SSMSP Zooplankton Sampling Protocol

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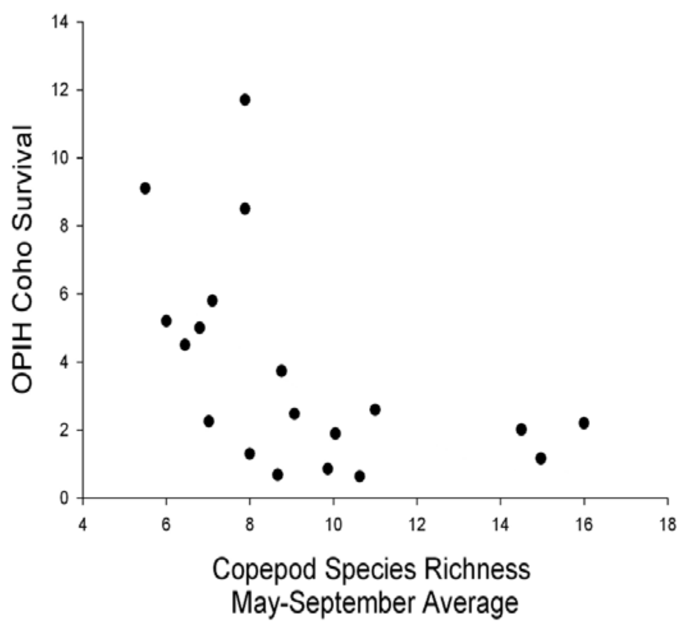
(Last updated by Julie E. Keister and Amanda K. Winans, 22 October 2015)

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.

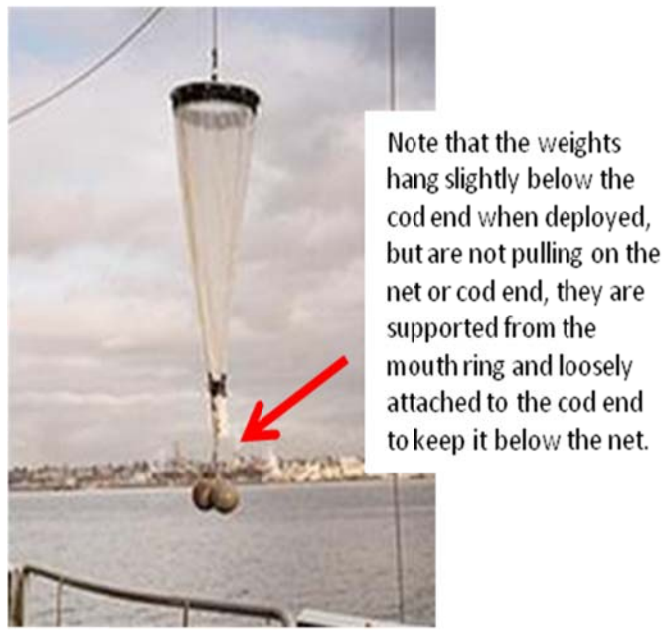
Ring and bongo (double ring) nets are described by their mouth diameter, mesh size, and their filtering ratio. Ring size is given in cm or m; mesh size in micrometers (microns, µm).

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.

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

Vertical net with weights attached



Weighting <u>vertical nets</u> 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.]

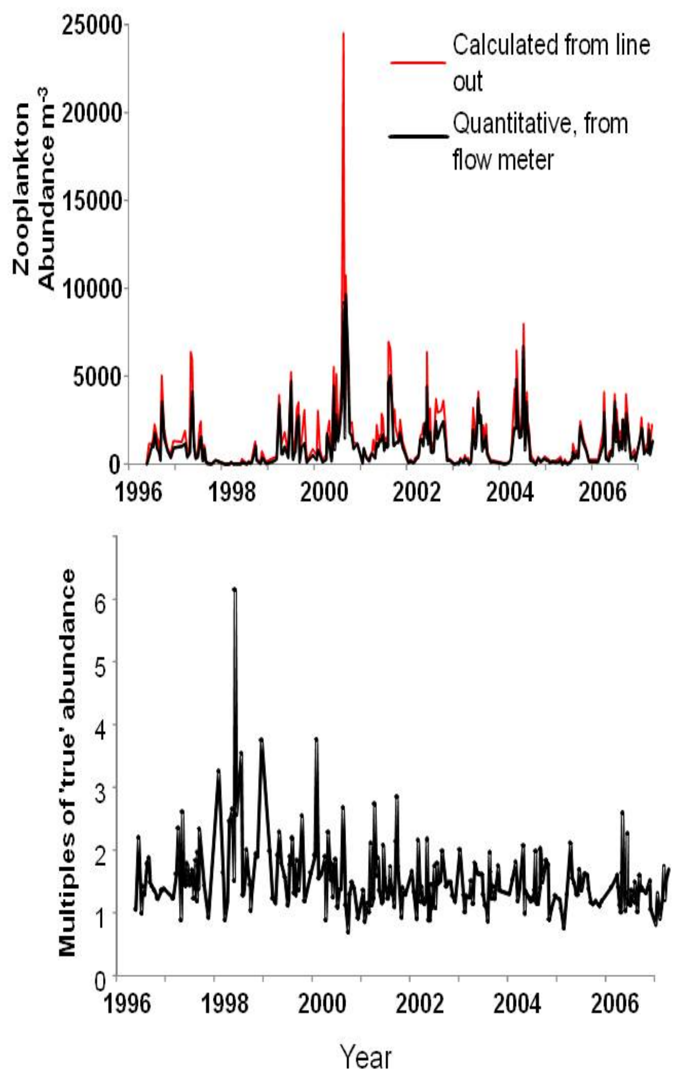
In calm weather <u>with a vertically-lifted net</u>, only enough weight to keep the cod end below the mouth of the net while dropping is needed (maybe 5 lbs). In rough conditions, if there's a strong wind or current, or if undertaking an oblique tow, more weight is needed (20+ lbs). The rougher the seas/current,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

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

2. Flow meters

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

There are many types of flow meters available. However, only a few types are suitable for measuring flow through a vertically-towed net. For vertical tows, the preferred model is a TSK flow meter (http://www.tsk-jp.com/tska/contact.html), which is the only flow meter we've found that is reliably accurate on vertical tows. The problem with most flow meters is that they spin when being deployed (while the net is going down) and retrieved, but not equally in both directions. The TSK style has a 'back-stop' to prevent spinning when going down backwards and a 3-point attachment so they don't flip upside down on 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.



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

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

Field Methods

- **Record** date, time, location, water depth, name of 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). ***<u>Work outside or in a hood and wear gloves while using formalin</u>*** (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.

Studies cited

Beaugrand, G., K. M. Brander, J. A. Lindley, S. Souissi, and P. C. Reid (2003), Plankton effect on cod recruitment in the North Sea, *Nature*, *426 (6967)*, 661-664, 10.1038/nature02164

Beaugrand, G., and P. C. Reid (2003), Long-term changes in phytoplankton, zooplankton and salmon related to climate, *Global Change Biol.*, *9 (6)*, 801-817,

Keister, J. E., E. Di Lorenzo, C. A. Morgan, V. Combes, and W. T. Peterson (2011), Zooplankton species composition is linked to ocean transport in the Northern California Current, *Global Change Biol.*, *17 (7)*, 2498-2511, 10.1111/j.1365-2486.2010.02383.x

Peterson, W. T. (2009), Copepod species richness as an indicator of long-term changes in the coastal ecosystem of the northern California Current, *CalCOFI Reports*, *50*, 73-81,

Peterson, W. T., and F. B. Schwing (2003), A new climate regime in northeast Pacific ecosystems, *Geophys. Res. Lett.*, 30 (17), doi:10.1029/2003GL017528

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:

<u>SSMSP Nisqually</u>

12 July 2014, 13:00
Ketron - deep
Bongo 335μm, 0-30m
flow: 1542 revs.

Jar 1 of 2

Wire Angle Table: Match up wire angle with target depth to find how many meters of line to put out.

Wire angle →	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
Target depth (m) ↓																
5	5	5	5	5	6	6	6	7	7	8	9	10	12	15	19	29
10	10	10	10	11	11	12	12	13	14	16	17	20	24	29	39	58
12	12	12	12	13	13	14	15	16	17	19	21	24	28	35	46	69
15	15	15	16	16	17	17	18	20	21	23	26	30	35	44	58	86
17	17	17	18	18	19	20	21	22	24	26	30	34	40	50	66	98
20	20	20	21	21	22	23	24	26	28	31	35	40	47	58	77	115
25	25	25	26	27	28	29	31	33	35	39	44	50	59	73	97	144
30	30	30	31	32	33	35	37	39	**42**	47	52	60	71	88	116	173
35	35	36	36	37	39	40	43	46	49	54	61	70	83	102	135	202
40	40	41	41	43	44	46	49	52	57	62	70	80	95	117	155	230
45	45	46	47	48	50	52	55	59	64	70	78	90	106	132	174	259
50	50	51	52	53	55	58	61	65	71	78	87	100	118	146	193	288
55	55	56	57	59	61	64	67	72	78	86	96	110	130	161	213	317
60	60	61	62	64	66	69	73	78	85	93	105	120	142	175	232	346
65	65	66	67	69	72	75	79	85	92	101	113	130	154	190	251	374
70	70	71	72	74	77	81	85	91	99	109	122	140	166	205	270	403
75	75	76	78	80	83	87	92	98	106	117	131	150	177	219	290	432
80	80	81	83	85	88	92	98	104	113	124	139	160	189	234	309	461
85	85	86	88	90	94	98	104	111	120	132	148	170	201	249	328	489
90	90	91	93	96	99	104	110	117	127	140	157	180	213	263	348	518
95	95	96	98	101	105	110	116	124	134	148	166	190	225	278	367	547
100	100	102	104	106	110	115	122	131	141	156	174	200	237	292	386	576
105	105	107	109	112	116	121	128	137	148	163	183	210	248	307	406	605
110	110	112	114	117	121	127	134	144	156	171	192	220	260	322	425	633
115	115	117	119	122	127	133	140	150	163	179	200	230	272	336	444	662
120	120	122	124	128	132	139	146	157	170	187	209	240	284	351	464	691
125	125	127	129	133	138	144	153	163	177	194	218	250	296	365	483	720
130	130	132	135	138	143	150	159	170	184	202	227	260	308	380	502	749
135	136	137	140	144	149	156	165	176	191	210	235	270	319	395	522	777
140	141	142	145	149	154	162	171	183	198	218	244	280	331	409	541	806
145	146	147	150	154	160	167	177	189	205	226	253	290	343	424	560	835
150	151	152	155	160	166	173	183	196	212	233	262	300	355	439	580	864
155	156	157	160	165	171	179	189	202	219	241	270	310	367	453	599	893
160	161	162	166	170	177	185	195	209	226	249	279	320	379	468	618	921

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.

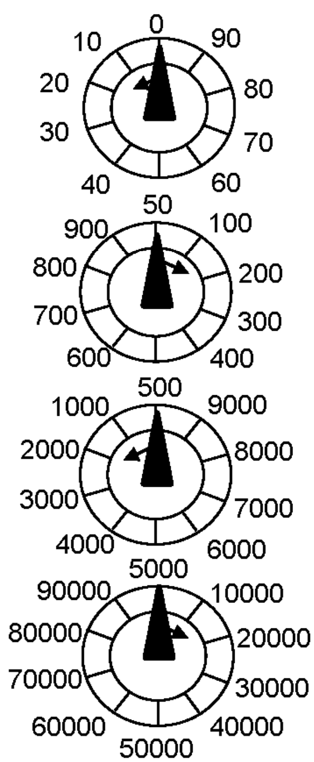
The TSK flow meter uses opposing gears that all rotate continuously when the propeller spins. There are several points to know to read them accurately.

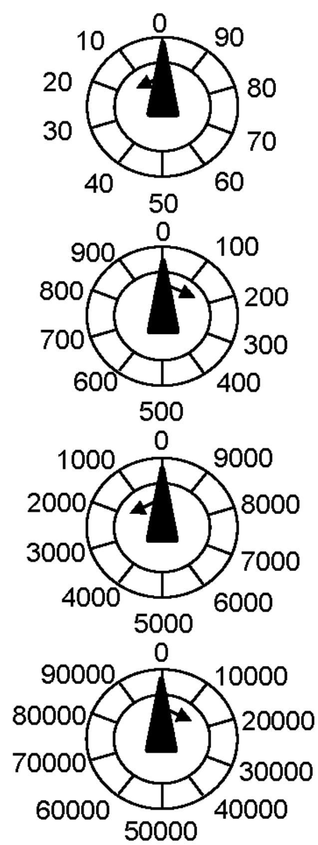
1) The flow meter should be reset PERFECTLY to zero on all dials before each deployment as shown below (rotate dials up to 0 by hand).

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.

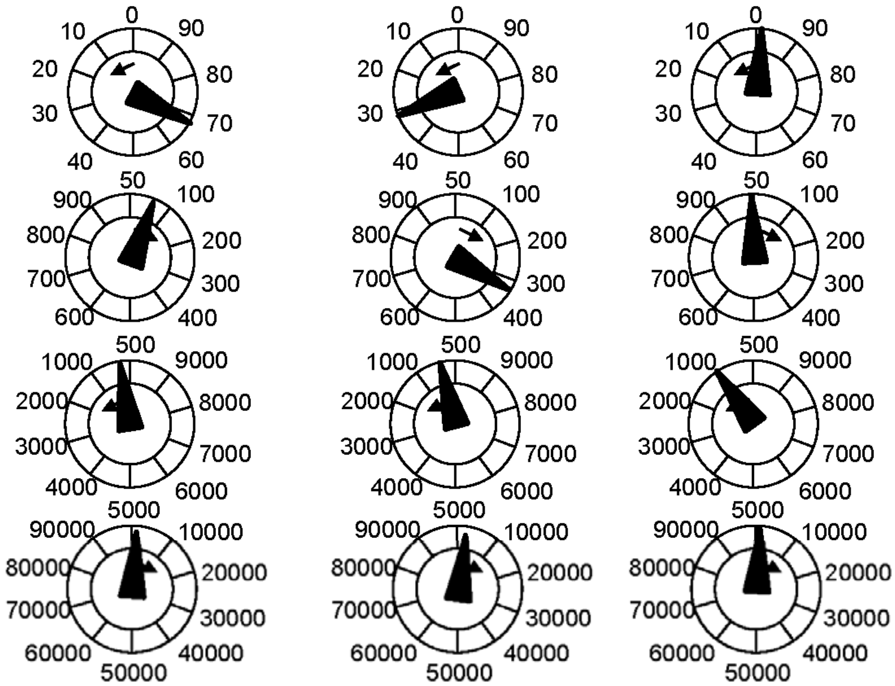
3) Start by reading the bottom dial and work up. See examples on next page. Because dials rotate continuously, every dial will show some reading after a tow, but a dial doesn't "count" until it's gone at least *past* its first tick (past 10 on the first dial, past 100, etc.). You will rarely if ever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

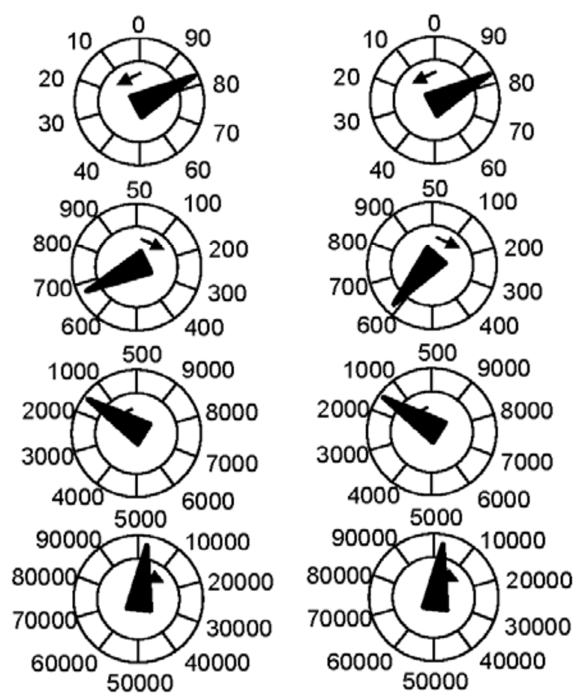
IMPORTANT -- Note these two slightly different readings.

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???**

SSMSP Zooplankton Monitoring

Collecting group:________________________ **Collector names:**_______________________

Collection Date:_________________________ **Station:**____________________________

Gear type:	**Bongos** 60-cm, 335-µm	**Bongos** 60-cm, 335-µm	**Bongos** 60-cm, 335-µm	**Vertical** 60-cm, 200-µm
Station ID				
Latitude				
Longitude				
Tow start time				
Tow end time				
Station Depth (m)				
Wire out (m)*				
Wire angle on deployment*				
Target tow depth (m)				
Sensor depth reading (m)				
Flow meter serial #				
Flow meter revs:START:				
END:				
TOTAL:				
Weather / sea state and winds:				
Comments (include engine RPMs for bongo tows):				

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