for fisheries and ecosystem management in Puget Sound.

2.2 Zooplankton as Indicators

Mesozooplankton species composition can be used as a sensitive and biologically relevant indicator of ecosystem and food web function (Beaugrand et al., 2003; Keister et al., 2011; Peterson, 2009). Zooplankton are ubiquitous and diverse, and populations are not directly

impacted by commercial fishing. In addition, unlike in the phytoplankton and microzooplankton, where population change occurs on the order of hours to days, mesozooplankton (hereafter, simply "zooplankton") lifecycles are typically on the order of several weeks to a year. Finally, different species can be associated with water masses of various origins (Keister et al., 2011). Zooplankton communities therefore reflect the integration of many factors over seasonally and annually relevant timescales; these factors include oceanographic and estuarine transport, primary productivity, temperature, salinity, and predation.

Depending on what ecosystem characteristics an indicator is intended to reflect, different portions of the diverse zooplankton assemblage can be assessed with targeted sampling methods (no single method captures the entire zooplankton assemblage). Copepods, which typically dominate zooplankton biomass, can be sampled effectively from the entire water column with a vertically towed net with 200 μm mesh. In the Northern California Current, multi-annual variability in copepod species composition from samples collected in this manner is closely related to long-term shifts in ocean transport patterns forced by climatic cycles (e.g., the Pacific Decadal Oscillation, PDO) (Keister et al., 2011). This relationship is in part due to the fact that copepods are too small to swim against ocean currents and are carried along with moving water masses. Different species can be highly indicative of subtropical (warm) water masses vs. boreal (cold) water masses in the Northern California Current. Part of KC's zooplankton program will involve a similar vertical-tow sampling technique, with sample analysis focused on detailed speciation and lifecycle staging of copepods. This effort will provide data on the zooplankton assemblage within the entire water column, from a maximum depth of 200 m to the surface, ensuring that even species with diel migration patterns will be captured during daytime sampling. These data will be used to calculate an indicator of general ecosystem and food web function ("ecosystem indicator") based on a multivariate index of zooplankton species composition, currently under development in Puget Sound (J. Keister, *pers. comm*., 2014). This indicator will provide insight into long-term climate-driven cycles and patterns of marine influence in the Puget Sound ecosystem.

Copepod species richness and biomass anomalies have also been related to salmon survival in the Northern California Current system (Peterson, 2009; Peterson and Schwing, 2003). This relationship may be due to the effect of the varying nutritional value of different copepod species on the efficiency of the marine food web. For example, cold-water (boreal) copepods must store more high-energy lipids to survive long winters and periods of diapause (Lee et al., 2006) and are therefore more nutritious prey. Juvenile salmonids transition from nearshore environments and are increasingly found feeding on zooplankton and small forage fish in offshore waters as they grow (e.g., by about mid-summer through fall for Chinook, Beamish et al., 1998). However, copepods are too small to make up a significant portion of the marine diet of juvenile salmonids; therefore, the copepod assemblage likely impacts their survival indirectly through other food-web linkages. Juvenile survival is predicted to be more tightly coupled to variability in the composition and abundance of larger zooplankton, such as amphipods, euphausiids, larval crustaceans, and larval fish, on which they prey directly (J. Keister, *pers. comm*., 2014; Duffy et al., 2010; Peterson et al., 2013).

Zooplankton in the size range preyed-upon by juvenile salmonids and forage fish are effectively sampled with a large mesh (335 µm) net towed obliquely (at an angle) behind a boat. This type of net can be pulled faster through the water, capturing larger zooplankton that may swim fast enough to escape a vertically towed net. Part of KC's zooplankton program will involve this oblique-tow sampling technique, which will be used to collect data on the zooplankton assemblage in the upper euphotic zone (0–30 m) where salmonids (as visual predators) primarily feed. Sample analysis will be focused on identifying broader taxonomic groupings of zooplankton. This effort will provide data for an indicator of offshore prey quality and quantity ("prey-field indicator"), currently under development in Puget Sound (J. Keister, *pers. comm*. 2014). This indicator is likely to be particularly useful for characterizing and predicting patterns of salmon survival and return in Puget Sound, where poor (low prey quantity or quality) early marine diet has been hypothesized to be a factor in reduced juvenile salmonid size/growth rate and thus ultimate survival (Greene et al., 2005; Duffy et al., 2010; Duffy and Beauchamp, 2011).

3.0 GOALS AND OBJECTIVES

The primary goal of this program is to expand KC's existing marine monitoring activities to include long-term monitoring of zooplankton species composition and abundance. These data will be used to further our understanding of the Puget Sound marine food web and to develop indicators of ecosystem and food web function that can be used for assessment of human and climate impacts and resource management. In order to meet this goal, the following objectives were developed:

1. Catalog and quantify the zooplankton present in the Central Basin of Puget Sound within KC boundaries.
2. Measure variability in community structure, abundance, and biomass over seasonal and interannual cycles.
3. Monitor for long-term changes compared to current baseline variability.
4. Provide data to be used by J. Keister (UW) for the development of a general indicator of Puget Sound ecosystem and food web function (the "ecosystem indicator") based on a multivariate index of zooplankton species composition.
5. Provide data to be used by SSMSP and J. Keister (UW) for the development of an indicator of prey quality and abundance for juvenile salmon based on an index of zooplankton taxonomic composition and biomass (the "prey-field indicator").
6. Use indicators to explore ecosystem and food web dynamics in the Central Basin of Puget Sound in conjunction with other KC monitoring datasets (phytoplankton, water quality). Interpret data in context of historical datasets on zooplankton community structure as available for Puget Sound and provide a yearly summary of the zooplankton assemblage and seasonal dynamics.
7. Provide data and analysis to internal (i.e., the KC Wastewater Treatment Division). and external interested parties.

4.0 PROGRAM ORGANIZATION AND RESPONSIBILITIES

The tasks involved in conducting the marine zooplankton monitoring program and the personnel responsible for those tasks are listed below.

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5.0 SAMPLING DESIGN

5.1 Temporal Aspects

Zooplankton sampling using vertically and obliquely towed nets will occur on routine ambient marine sampling events on the King County Environmental Laboratory's (KCEL) *R/V Liberty*. These sampling events occur twice monthly from February through November. Sampling occurs once monthly in December and January to allow time for vessel and instrument maintenance. All net tows are conducted during daylight hours.

5.2 Spatial Aspects

From the set of 12 stations regularly sampled by boat for ambient marine monitoring by the KCEL, four zooplankton sampling locations (Figure 1, Table 1) were chosen to provide broad spatial coverage in the Central Basin of Puget Sound (KC waters). Locations were chosen from the set of stations that are also sampled for quantitative taxonomic analysis of phytoplankton (see the Marine Phytoplankton Monitoring SAP, King County, *in prep*.) in order to provide a complementary dataset that enables more complete analysis of the entire planktonic food web. Two stations, KSBP01 (Jefferson Head) and NSEX01 (East Passage) roughly represent the north and south extent of KC marine waters. KSBP01 is close to the UW Ocean Remote Chemical Analyzer (ORCA) buoy off Point Wells, which collects water quality depth profiles at high temporal resolution (up to once every two hours). Finally, LSNT01 and nearby PWBONGO (off of Fauntleroy) were chosen as stations midway between KSBP01 and NSEX01. These stations are near the KC water quality monitoring buoy, which collects water quality data at a depth of 1 m, every 15 min.

Vertical tows will take place at three stations: KSBP01, NSEX01, and LSNT01. The more time-consuming oblique tows will take place only at LSNT01 and PWBONGO. This pair of oblique tows will provide a more complete picture of the zooplankton community along a depth gradient, allowing comparison of mid-channel (LSNT01, 210 m deep) and near-shore locations (PWBONGO, 40 m deep).

This program is currently limited by time constraints because the zooplankton samples are collected along with samples for many other parameters. However, the number of samples collected may be adjusted in the future as time pressures lessen, such as with the procurement of a faster sampling boat.

5.3 Measured Parameters

In addition to quantitative taxonomic analysis of zooplankton (see 7.0 Sample Analysis), concurrent sampling of other parameters relevant to the zooplankton program is described below (at stations specified in Table 1). Details of these activities can be found in separate program SAPs or the Marine Monitoring SAP (King County, *in prep*.).

Figure 1. Marine monitoring stations sampled for zooplankton and other relevant parameters.

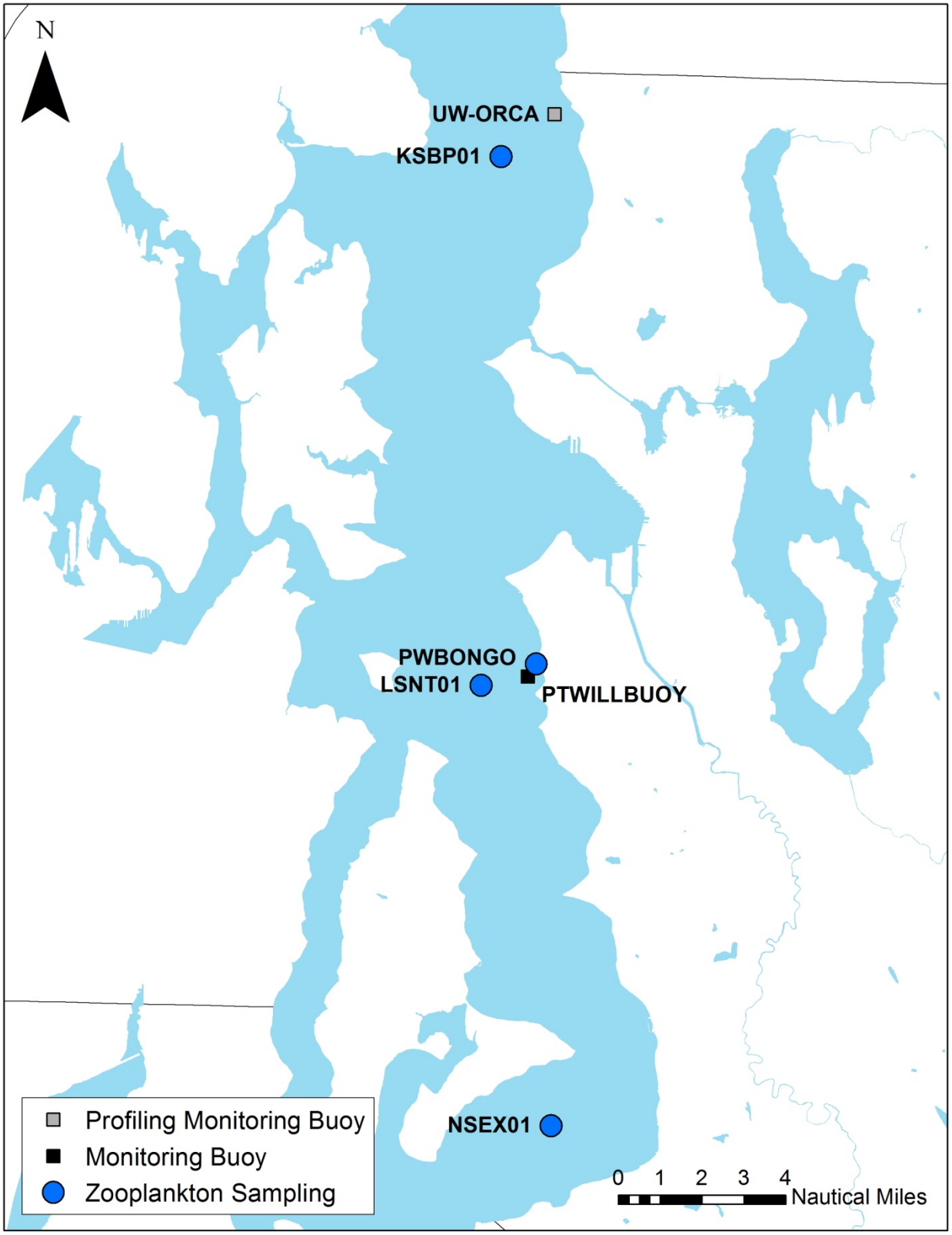

Table 1. Description of stations sampled for zooplankton and other relevant parameters.

Locator	Description	Latitude, Longitude	Bottom depth (m)	Zooplankton tows	Other relevant parameters
KSBP01	Jefferson Head North Central Basin	47º 44'38.25", -122º 25'41.41"	276	Vertical	CTD profiles Conventionals Phytoplankton
LSNT01	Fauntleroy Mid Central Basin	47º 32'00.00", -122º 26'00.00"	210	Vertical Oblique	CTD profiles Conventionals Phytoplankton
PWBONGO	Fauntleroy Mid Central Basin	47º 32'32.10", -122º 24'04.30"	40	Oblique	None
NSEX01	East Passage South Central Basin	47º 21'31.02", -122º 23'13.49"	178	Vertical	CTD profiles Conventionals Phytoplankton
PTWILLBUOY	Point Williams Buoy Mid Central Basin	47º 32'13.79", -122º 24'22.02"	175	None	Autonomous instruments (temperature, salinity, dissolved oxygen, chlorophyll fluorescence, turbidity, and nitrate)
UW-ORCA	Point Wells ORCA Buoy North Central Basin	47º 45'40.20", -122º 23'49.80"	100	None	Autonomous CTD profiles

CTD (Conductivity, Temperature, and Depth) Profiler: Profiles are taken of the entire water column down to 5 m above the bottom depth. Sensors measure conductivity (salinity), temperature, depth, dissolved oxygen, chlorophyll fluorescence, photosynthetically active radiation (PAR), and transmissivity.

Conventionals: Discrete water samples are taken from Niskin bottles on the sampling rosette for conventional water quality analyses at regular depth intervals. Nutrients (ammonium nitrogen, nitrite + nitrate nitrogen, orthophosphate phosphorus, and silica) and total suspended solids are analyzed from samples taken at all depths, while total nitrogen is analyzed from samples at the 1-m depth only. Chlorophyll-*a* is analyzed from samples taken in the euphotic zone at 1 m, 15 m, 25 m, and 35 m. At KSBP01 and NSEX01, samples are also taken at the estimated chlorophyll maximum layer, which is chosen upon inspection of the chlorophyll fluorescence profile at one of 2.5, 3.5, 5.5, 8, or 10 m.

Phytoplankton: Discrete water samples are taken from the Niskin bottles for analysis of phytoplankton abundance and biomass by taxonomic group (to the genus level where possible). Samples are taken from 1 m at all stations, and at the estimated chlorophyll maximum layer at KSBP01 and NSEX01. Samples are quantitatively analyzed live with a FlowCAM instrument and image analysis software for taxonomic identification. In addition, phytoplankton are identified (but not quantified) down to the species or higher taxonomic level using a compound microscope.

Autonomous Instruments: The Point Williams buoy is equipped with a YSI sonde and a Satlantic optical nitrate sensor that measure the following parameters at a depth of 1 m every 15 min: temperature, salinity, dissolved oxygen, chlorophyll fluorescence, turbidity, and nitrate. The YSI sonde is replaced with a cleaned and calibrated sonde on a monthly basis (less frequently in winter), at which time water samples for sensor validation are collected (salinity, dissolved oxygen, and nitrate) (see the Marine Monitoring Moorings SAP, *in prep*.) The UW Point Wells ORCA buoy is equipped with SeaBird instruments collecting full depth profiles of temperature, salinity, chlorophyll fluorescence, and dissolved oxygen. Profiles are collected at variable frequency dependent on solar-powered battery capacity: approximately two per day in winter and every two hours in summer. Discrete water samples are also collected for sensor validation (dissolved oxygen and chlorophyll) every 3–5 weeks (Newton and Devol, 2012).

6.0 SAMPLING PROCEDURES

The Field Science Unit of the KCEL will be responsible for collecting all samples and will follow standardized protocols developed by J. Keister in collaboration with William Peterson, Cheryl Morgan, and Marc Trudel (Keister, 2014). The protocols presented below are consistent with other protocols being used in marine zooplankton monitoring programs in Puget Sound and other regions, allowing for meaningful comparisons between datasets.

All sampling will be done from KC's R/V *Liberty*. Details on procedures for vessel station positioning, shipboard health and safety considerations, and other general vessel operations can be found in the Marine Monitoring SAP (King County, *in prep*.).

6.1 Equipment

Vertical tows are performed with a 60 cm diameter ring net with 200 µm mesh and a length:width ratio of 5:1. A 11.4 cm diameter by 15.2 cm length cod end with the same mesh size is used to concentrate and collect the sample. A TSK-style flow meter mounted in the mouth of the net is used to determine the volume of water sampled, which is necessary for quantitative abundance and biomass measurements. The TSK-style flow meter is most suitable for a vertically towed net because it only spins while the net is being retrieved from the bottom depth. The net is weighted with a 5 lb weight (or more as necessary) attached by a bridle hanging down from the mouth of the net. The cod end is tied to the weight tightly enough to hold the cod end below the net (to avoid tangling), but loosely enough so that the weight is not directly pulling on the cod end and stretching the net.

Oblique tows are performed with a set of two 60 cm diameter nets mounted side-by-side on a frame (a "bongo" double-net). The nets each have 335 µm black mesh and 11.4 cm diameter by 30.5 cm length cod ends with the same mesh size. A torpedo-style flow meter is mounted in the mouth of one of the nets, and a Sensus Ultra depth sensor is attached inside the bongo frame. Weights are attached to the frame between the mouths of the two nets. Total weight for the bongo ranges from 30 lbs (typical) to 50+ lbs (for rough conditions or strong currents).

6.2 Net Deployment

Prior to deploying for the first time each field day, nets are checked for holes, tangles, and loose fittings, and weights are attached. Prior to each deployment, the flow meter must be reset to zero (TSK model) or the initial flow meter count recorded (torpedo model).

During vertical tows, the ring net is allowed to sink to 5 m above the bottom (to a maximum depth of 200 m). If the line angle is not vertical, the line out must be increased to achieve the target depth as calculated in Table A1. Departures from vertical will be estimated and noted on the field sheet. The net is then immediately hauled to the surface at a rate of 30 m/min using the vessel's hydraulic winch.

During oblique tows, the bongo net is deployed at the surface, towed down to 30 m, and hauled back up to the surface using the vessel's hydraulic winch (a 'double oblique' tow). The net is towed at 1.5-2 knots with a 30 m/min wire payout speed and a wire angle of 45°. The winch operator may adjust the rate of wire payout as necessary to maintain a 45° wire angle (and thus an accurate net depth). The captain may also adjust the vessel speed slightly to achieve the target angle, but never below 1.5 knots in order to prevent fast-swimming organisms from being under-sampled. Departures from a 45° angle are noted on the field sheet. Depths recorded with the depth sensor are used to adjust future tows as necessary. When conditions permit, the vessel should tow the net across a bathymetry contour (constant bottom depth). The sample will be collected from the side of the bongo double-net with the flow meter, which gives the most accurate filtration volume for the sample. The second sample will be discarded unless desired as a duplicate sample.

The amount of weight attached to both nets is adjusted as necessary for the station conditions. In rough conditions or where there are strong currents, more weight is added to ensure the net samples deep enough. Weight should be reduced (particularly for oblique tows) to prevent the net from sampling too deep in calm conditions.

Immediately upon retrieval of the net for either type of tow, the flow meter is read and recorded and the value checked to confirm that the flow meter was spinning and that the flow rate was within the expected range for the net deployment (Appendix A).

6.3 Sample Processing

Nets are gently rinsed from the outside with a seawater hose immediately upon retrieval to concentrate the sample in the cod end. Special attention is paid to rinsing seams, where organisms can be trapped. Nets are visually inspected to ensure all plankton are rinsed into the cod end. The cod end is removed once the sample has drained to below the top of the cod end. The sample is further concentrated (but kept suspended in enough seawater to be pourable) in the cod end or in a sieve of equal mesh size, and the contents are then thoroughly rinsed into a sample jar. The cod end or sieve is kept over or inside a bucket or basin at all times to catch any sample that spills (spills are rinsed into and concentrated through the sieve and then rinsed into the sample jar). The smallest sample jar necessary is used, a minimum of ~250 ml. If biomass is high (more than half the jar volume), the sample is moved to a larger jar or split into two jars. If splitting the sample is necessary, the sample is poured into a large container (bucket) and equal volumes are distributed into two containers, a small amount at a time, mixing well before each pour. At least ¼ of the sample is preserved. Any large jellies (mainly Scyphomedusae) are rinsed and removed from the sample before fixation. These are counted, identified to the lowest practical taxonomic level (Appendix B), measured (bell-width, representative measurements only if they are numerous), and discarded.

Samples are fixed and preserved immediately after processing with baking soda-buffered formalin (pH 8.2) at a final concentration of 5% (i.e., 35 ml of buffered formalin for a 700 ml sample jar). Once formalin has been added, sample jars are topped off to the bottom of

the threads with seawater, capped tightly, and swirled to mix. Formalin is prepared ahead of time by adding baking soda in excess, mixing, and letting the mixture stand for 24 hr to allow the solution to saturate and the extra buffer to precipitate out. The formalin is then dispensed into a squeeze bottle with a measuring reservoir for field use.

Samples will be stored in a cool, dark place at KCEL until delivery in batches to J. Keister's laboratory at UW, within 45 days of collection.

6.4 Sample Documentation and Field Sheets

Sample jars will be labeled after each tow by affixing a label onto the side of the jar and the lid (Appendix A). The label will include the following information: date, time, station locator, net mesh size, net ring size, type of tow (vertical/oblique), depth of tow, flow meter start and end readings, split fraction if the sample was split, and "SSMSP" if the sample is from an oblique tow. If pre-made labels are used, they must be carefully checked for accuracy when affixed to the sample container and use waterproof ink that will not fade for 20+ years.

A field sheet will be completed on each sampling day, which contains all of the above information for each sample as well as the wire angle for each tow, weather notes, wind speed, current speed and direction, vessel speed, and anything unusual about the sampling event (Appendix A). Original field sheets are filed and retained by KCEL. Copies of field sheets are provided to UW with delivery of corresponding samples.

6.5 Sample Chain of Custody

Standardized KC procedures for sample chain of custody will be followed from the time at which each sample is collected. While in the field, all samples will be under direct possession and control of the KC Field Sciences Unit staff. For chain of custody purposes, the R/V *Liberty* is considered a "controlled area." Each day, all sample information will be recorded on a chain of custody form (Appendix A). This form will be completed in the field and will accompany all samples during transport and delivery to KCEL each day. The samples will be stored in a secure location at KCEL. When batches of samples are delivered to UW, they will be accompanied by chain of custody forms, which will be signed by UW personnel upon receipt. Original copies of chain of custody forms will be archived in KC's program file.

7.0 SAMPLE ANALYSIS

Samples will be analyzed by expert zooplankton taxonomists at UW using standardized protocols within four months of the date of collection. Vertical and oblique tow samples are analyzed using different protocols based on the types of organisms captured and to achieve the program objectives of obtaining data for two different indicators. Safety procedures for working with formalin are followed (see 10.0 Health and Safety).

7.1 Vertical Tow Samples

1) The sample is first poured through a sieve in a funnel draining to a 5% formalin container. The 5% formalin is retained and used for re-preserving the samples after analysis. The sample is then rinsed with filtered seawater and poured from the sieve into a clear dish and examined over a light table. Very rare (fewer than 30 in the sample) or large (greater than 1 cm) organisms are removed from the sample. These are identified, counted, and measured. If the number of zooplankton selected in this way is large, this subsample is quantitatively split and only half or one quarter is analyzed. Abundance (see 7.1.3 Data End-Points) for these organisms is calculated from the volume of water filtered by the net and the split fraction, if used.
2) The rest of the sample is rinsed into a graduated cylinder to settle. The volume of settled zooplankton is then estimated so that an appropriate dilution for analysis can be made. If there are large clumps of gelatinous zooplankton or phytoplankton, the volume is estimated visually with and without these clumps.
3) The entire sample is then quantitatively diluted into a large flask with filtered seawater to typically 5-10× the volume of the settled zooplankton without clumps. Higher dilutions are used if the sample is very concentrated.
4) The diluted zooplankton sample is then quantitatively subsampled 2-3 times with a 1 mL Stempel pipette (total subsample volumes 1-3 mL each, depending on concentration), mixing well each time and making efforts to representatively include fast sinkers such as pteropods in the subsamples.
5) Each subsample is analyzed under a dissecting microscope with a Bogorov or similar counting chamber. A total of 200-500 organisms are counted for each sample. Zooplankton are counted and identified to the species level where possible, focusing particularly on calanoid copepods. Large copepods (*Calanus* spp. and larger) are also measured and differentiated by larval stage and sex. Other zooplankton, such as pteropods, amphipods, euphausiids, and crab larvae, are also identified to species and measured. If there are too many individuals in a single taxonomic group to measure, a representative subset of 30 are measured. For details, see Appendix C. Abundance (see 7.1.3 Data End-Points) for these organisms is calculated from the dilution factor, the total volume subsampled, and the volume of water filtered by the net.
6) A single, larger subsample of 10–40 mL (depending on concentration) is taken from the diluted zooplankton sample with a 10 mL Stempel pipette. Organisms that are 2-

5 mm in size which have not been found in other subsamples are identified and measured. Abundance is calculated as in 5) but from this larger subsample volume.

7) Finally, all components of the sample are mixed back together, sieved, and re-preserved in seawater with 5% formalin for storage.

7.2 Oblique Tow Samples

1) The sample is first poured through a sieve in a funnel draining to a 5% formalin container. The 5% formalin is retained and used for re-preserving the samples after analysis. The sample is then rinsed with filtered seawater and poured from the sieve into a clear dish and examined over a light table. Large organisms that are rare (fewer than 30 in the sample) are removed. These are identified and counted, and some taxa are measured (see Appendix D). Abundance (see 7.1.3 Data End-Points) for these organisms is calculated from the volume of water filtered by the net.
2) The rest of the sample is then well mixed and quantitatively split with a Folsom splitter to typically between one eighth and one half of its volume, depending on the density of organisms. Splits as small as 1/32 are used for very dense samples. Large organisms (if there are fewer than 30 in the split) are removed and quantified as in step 1. Abundance (see 7.1.3 Data End-Points) for these large, rare organisms is calculated from the volume of water filtered by the net and the split fraction.
3) One split is then sieved and rinsed into a graduated cylinder for quantitative dilution. The split is measured and rinsed into a 250 or 500 mL jar (larger if necessary). Additional filtered seawater is added to the jar as needed to achieve a reasonable organism density. The volume in the graduated cylinder and the volume of all water added to the jar are recorded for calculation of the final dilution factor.
4) The diluted split is mixed well and subsampled with a 10 mL Stempel pipette. A total of 200 organisms are counted under a dissecting microscope from a single 10 or 20 mL subsample using a Bogorov counting chamber. Zooplankton are counted and identified mainly to genus or broader taxonomic grouping. Larval stage is also determined for some zooplankton, such as crab larvae and euphausiids. Many taxa are also measured. If there are too many individuals in a single taxonomic group to measure, a representative subset of 30 are measured. However, all larval fish are measured. For details, see Appendix D. Abundance (see 7.1.3 Data End-Points) for these organisms is calculated from the split fraction, the dilution factor, the total volume subsampled, and the volume of water filtered by the net.
5) Finally, all components of the sample except for larval fish are mixed back together, sieved, and re-preserved in the recycled 5% formalin for storage. Larval fish and fish eggs are retained and preserved separately (in 70% ethanol for larvae, 5% formalin for eggs) for possible analysis by other researchers.

At the end of each sampling year, UW and KC will determine what samples are to be kept in storage at UW or disposed of by UW. However, samples will be retained for at least a year after analysis, or longer if space is available.

7.3 Data End-Points

The abundance of each taxonomic category (number of organisms/L) is calculated from the following volume measurements, as applicable according to the splitting/dilution procedures described above: the split fraction, total volume subsampled, sample or subsample dilution factor, and volume of seawater filtered by the net as determined from the flow meter. The biomass/L of measured organisms is also calculated from the same volume measurements and using established length-weight relationships (e.g., Chisholm and Roff, 1990).

8.0 DATA QUALITY OBJECTIVES

The data quality objectives for the zooplankton monitoring program are to collect data that are sufficiently precise, accurate, representative, complete, and comparable to meet the program objectives outlined above.

8.1 Precision

Precision, or the repeatability of a measurement dependent upon random error, is determined from occasional analysis of duplicate subsamples taken from the same sample by UW taxonomists. Field time constraints generally will not allow replicate samples to be taken from separate tows for the purpose of estimating sampling precision. As the budget allows, duplicate samples will occasionally be taken from both sides of the double bongo net, which will allow estimates of sampling precision for oblique tows. The sampling and analysis protocols used in this program are similar to protocols that have been used for many years by other zooplankton researchers, so published data can also be used for estimates of precision.

8.2 Accuracy and Bias

Accuracy, or the closeness of a sample mean to the true population mean, is affected by both systematic and random errors. Bias is a measure of the difference, due to systematic error, between a sample mean and the true population mean. Care will be taken to reduce individual taxonomist bias by providing consistent training, using standardized species identification criteria, and performing occasional comparisons of data from a single subsample analyzed by multiple taxonomists.

8.3 Representativeness

Representativeness is the degree to which sample data accurately and precisely estimate the value of a parameter for the population of interest (in this case, the zooplankton of the Central Basin of Puget Sound). The location and number of samples that will be collected each year is limited by personnel time and the program budget. Zooplankton populations are known to be quite spatially patchy, which must be a consideration when interpreting data from a relatively small number of stations. However, this program's sampling plan will achieve at least coarse-resolution estimates of the zooplankton community and its spatial variability in the Central Basin and along a depth gradient. Zooplankton community and abundance changes occur on the order of weeks to months rather than days (unlike phytoplankton); therefore the sampling frequency used in this program (twice monthly) should achieve a good estimate of seasonal patterns. The volume of water filtered by the nets is sufficient to collect a representative sample of numerically dominant zooplankton taxa, but rare species are likely to be missed.

Formalin is an effective fixing agent for most zooplankton, although gelatinous or very delicate species are not well preserved and thus will not be as well represented in the data.

The number of organisms subsampled and analyzed, which was chosen based on J. Keister's prior experience and published recommendations (e.g., Sell and Evans, 1982), is large enough to be representative of the whole sample. Samples are well mixed prior to subsampling to further ensure representativeness.

8.4 Completeness

Completeness is the total number of samples for which acceptable data are generated compared to the total number of samples submitted for analysis. Adhering to standardized sampling and analytical protocols will aid in providing a complete set of data for each sampling year. If 100% completeness is not achieved, the program team will evaluate whether additional samples can be collected and analyzed within time and budget constraints. However, due to the seasonal nature of this sampling effort, sampling at a later date cannot directly replace lost data.

8.5 Comparability

Comparability is the confidence with which one data set can be compared to another, either over time or between research groups. This objective will be achieved by standardizing protocols for collecting and analyzing samples and for validating and reporting data. Changes over time to the standardized protocols presented in this SAP will be minimized to ensure comparability over the entire time series. Coordination and consistent training with organizations doing similar work will be performed to ensure this dataset is usable by many organizations for multiple purposes.

9.0 DATA MANAGEMENT AND ANALYSIS

All field and sampling records and chain of custody documents will be archived according to KCEL policy for a period of 10 years from the date the samples were collected.

An annual report, including any appropriate figures and analyses, and all quality-controlled and validated data will be provided to KC by UW within four months of completing the analysis of samples collected within a given year. Data from samples funded by SSMSP will also be provided to SSMSP by J. Keister. J. Keister will notify the KC program manager prior to data being used for other publications or distribution.

Data will be provided to KC in the form of Microsoft Excel spreadsheets. The KC Marine and Sediment Assessment Group will review the report and accompanying raw data. After validation, these data will be input on a yearly basis to a relational database constructed in Microsoft Access. At a later date, a Microsoft SQL Server database will be developed to more permanently house this data alongside phytoplankton data (see Phytoplankton Monitoring SAP, King County, *in prep*.). Ultimately, this database will be made publicly accessible via a query-able web portal on the KC website.

Data will be statistically analyzed by the KC Marine and Sediment Assessment Group using univariate and multivariate techniques to investigate variability between sites and over seasonal and annual (and eventually, multiannual) time scales. KC water quality, weather, oceanographic, and phytoplankton data will be considered alongside the zooplankton data and used to generate testable hypotheses, interpret results, and inform an improved understanding of lower trophic-level ecology in the Central Basin of Puget Sound.

Data and analysis will be published by the KC Marine and Sediment Assessment Group in written summaries for the KC website (yearly) and water quality reports (every five years). Data may also be published by the KC Marine and Sediment Assessment Group in peer-reviewed journals.

10.0 HEALTH AND SAFETY

Details on general health and safety considerations for field work and vessel operations can be found in the Marine Monitoring SAP (King County, *in prep*.). A hazard specific to this program is the use of formalin (37% formaldehyde) for sample fixation. Formalin is a flammable and toxic liquid and vapor. It can cause burns on contact with skin or by inhalation and is carcinogenic, among other hazards. All personnel using formalin will be trained in its safe use, storage, and spill cleanup, and will be familiarized with its material data safety sheet (MSDS) (see Fisher Scientific - Formalin MSDS). In the field, personnel will wear gloves and work only in well-ventilated areas on deck when dispensing formalin into a sample. Personnel will wear gloves and goggles and work under a fume hood when manipulating formalin-fixed samples in the lab. Absorbent pads are provided in all work areas with formalin in case of a spill. Formalin waste is stored in hazardous waste containment for appropriate recycling or disposal.

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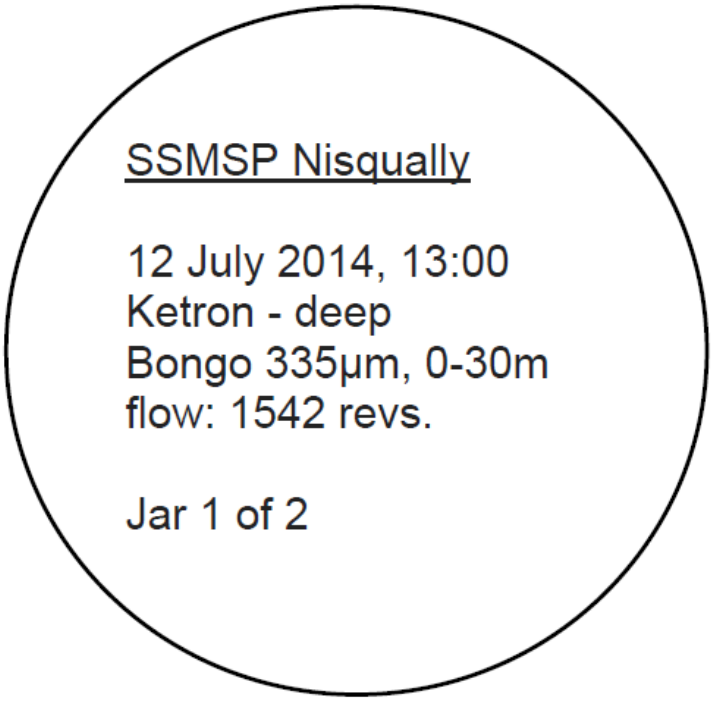
APPENDIX A: FORMS AND REFERENCES

Jar Labeling:

SSMSP Group Name
Date, Time (please write out the month)
Station & depth (or #) (You can use abbreviations if they're standardized)
Net type and mesh size, depth towed
flow meter reading
Jar # out of total (if more than 1 jar)

Example:



Zooplankton Monitoring

Collecting group:_____King County_____ **Collector names:**_____

Collection Date:_____

Gear type:	**Bongos** 60-cm, 335-µm	**Bongos** 60-cm, 335-µm	**Vertical** 60-cm, 200-µm	**Vertical** 60-cm, 200-µm	**Vertical** 60-cm, 200-µm
Station ID	**LSNT01**	**PWBONGO**	**LSNT01**	**NSEX01**	**KSBP01**
Latitude					
Longitude					
Tow start time					
Tow end time					
Station Depth (m)					
Wire out (m)*					
Wire angle on deployment* (estimated)					
Target tow depth (m)					
Flow meter serial #			TSK : 7283	TSK : 7283	TSK : 7283
Flow meter reading start					
Flow meter reading end					
Weather / sea state and winds:					
Comments:					

*Adjust line out using wire angle table. Record wire angle while deploying net. For vertical nets, indicate angle off 0 (straight up and down).

CHAIN OF CUSTODY RECORD

King County Zooplankton
Contact: Kim Stark (206) 477-4829
201 S. Jackson St., Suite 600 Seattle, WA 98104

Page ____ of _____

Sample ID	Collect Date	No. of Containers	Replicate	Comments

	total containers this page
	total all pages

RELINQUISHED BY Signature	Date
Printed Name	Time
Organization	

RECEIVED BY Signature	Date
Printed Name	Time
Organization	

Table A1: Calculate line out (m) required for target depth based on wire angle during net tows. Target wire angle and depth for oblique tows is in red.

Wire angle →	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**	**45**	**50**	**55**	**60**
Target depth (m) ↓												
5	5	5	5	5	6	6	6	7	**7**	8	9	10
10	10	10	10	11	11	12	12	13	**14**	16	17	20
15	15	15	16	16	17	17	18	20	**21**	23	26	30
20	20	20	21	21	22	23	24	26	**28**	31	35	40
25	25	25	26	27	28	29	31	33	**35**	39	44	50
30	**30**	**30**	**31**	**32**	**33**	**35**	**37**	**39**	**42**	**47**	**52**	**60**
35	35	36	36	37	39	40	43	46	**49**	54	61	70
40	40	41	41	43	44	46	49	52	**57**	62	70	80
45	45	46	47	48	50	52	55	59	**64**	70	78	90
50	50	51	52	53	55	58	61	65	**71**	78	87	100
55	55	56	57	59	61	64	67	72	**78**	86	96	110
60	60	61	62	64	66	69	73	78	**85**	93	105	120
65	65	66	67	69	72	75	79	85	**92**	101	113	130
70	70	71	72	74	77	81	85	91	**99**	109	122	140
75	75	76	78	80	83	87	92	98	**106**	117	131	150
80	80	81	83	85	88	92	98	104	**113**	124	139	160
85	85	86	88	90	94	98	104	111	**120**	132	148	170
90	90	91	93	96	99	104	110	117	**127**	140	157	180
95	95	96	98	101	105	110	116	124	**134**	148	166	190
100	100	102	104	106	110	115	122	131	**141**	156	174	200
105	105	107	109	112	116	121	128	137	**148**	163	183	210
110	110	112	114	117	121	127	134	144	**156**	171	192	220
115	115	117	119	122	127	133	140	150	**163**	179	200	230
120	120	122	124	128	132	139	146	157	**170**	187	209	240
125	125	127	129	133	138	144	153	163	**177**	194	218	250
130	130	132	135	138	143	150	159	170	**184**	202	227	260
135	136	137	140	144	149	156	165	176	**191**	210	235	270
140	141	142	145	149	154	162	171	183	**198**	218	244	280
145	146	147	150	154	160	167	177	189	**205**	226	253	290
150	151	152	155	160	166	173	183	196	**212**	233	262	300
155	156	157	160	165	171	179	189	202	**219**	241	270	310
160	161	162	166	170	177	185	195	209	**226**	249	279	320
165	166	168	171	176	182	191	201	215	**233**	257	288	330
170	171	173	176	181	188	196	208	222	**240**	264	296	340
175	176	178	181	186	193	202	214	228	**247**	272	305	350
170	171	173	176	181	188	196	208	222	**240**	264	296	340
180	181	183	186	192	199	208	220	235	**255**	280	314	360
185	186	188	192	197	204	214	226	242	**262**	288	323	370
190	191	193	197	202	210	219	232	248	**269**	296	331	380
195	196	198	202	208	215	225	238	255	**276**	303	340	390
200	201	203	207	213	221	231	244	261	**283**	311	349	400

Reading a TSK flow meter:

Before using the flow meter, please study these instructions carefully. Misread flow readings are remarkably common and result in big errors in abundance and biomass calculations.

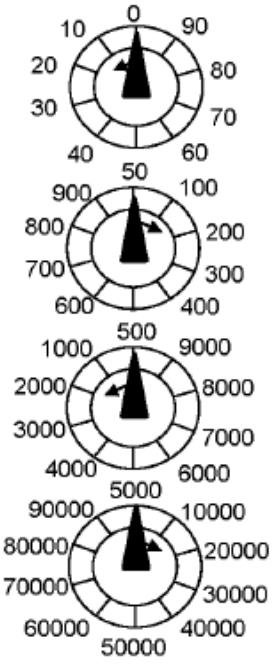
The TSK flow meter uses opposing gears that all rotate continuously when the propeller spins. There are several points to know to read them accurately.

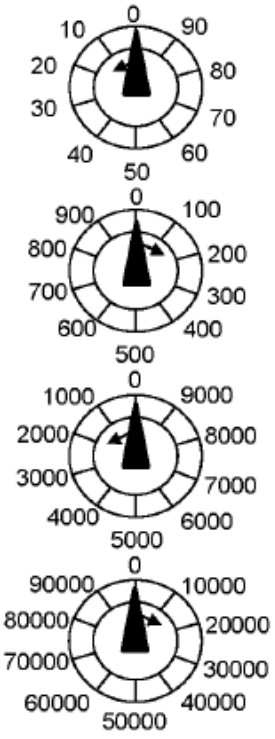
1) The flow meter should be reset PERFECTLY to zero on all dials before each deployment as shown below (rotate dials up to 0 by hand).

2) Although the meter shows the dial numbering on the LEFT below, they should show the numbering as on the RIGHT (note the added 0s at the zero position of each dial). I.e., each dial starts at 0 and rotates continuously toward higher numbers.

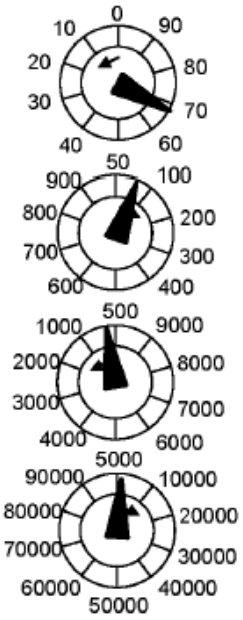
3) Start by reading the bottom dial and work up. See examples on next page. Because dials rotate continuously, every dial will show some reading after a tow, but a dial doesn't "count" until it's gone at least *past* its first tick (past 10 on the first dial, past 100, etc.). You will rarely if ever get a reading from the bottom dial: most readings for vertical tows will be between 100-1500 revolutions.

4) Procedurally, the net must be lifted at a fast enough rate for the flow to depress the backstop. If you get anomalously low readings compared to normal, then try to watch for a spinning propeller when retrieving. Always record the serial number (on outer flap) once per trip to match with the calibration.

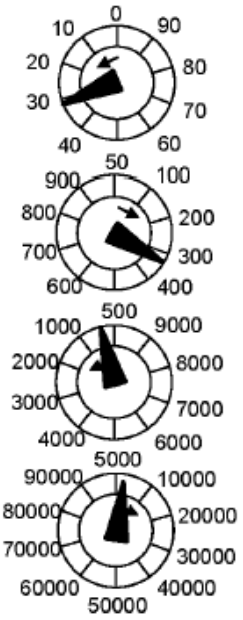




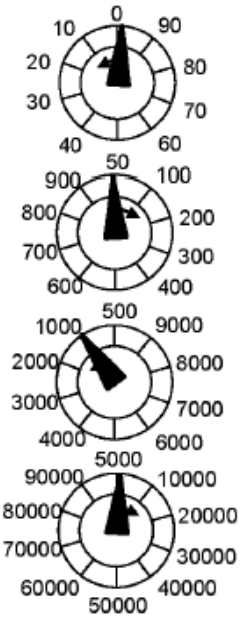
Some examples:



68 revs.



332 revs.



999 revs.

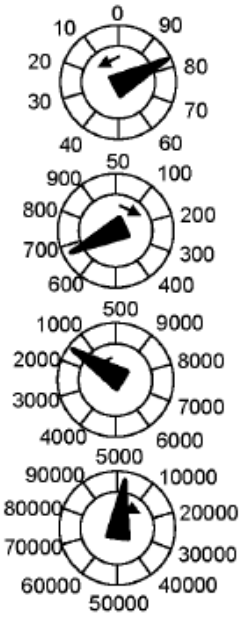
IMPORTANT -- Note these two slightly different readings.

The one on the *left* is **1682**.

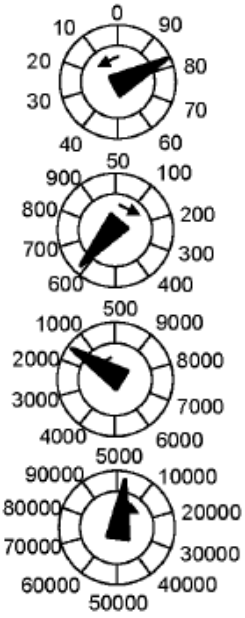
But the reading on the *right* is <u>not possible</u> and means that <u>the dials were not all perfectly zeroed before deployment</u>.

What's wrong with it? The top two dials are inconsistent – for a reading of 82 on the top dial to be correct, the reading on the 2nd dial should either be *almost* to the 600 or *almost* to the 700, not just past 600.

So what's the correct reading? That's very hard to tell, and emphasizes the importance of zeroing perfectly to begin with. In this case, the higher-order dial is probably the one that's off because it would be caused by a smaller mistake when zeroing I.e., the actual reading is probably **1582**. You will have to use some judgment when there's an error like this, so it's best to draw the dial positions on the log sheet and interpret in whichever way is *most likely* given other readings for similar tow depths and the smallest probably zeroing error.



1682

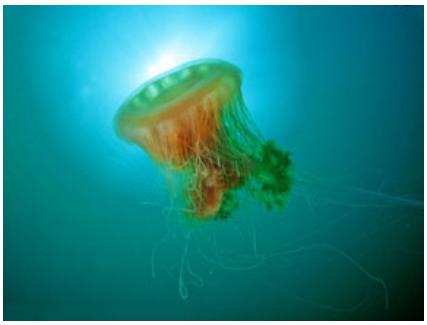


1???

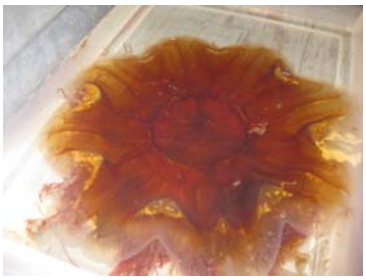
APPENDIX B: IDENTIFYING COMMON SCYPHOZOA

Aurelia labiata (Moon Jelly): Translucent white with 4 horseshoe-shaped stomach pouches on top of bell. Tentacles are short. Bell up to 40 cm in diameter.

Phacellophora camtschatica (Fried Egg Jellyfish): Center is yellow, with rest of bell clear, whitish, or very pale yellow. Bell up to 60 cm in diameter, and has 16 lobes.



Cyanea capillata (Lion's Mane Jellyfish): Deep brick red to purplish. Younger ones can be yellowish-brown. Bell up to 2 m in diameter. You will probably only see it up to 30-40 cm. The bell has 8 distinct lobes (different from *Phacellophora*). Looks like an 8-pointed star when lying flat.



Adapted by A. Winans from:
JelliesZone.com and Wrobel, D., and C. Mills. 1998. Pacific Coast Pelagic Invertebrates: A Guide to the Common Gelatinous Animals. Sea Challengers and Monterey Bay.

APPENDIX C: VERTICAL TOW TAXONOMIC ANALYSIS

Table C1. Organisms and organism larval stages identified in vertical tow samples. Organisms are identified to the species level unless otherwise specified in "Notes". Measurements are copepod prosome length, crab carapace length, jelly (Cnidaria) bell-width and/or height, or other length measurement.

Functional Group	Genera	Life-Cycle Stage/Sex		Measured?	Notes
Copepoda - Calanoida	*Calanus*	Copepodite	1 - 5	Y	
		Adult	Female, Male	Y	
	Metridia	Copepodite	1 - 5	N	
		Adult	Female, Male	N	
Copepoda - Cyclopoida	All	Copepodite	-	N	Identified to genus only
		Adult	-	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	Y	
		Juvenile	-	Y	
		Adult	-	Y	
Decapoda - crabs	All	Zoea	1-5	Y	
		Megalopa	-	Y	
Decapoda - other	All	Not staged	-	Y	Identified to lowest practical taxonomic level, to species when common
Amphipoda	All	Not staged	-	Y	
Pteropoda	All	Not staged	-	Y	
Chaetognatha	All	Not staged	-	Y	Not identified further
Cnidaria - jellies	All	Not staged	-	Y	Large specimens identified to species, others to lowest practical taxonomic level with representative drawing. Siphonophora also identified to zooid type (medusa or polyp). When numerous, only representative measurements are taken.
Ctenophora - jellies	All	Not staged	-	Y	Large specimens identified to species, others to lowest practical taxonomic level with representative drawing. When numerous, only representative measurements are taken.
Appendicularia	All	Not staged	-	N	Identified to genus only
Other	-	Not staged	-	N	Identified to lowest practical taxonomic unit, to species when common

APPENDIX D: OBLIQUE TOW TAXONOMIC ANALYSIS

Table D1. Taxonomic categories used in analysis of oblique tow samples. Organisms in each group are identified to species or higher level, as listed. "Stages counted" lists the analyzed life-cycle stages of each taxa; unlisted stages (those <2.5 mm) are not counted or measured. C5: stage 5 copepodite (juvenile copepod). F2: stage 2 furcilia (larval euphuasiid). Measurements are copepod prosome length, crab carapace length, jelly (Cnidaria) bell-width, or other length measurement needed to speciate or convert length to biomass using regression relationships.

Phylum/ Subphylum		Mid-Level Taxonomic Grouping		Species or Genus	Lowest Taxonomic Level	Stages Counted	Measured?	Staged?
Arthropoda	Crustacea	Amphipoda	Gammaridae	*Cyphocaris challengeri*	Species	All	Y	N
				Corophium	Genus	All	Y	N
				Other	Family	All	Y	N
			Hyperiidae	*Primno macropa*	Species	All	Y	N
				Parathemisto	Genus	All	Y	N
				Hyperoche	Genus	All	Y	N
				Hyperia	Genus	All	Y	N
				Other	Family	All	Y	N
			Caprellidae		Family	All	Y	N
		Copepoda	Calanoida	*Epilabidocera longipedata*	Species	C5 & Adults	Y	N
				Paraeuchaeta	Genus	C5 & Adults	Y	N
				Neocalanus	Genus	C5 & Adults	Y	N
				Paraeuchaeta	Genus	C5 & Adults	Y	N
				Eucalanus	Genus	C5 & Adults	Y	N
				Calanus	Genus	C5 & Adults	Y	N
			Harpacticoida		Order	C5 & Adults	Y	N
			Other		Subclass	C5 & Adults	N	N

Phylum/ Subphylum		Mid-Level Taxonomic Grouping			Species or Genus	Lowest Taxonomic Level	Stages Counted	Measured?	Staged?
Arthropoda	Crustacea	Decapoda (crabs)	Brachyura	Cancridae	*Cancer antennarius*	Species	All	N	Y
					Cancer magister	Species	All	N	Y
					Cancer oregonensis	Species	All	N	Y
					Cancer gracilis	Species	All	N	Y
					Other	Family	All	N	Y
				Majidae	*Chionoecetes tanneri*	Species	All	N	Y
					Oregonia gracilis	Species	All	N	Y
					Other	Family	All	N	Y
				Xanthidae	*Lophopanopeus bellus*	Species	All	N	Y
					Other	Family	All	N	Y
				Pinnotheridae	*Fabia subquadrata*	Species	All	N	Y
					Pinnixa	Genus	All	N	Y
					Other	Family	All	N	Y
				Varunidae	*Hemigrapsus oregonensis*	Species	All	N	Y
				Epialtidae	*Pugettia*	Genus	All	N	Y
				Other		Infraorder	All	N	Y
			Anomura	Paguroidea	*Orthopagurus schmitti*	Species	All	N	Y
					Pagurus hirsutiusulus	Species	All	N	Y
					Pagurus ochotensis	Species	All	N	Y
					Other	Superfamily	All	N	Y
				Porcellanidae	*Petrolisthes cinctipes*	Species	All	N	Y
					Petrolisthes eriomerus	Species	All	N	Y
					Pachycheles	Genus	All	N	Y
					Other	Family	All	N	Y
				Lithodidae	*Lopholithodes*	Genus	All	N	Y
				Galatheoidea		Superfamily	All	N	Y
				Other		Infraorder	All	N	Y

Phylum/ Subphylum		Mid-Level Taxonomic Grouping			Species or Genus	Lowest Taxonomic Level	Stages Counted	Measured?	Staged?
Arthropoda	Crustacea	Decapoda (shrimp)	Cumacea			Order	Larval - Adult	Y	N
			Mysida	Mysidae	*Orientomysis hwanhaiensis*	Species	Larval - Adult	Y	N
					Alienacanthomysis macropsis	Species	Larval - Adult	Y	N
					Archaeomysis grebnitzkii	Species	Larval - Adult	Y	N
					Pacifacanthomysis nephrophthalma	Species	Larval - Adult	Y	N
					Exacanthomysis davisi	Species	Larval - Adult	Y	N
					Neomysis mercedis	Species	Larval - Adult	Y	N
					Other	Family	Larval - Adult	Y	N
			Caridea	Crangonidae		Family	Larval - Adult	Y	N
				Hippolytidae		Family	Larval - Adult	Y	N
				Pandalidae		Family	Larval - Adult	Y	N
				Pasiphaeidae		Family	Larval - Adult	Y	N
				Alpheidae		Family	Larval - Adult	Y	N
				Upogebiidae		Family	Larval - Adult	Y	N
				Other		Infraorder	Larval - Adult	Y	N
			Axiidae	Callianassidae	*Neotrypaea californiensis*	Species	Larval - Adult	Y	N
					Other	Family	Larval – Adult	Y	N
		Euphausiacea (krill)			*Euphausia pacifica*	Species	All	F2 - Adults	Y
					Thysanoessa longipes	Species	All	F2 - Adults	Y
					Thysanoessa raschii	Species	All	F2 - Adults	Y
					Thysanoessa spinifera	Species	All	F2 - Adults	Y
		Cirripedia (barnacles)				Infraclass	All	N	N
		Cladocera			*Podon*	Genus	All	N	N
					Evande	Genus	All	N	N
		Isopoda				Order	Larval - Adult	Y	N
		Ostracoda				Class	All	N	N
		Arachnida (terrestrial spiders)				Class	All	Y	N

Phylum/ Subphylum	Mid-Level Taxonomic Grouping			Species or Genus	Lowest Taxonomic Level	Stages Counted	Measured?	Staged?
Mollusca	Cephalopoda	Teuthida		*Doryteuthis opalescens*	Species	All	Y	N
				Other	Order	All	Y	N
		Octopoda			Order	All	Y	N
	Gastropoda	"Pteropoda"		*Clione limacine**	Genus	All	Y	N
				*Limacina helicina**	Genus	All	Y	N
				*Clio pyramidata**	Genus	All	Y	N
				Other	Polyphyletic	All	N	N
		Other			Class	All	N	N
	Bivalvia				Class	All	Y	N
	Other				Phylum	All	N	N
Chordata	Tunicata	Ascidiacea			Class	All	N	N
		Larvacea			Class	All	N	N
	Actinopterygii (ray-finned fishes)				Class	Egg - Juvenile	Y	N
Cnidaria (jellies)	Hydrozoa	Leptomedusae		*Aequora victoria**	Genus	All	N	N
		Siphonophorae	Physonectae	*Muggiaea atlantica**	Genus	All	N	N
				Other	Suborder	All	N	N
			Calycophorae	*Nanomia bijuga**	Genus	All	N	N
				Other	Suborder	All	N	N
		Other			Class	All	N	N
	Scyphozoa			*Aurelia labiata**	Genus	All	N	N
				Other	Class	All	Y	N
Ctenophora (jellies)				*Pleurobrachia bachei**	Genus	All	N	N
				Beroe	Genus	All	N	N
Protozoa				*Noctiluca*	Genus	All	N	N
Bryozoa					Phylum	All	N	N
Chaetognatha					Phylum	All	Y	N
Echinodermata					Phylum	All	N	N
Nemertea (worms)				*Pilidiophora*	Genus	All	N	N
Annelida (worms)	Polychaeta			*Tomopteris*	Genus	Adult	Y	N
				Other	Class	All	Y	N
Various	Eggs				N/A	All	Y	N

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