

## **Calibration**

### **Introduction**

Within the realization of the project “POSEIDON-II”, a large tank was constructed by Fugro-Oceanor under counseling by Vassilis Zervakis, of the Dept. of Marine Sciences of the University of the Aegean, and delivered to the Institute of Oceanography of the Hellenic Centre for Marine Research. The design of the tank was based on the previous experience obtained by H.C.M.R. staff in using a self-constructed tank for calibrations in the framework of the “POSEIDON-I” project.

The tank designed for the “POSEIDON-II” calibrations is large. The reasons for designing a large tank are:

- it allows the calibrations of many instruments simultaneously,
- it is highly thermally stable, due to the large mass of water it contains
- it can be used not only for “POSEIDON-II” project instruments, but also for the largest CTDs and current-meters used by the H.C.M.R.

### **Characteristics of the T/C tank**

The new T/C calibration tank is larger than the previous one. It has a cylindrical shape, with an inner diameter of about 122 cm and an inner height of 120cm, allowing all the temperature / salinity measuring instruments of H.C.M.R. to be immersed in it. The tank is made of PVC walls and polyurethane filling, and the 9-cm thick walls provide excellent heat insulation. It is equipped with a 2000 W heating element and an electric motor equipped with a propeller for the efficient homogenization of the water in the tank.

### **The need for calibration**

All sensors are only as good as their calibration. A good sensor will produce poor results with poor calibration, but a poor sensor will only provide relatively poor data with the best calibration. Good measurements require both good sensors and good calibrations. A good sensor requires both good static and

dynamic calibration to obtain optimum results. A major part of any calibration is a sensor's past calibration and performance history.

### Temperature calibration

For the calibration of temperature sensors (thermistors) we use the SBE-35 Deep Ocean Standards Thermometer as a reference source.

Deep Ocean Standards Thermometer <i>SBE 35</i>	Temperature (ITS-90)	Range -5 to +35 °C	Accuracy 0.001 °C	Resolution 0.000025 °C
---	-------------------------	-----------------------	----------------------	---------------------------

Important considerations in temperature measurements we must keep in mind.

Pressure sensitivity: Thermistors and PRTs are also pressure sensitive, so to eliminate this effect the sensing elements are put into pressure protected tubes. However, this increases the mass of the sensor and reduces the frequency response.

Self-heating: It takes energy to make a measurement. Thus the thermistor is heated by the current required to make the measurement, and the measurement may be contaminated. With the thermistor controlled Wien bridge oscillator, about 75 milliwatts is used in the sensor, but a minimum of about  $10^{-7}$  watts is dissipated in the thermistor itself, to reduce self heating error to less than 0.000.1 °C.

External heating: In atmospheric temperature measurements, care must be taken to keep the direct heating from any source to a minimum.

Sensor Signal to Noise Ratio - drift, resolution, repeatability. The ability of a sensor to measure the environment is best shown by its signal to noise ratio. The full sensor noise spectrum is seldom given by manufacturers, but instead they refer to such quantities as drift, resolution and repeatability. In reality the thing you want is to plot the signal being measured against the noise of the sensor. Normally geophysical signals have higher energy at low frequency and lower energy at higher frequency. The low frequency part of the sensor noise, is often referred to as "drift" or long-term stability, and is also reflected in the parameter known as accuracy. The high frequency part of the sensor

noise spectrum is related to “repeatability” and resolution or short-term stability. In general we use:

Accuracy - represents the ability of a sensor to measure a signal relative to some absolute standard. A temperature sensor might be specified as having a range of  $-5^{\circ}\text{C}$  to  $+25^{\circ}\text{C}$  with an accuracy of  $0.01^{\circ}\text{C}$  over one year. This means that in this temperature range, the sensor has a low drift (generally due to aging of components), so that within a year's time, the reading typically will change by less than  $0.01^{\circ}\text{C}$ .

Resolution - the ability of a sensor to see differences or changes in signal. Generally the resolution is smaller than the accuracy. The sensor can change slowly with time, and have a poor accuracy, but still have a good resolution.

Repeatability - the ability of a sensor to duplicate a previous output with the same input. This is related to accuracy and it depends on the sampling time frame.

For the Sea Bird temperature sensor used on the HCMR, we can look at the differences from calibration to calibration relative to the same standard (SBE-35). Keeping a track of these differences we can evaluate the sensors performance.

## **Conductivity calibration**

Modern technology allows us to measure the electrical conductivity of seawater and calculate salinity from conductivity assuming standard ionic composition of seawater and hence the conductivity of seawater does not change significantly. (Note this is the assumption in all measurements of salinity, i.e. that the ionic composition of the dissolved solids does not change.) This will probably be violated in estuaries where the salinity results from the mixing of fresh water with seawater at the mouth of the estuary, and the dissolved solids brought down the river. There are two ways that are currently used to measure the conductivity of seawater.

1. Inductive - Here two coils (transformers) are coupled by a tube containing sea water. An AC signal is applied to one coil, and the amount of signal transferred to the other depends on the electrical properties of the fluid connecting the two coils. The dimensions of this tube must be critically controlled, and must not change. There

also must be no bubbles or other materials in the cell, which do not have the conductivity of seawater.

2. Direct electrode measurement. The method used in laboratory salinometers and in the Neil Brown Mark III and V and the Sea Bird CTDs. It is based on the simple and then in the cross terms, to fit the observed laboratory data, i.e.

$$\begin{aligned}
 S = & a + bT + cT^2 + dT^3 + \dots \\
 & + eC + fC^2 + GC^3 + \dots \\
 & + hP + iP^2 + jP^3 + \dots \\
 & + kTC + lTC^2 + \dots \\
 & + mT^2C + \dots \\
 & + \dots
 \end{aligned}$$

The standard scale is the Practical Salinity Standard and called PSS-78, which was adopted by the oceanographic community in 1978. The UNESCO reports give background papers, descriptions, FORTRAN program listings and test tables for the PSS-78. For a very rough approximation over typical values found in the open ocean, we can reduce the complicated power expansion of the accurate equation to

$$S = 3.55 + 10.2 \cdot C - 0.73 \cdot T$$

which is not a function of pressure to the first order. So for  $T=10.0^\circ\text{C}$ ,  $P=0.0$  dbars and  $C=3.808$  S/M, the full equation gives  $S=35.00$  ppt. The above approximation yields  $S=35.092$  ppt. Inverting the above approximation, it is observed that conductivity is a stronger function of temperature than salinity.

$$C = 0.098 \cdot S + 0.0716 \cdot T - 0.348$$

In the ocean, the temperature (range 0 to  $30^\circ\text{C}$ ) fluctuates more than salinity (range 30 to 40 PSU and typically between 33 and 35 PSU), so a conductivity record tends to look very much like a temperature record.

The Sea Bird conductivity sensor (used on HCMRs moorings and CTDs) uses a Beckman three-electrode conductivity cell as the variable resistance element in an AC Wien Bridge Oscillator. The two outside cells are at ground and the middle cell is the varying electrode, so the cell is symmetrical. The AC signal prevents electrode polarization. The measurement is contained entirely within the cell so the dimension of the cell is the critical factor. All electrodes

suffer from fouling, such biological growth, oils, etc. In moored applications, this is the limiting factor in observations. With longer monitoring programs being required, it is important to make time series observations of salinity (so we can calculate density, etc.), and we can't do long term conductivity observations as easily as temperature or pressure measurements.

Drift - The drift of a conductivity sensor is generally due to, (1) electronic component aging, (2) electrode fouling, (3) dimensional changes in the cell geometry, and (4) non-conducting material in the cell. Generally the electronic drift is smooth and smaller than other factors. The fouling problem is the limiting factor in making conductivity measurements (especially moored observations) in the ocean environment.

- a. Warm equatorial waters are conducive to calcium carbonate formation inside the cells. A mild solution of HCl will dissolve this and bring the cell back into calibration.
- b. Electrodes are platinized to increase the area of the electrode in contact with seawater. If the area is large, then this drops out of the equation, leaving only the basic seawater conductivity. The platinum black used is a very spongy material, which allows a large surface area of platinum to be in good electrical contact with the seawater. However, any mechanical damage (such as cleaning the cell with a brush) will change the cell constant, and the readings.
- c. Biology, especially in warm surface waters, is looking for a place to grow, and will contaminate the sensor in two ways. By growing around the cell openings, it will block the water flow and hence affect the reading. By growing in the cell, the dimension will be changed and material in the cell with conductivity other than that of seawater will affect the reading. These effects are reduced in moored sensors by the addition of TriButyl Tin poison tubes on each end of the cells. Occasionally a CTD sensor will be temporarily fouled with a jellyfish or other small piece of biological material, and may even require mechanical cleaning.
- d. If a wet electrode cell is allowed to dry out with salt water in it, salt crystals will be formed in the platinizing and reduce the area that is in contact with the seawater. When the cell is wet again, the area of the electrodes are changed, and these salt crystals affect the readings until they have dissolved, which can take a surprising amount of time (order of weeks). Therefore, Sea Bird recommends that the user keep a Tygon tube on the conductivity cell, keeping it moist.

- e. Sediment in the conductivity cell. Sediment is generally small pieces of minerals such as quartz, and as such non-conductive. The conductivity cell measures the flow of current per square unit of cell cross-section, and when this area is blocked by a non-conducting material, the conductivity drops. a typical bottom moored record will show negative going spikes due to the effects of suspended sediments.

The calibration of conductivity sensors at HCMR takes place in the same thermo-isolated tank as temperature calibration with discrete gradients for both parameters. In order to achieve that fresh sea water is collected and then with the use of the tanks heater the upper predefined limit of temperature and salinity is reached. Then with the use of crushed ice both parameters decrease down to the lower limit. During this multistep procedure the physical properties of the seawater are monitored and data and water samples are collected. The samples are later analyzed in a high precision salinometer and conductivity/salinity sensors data are fitted to the samples values.

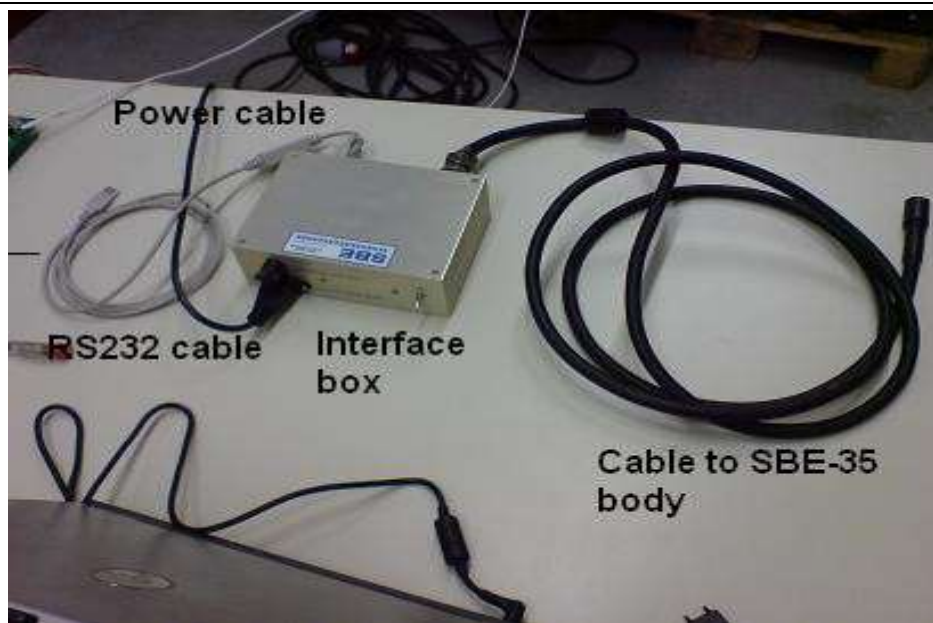
## T- C Calibration Procedure Protocol

### Preparation: Day before

1. Ice supply for T-C gradients pending from the desired calibration ranges. At least 300 kgr per experiment must be available. (The collection from the ice-making machine should start at least 2 WEEKS before the experiment the ice bags are stored in the freezer outside the lab).
2. Filling up the tank with local seawater (1100-1200 lt).
3. Testing the functionality of the stirrer and heater. Stirrer and heater should be turn on from the previous day not only to test their functionality but to increase the water temperature to the predefined calibration data range. This procedure sometimes takes hours.
4. Setting up and testing the reference sensors, logging files and monitor screens. **(Note 1)**
5. Decide the calibration data range. **(Note 2)**
6. Configure the instruments to be calibrated. **(Note 3)**

### Note 1.

The SBE-35 Deep Ocean Standards Thermometer comes with an interface box, a cable to connect the interface box with the thermometer body, another cable to connect with a standard RS232 serial port. We connect the RS232 cable to the connector of the interface box and the corresponding port of a PC equipped with the Seaterm program of Seabird Electronics. If the PC does not have a serial port, it is imperative to find and install a USB to serial converter.



SBE 35 interace box and connectors.

After the connection we turn the thermometer interface box ON and we load Seaterm. The SBE 35 starts logging with the RUN command and data are presented in real time through the Seaterm terminal window. Because the data string recorded by Seaterm (with capture tab On) does not include a time stamp we use Teraterm (freeware software) too, in order to have the exact time of the measurement attached to the SBE 35 data string.

The number of acquisition cycles per sample is user-programmable (N Cycles). Increasing the number of cycles per sample increases the time to acquire the sample, while reducing the RMS temperature noise from the sensor. In our experiments we used NCYCLES = 8, thus the sampling rate of our instrument was every 11.5 seconds resulting to 5 measurements per minute.

The secondary sensors used for monitoring in real time the temperature, conductivity and salinity are a SBE 37 SIP and an Aanderaa 3919B both with serial interface. They are connected to a different pc and set to transmit real time data with sampling interval close to the SBE 35.



Date	Time	Depth	Parameter	Value
Tue Jul 26	16:19:09.062 2011	3919	Conductivity	51.401
Tue Jul 26	16:19:09.062 2011	3919	Temperature	22.056
Tue Jul 26	16:19:09.062 2011	3919	Salinity	36.092
Tue Jul 26	16:19:39.046 2011	3919	Conductivity	51.401
Tue Jul 26	16:19:39.046 2011	3919	Temperature	22.056
Tue Jul 26	16:19:39.046 2011	3919	Salinity	36.094
Tue Jul 26	16:20:09.046 2011	3919	Conductivity	51.402
Tue Jul 26	16:20:09.046 2011	3919	Temperature	22.056
Tue Jul 26	16:20:09.046 2011	3919	Salinity	36.093
Tue Jul 26	16:20:24.046 2011	3919	Conductivity	51.404
Tue Jul 26	16:20:24.046 2011	3919	Temperature	22.056
Tue Jul 26	16:20:24.046 2011	3919	Salinity	36.095
Tue Jul 26	16:20:39.046 2011	3919	Conductivity	51.402
Tue Jul 26	16:20:39.046 2011	3919	Temperature	22.058
Tue Jul 26	16:20:39.046 2011	3919	Salinity	36.092
Tue Jul 26	16:20:54.046 2011	3919	Conductivity	51.403
Tue Jul 26	16:20:54.046 2011	3919	Temperature	22.058
Tue Jul 26	16:20:54.046 2011	3919	Salinity	36.093
Tue Jul 26	16:21:09.046 2011	3919	Conductivity	51.402
Tue Jul 26	16:21:09.046 2011	3919	Temperature	22.058
Tue Jul 26	16:21:09.046 2011	3919	Salinity	36.092
Tue Jul 26	16:21:24.046 2011	3919	Conductivity	51.404
Tue Jul 26	16:21:24.046 2011	3919	Temperature	22.058
Tue Jul 26	16:21:24.046 2011	3919	Salinity	36.094
Tue Jul 26	16:21:39.046 2011	3919	Conductivity	51.402
Tue Jul 26	16:21:39.046 2011	3919	Temperature	22.058
Tue Jul 26	16:21:39.046 2011	3919	Salinity	36.092
Tue Jul 26	16:21:54.046 2011	3919	Conductivity	51.404
Tue Jul 26	16:21:54.046 2011	3919	Temperature	22.058
Tue Jul 26	16:21:54.046 2011	3919	Salinity	36.094
Tue Jul 26	16:22:09.046 2011	3919	Conductivity	51.403
Tue Jul 26	16:22:09.046 2011	3919	Temperature	22.058
Tue Jul 26	16:22:09.046 2011	3919	Salinity	36.093
Tue Jul 26	16:22:24.046 2011	3919	Conductivity	51.403
Tue Jul 26	16:22:24.046 2011	3919	Temperature	22.058
Tue Jul 26	16:22:24.046 2011	3919	Salinity	36.093
Tue Jul 26	16:22:39.046 2011	3919	Conductivity	51.402
Tue Jul 26	16:22:39.046 2011	3919	Temperature	22.058
Tue Jul 26	16:22:39.046 2011	3919	Salinity	36.092
Tue Jul 26	16:22:54.046 2011	3919	Conductivity	51.403
Tue Jul 26	16:22:54.046 2011	3919	Temperature	22.059
Tue Jul 26	16:22:54.046 2011	3919	Salinity	36.092
Tue Jul 26	16:23:09.046 2011	3919	Conductivity	51.402
Tue Jul 26	16:23:09.046 2011	3919	Temperature	22.059
Tue Jul 26	16:23:09.046 2011	3919	Salinity	36.091
Tue Jul 26	16:23:24.046 2011	3919	Conductivity	51.406
Tue Jul 26	16:23:24.046 2011	3919	Temperature	22.059
Tue Jul 26	16:23:24.046 2011	3919	Salinity	36.094
Tue Jul 26	16:23:39.046 2011	3919	Conductivity	51.404
Tue Jul 26	16:23:39.046 2011	3919	Temperature	22.059
Tue Jul 26	16:23:39.046 2011	3919	Salinity	36.093
Tue Jul 26	16:23:54.046 2011	3919	Conductivity	51.404
Tue Jul 26	16:23:54.046 2011	3919	Temperature	22.059
Tue Jul 26	16:23:54.046 2011	3919	Salinity	36.093
Tue Jul 26	16:24:09.046 2011	3919	Conductivity	51.404
Tue Jul 26	16:24:09.046 2011	3919	Temperature	22.059
Tue Jul 26	16:24:09.046 2011	3919	Salinity	36.093
Tue Jul 26	16:24:24.031 2011	3919	Conductivity	51.404
Tue Jul 26	16:24:24.031 2011	3919	Temperature	22.059
Tue Jul 26	16:24:24.031 2011	3919	Salinity	36.093
Tue Jul 26	16:24:39.031 2011	3919	Conductivity	51.406
Tue Jul 26	16:24:39.031 2011	3919	Temperature	22.059
Tue Jul 26	16:24:39.031 2011	3919	Salinity	36.094
Tue Jul 26	16:24:54.031 2011	3919	Conductivity	51.406
Tue Jul 26	16:24:54.031 2011	3919	Temperature	22.059
Tue Jul 26	16:24:54.031 2011	3919	Salinity	36.093
Tue Jul 26	16:25:09.031 2011	3919	Conductivity	51.404
Tue Jul 26	16:25:09.031 2011	3919	Temperature	22.059
Tue Jul 26	16:25:09.031 2011	3919	Salinity	36.093
Tue Jul 26	16:25:24.031 2011	3919	Conductivity	51.406
Tue Jul 26	16:25:24.031 2011	3919	Temperature	22.059
Tue Jul 26	16:25:24.031 2011	3919	Salinity	36.094
Tue Jul 26	16:25:39.031 2011	3919	Conductivity	51.404
Tue Jul 26	16:25:39.031 2011	3919	Temperature	22.059
Tue Jul 26	16:25:39.031 2011	3919	Salinity	36.093
Tue Jul 26	16:25:54.031 2011	3919	Conductivity	51.406
Tue Jul 26	16:25:54.031 2011	3919	Temperature	22.059
Tue Jul 26	16:25:54.031 2011	3919	Salinity	36.094
Tue Jul 26	16:26:09.031 2011	3919	Conductivity	51.407
Tue Jul 26	16:26:09.031 2011	3919	Temperature	22.061
Tue Jul 26	16:26:09.031 2011	3919	Salinity	36.093
Tue Jul 26	16:26:24.031 2011	3919	Conductivity	51.404
Tue Jul 26	16:26:24.031 2011	3919	Temperature	22.061
Tue Jul 26	16:26:24.031 2011	3919	Salinity	36.091
Tue Jul 26	16:26:39.031 2011	3919	Conductivity	51.407
Tue Jul 26	16:26:39.031 2011	3919	Temperature	22.061
Tue Jul 26	16:26:39.031 2011	3919	Salinity	36.093
Tue Jul 26	16:26:54.031 2011	3919	Conductivity	51.406
Tue Jul 26	16:26:54.031 2011	3919	Temperature	22.061
Tue Jul 26	16:26:54.031 2011	3919	Salinity	36.092
Tue Jul 26	16:27:09.031 2011	3919	Conductivity	51.406
Tue Jul 26	16:27:09.031 2011	3919	Temperature	22.061
Tue Jul 26	16:27:09.031 2011	3919	Salinity	36.092
Tue Jul 26	16:27:24.031 2011	3919	Conductivity	51.407
Tue Jul 26	16:27:24.031 2011	3919	Temperature	22.061
Tue Jul 26	16:27:24.031 2011	3919	Salinity	36.093

Print screen from the Aanderaa 3919B using TerraTerm to log data.

NOTICE: PCs that will be used during the experiment should be fully synchronized. Differences in time can affect the quality of the data.

## Note 2.

In order to minimize the calibration time, we can plan our procedure in such a way as to cover both the temperature and conductivity ranges that would keep our measurements within calibration range. Thus, the first thing we need to do is to define the calibration ranges for temperature and salinity.

For temperature, just a fast review of the bibliography reveals that a range from 11 to 28 degrees C easily covers the whole range of temperatures found in the open seas around Greece. For conductivity, it is slightly more complicated: One has to consider the minimum (11°C) and maximum (28 °C) temperatures, minimum (31) and maximum (39.6) salinities and minimum (1dbar) and maximum (5000 dbar) pressures, and then calculate the range of conductivities that these values determine. The minimum conductivity is estimated using the minima of all the other parameters, and the maximum accordingly. Using the above values, we find a minimum conductivity of 3.50 S m<sup>-1</sup> and a maximum conductivity of 6.47 S m<sup>-1</sup>. These are the values defining the conductivity range we have to cover in the tank.

These ranges can be compute it using the SeaCalc by Seabird and for further planning of the experiment one should the “Calibration\_planning.xls” created by Prof. Vassilis Zervakis. Using this methodology it is possible for the calibration range to change to any desired limits.

SeaCalc II

Practical Salinity | Absolute Salinity |

Use this tab to calculate Practical Salinity, as defined by the 1978 Practical Salinity Scale (PSS 1978).

Pressure [dbar]	0.000	Depth [salt water, m] = 0.000
Temperature [ITS-68, deg C]	28.006720	Depth [fresh water, m] = 0.000
Temperature [ITS-90, deg C]	28.000000	Density [sigma-t, Kg/m <sup>3</sup> ] = 24.65272
Conductivity [S/m]	6.047613	Density [sigma-theta, Kg/m <sup>3</sup> ] = 24.65272
Practical Salinity [PSU]	38.000000	Density [sigma-ref p, kg/m <sup>3</sup> ] = 24.65272
Reference Pressure [dbar]	0.00	Potential Temperature [ITS-68, deg C] = 28.00672
Latitude [deg]	35.0	Sound Velocity [Chen-Millero, m/s] = 1544.499
		Sound Velocity [Wilson, m/s] = 1544.885
		Sound Velocity [Delgrossi, m/s] = 1544.407
		Specific Volume Anomaly [10 <sup>-8</sup> * m <sup>3</sup> /Kg] = 327.838
		Oxygen Saturation, Weiss [ml/l] = 4.421
		Gravity [m/s <sup>2</sup> ] = 9.797328

Calculate Exit Βοήθεια

SeaCalc print screen

### Note 3

First of all the instruments to be calibrated must be synchronized with one of the lab pc and previous data must be already downloaded leaving the memory empty. Secondary an appropriate sampling frequency should be set; usually we use a time interval of 15 sec which is close to the SBE 35 interval. In reality the SBE 37 always averages 4 A/D cycles per sample and SBE 16 plus provides an option for the averaging cycles per sample (we choose again 4 A/D cycles per sample).

```
SBE16_5039_rawhex.cnv - Σημειωματάριο
Αρχείο  Επεξεργασία  Μορφή  Προβολή  Βοήθεια
* Sea-Bird SBE16plus Data File:
* Filename = C:\Documents and Settings\calib01\Desktop\Calibration_26_July2011\5039_files\SBE16_5039_rawhex.hex
* Software Version 1.57
* Temperature SN = 5039
* Conductivity SN = 5039
* System Upload Time = Jul 27 2011 14:01:42
* ds
* s
* SBE 16plus-IM V 1.2c SERIAL NO. 5039 27 Jul 2011 14:00:02
* vbatt = 11.8, vlith = 8.4, ioper = 49.4 ma, ipump = 99.5 ma,
* status = not logging
* sample interval = 15 seconds, number of measurements per sample = 4
* samples = 1293, free = 837567
* run pump during sample, delay before sampling = 2.0 seconds
* battery cutoff = 7.5 volts
* pressure sensor = none
* SBE 38 = no, Ext Volt 0 = no, Ext Volt 1 = no, Ext Volt 2 = no, Ext Volt 3 = no
* output format = raw HEX
* S>
* cal
* SBE 16plus-IM V 1.2c SERIAL NO. 5039 27 Jul 2011 14:00:36
* temperature: 07-apr-07
* TA0 = 1.236299e-03
* TA1 = 2.563994e-04
* TA2 = 6.912602e-07
* TA3 = 1.193900e-07
* TOFFSET = 0.000000e+00
* conductivity: 07-apr-07
* G = -1.018416e+00
* H = 1.593051e-01
* I = -5.900539e-04
* J = 7.029523e-05
* CF0 = 2.536905e+03
* CPCOR = -9.570000e-08
```

CTD 16 plus configuration

## T- C calibration experiment

The practices followed during the HCMRs T-C calibration experiment are described above as a multi-step procedure.

Start the reference thermometer and the monitor sensors, in order to log temperature and conductivity. Sensors should be placed to the center of the tank. The tank has to be warmed up to the desired temperature .THE MIXING MOTOR SHOULD ALWAYS BE ON WHEN HEATER IS ON.

1. Beginning of the experiment. Starting to log the procedure in a excel file.
2. Stirrer Motor in ON Mode
3. Heater in ON Mode
4. Measure the Tank temperature
5. Once the desired temperature and conductivity values have been reached, STOP the heater but leave the motor running.
6. Start the instruments to be calibrated and obtain 5 minutes readings in the air.

7. Close the stirrer and place the instruments inside the tank.
8. Open the stirrer until the water is fully homogenized.
9. Wait for the water to get fully homogenized. The criterion for a fully homogenized tank is based on the standard deviation of the measurements of the SBE- 35 thermometer, which provides continuous real-time measurements of the tank's temperature. **(Note 1)**
10. Note the reference values for Temperature, Conductivity, Salinity
11. First calibration point
12. Reducing the stirrers rotation speed (frequency=18)
13. Beginning of the 5 minute time window. Note the reference values for Temperature, Conductivity and Salinity.
14. Take 5-min long measurements
15. End of the 5 minute time window. Note the reference values for Temperature, Conductivity and Salinity.
16. Sample for salinity .Rinse 3 times and fill a salinity bottle with water from the tank. Close the bottle firmly, and mark it with the characteristic sample number for identification. Use empty bottles - do not spill foreign water into the tank.
17. Removal of water from the tank. An amount between 15-30 lts must be removed in order to reach the next calibration point.
18. Increasing the stirrers rotation speed (frequency=24)
19. Adding crushed ice in the tank in order to reach the next calibration point.
20. Wait for the water to get fully homogenized within the predefined calibration data steps. When it is then you have reached the next Calibration point.

21. Second calibration point
22. Repeat procedure described in steps 10-22 for all your calibration points.  
Total 12-15 calibration points are needed in order to cover the desired measuring range.
23. Upon finishing the procedure with the last calibration point stirrer goes to OFF mode.
24. Carefully remove all instruments from the tank (reference sensors also), stop logging, download the data and wash them with fresh water
25. Empty the tank and wash it with fresh water
26. Set the salinity bottles aside to obtain room temperature, as well as the SSW bottle
27. Turn on the salinometer for next day's use, as it requires being on for at least eight hours before use.
28. End of the experiment.

### Note 1

The output data string of the SBE- 35 thermometer looks like this

197.20 1047481 289795.4 15 35 29 289955.4 22.654745

Where

- 197.20 = average of raw **reference** zero readings taken during a measurement
- 1047481 = average of raw **reference** resistor full scale readings taken during a measurement
- 289795.4 = average of raw thermistor readings taken during a measurement
- 15 = (maximum – minimum) raw **reference** zero reading during a measurement (provides a measure of the amount of variation during the measurement)



- 35 = (maximum – minimum) raw **reference** resistor full scale reading during a measurement (provides a measure of the amount of variation during the measurement)
- 29 = (maximum – minimum) raw thermistor reading during a measurement (provides a measure of the amount of variation during the measurement)
- 289955.4 = average raw thermistor reading, corrected for zero and full scale reference readings
- 22.654745 = average corrected raw thermistor reading, converted to engineering units (°C [ITS-90])

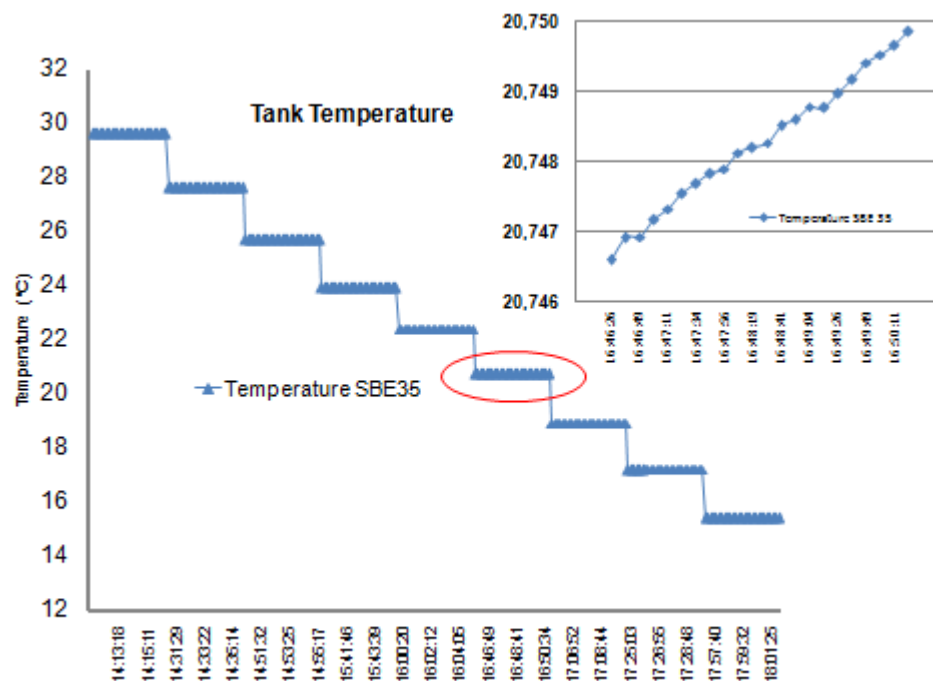
This 6th column of the data string is an indicator of the homogeneity of the water mass inside the tank.

teratermsbe35.log - Σημειωματάριο										
Αρχείο	Επεξεργασία	Μορφή	Προβολή	Βοήθεια						
[Tue Jul 26 14:22:34.885 2011]	198.02	1047500	235825.5	15	19	15	235914.1	27.206969		
[Tue Jul 26 14:22:46.131 2011]	198.02	1047499	235829.8	13	16	15	235918.6	27.206474		
[Tue Jul 26 14:22:57.378 2011]	198.43	1047500	235830.0	12	16	14	235918.2	27.206519		
[Tue Jul 26 14:23:08.625 2011]	197.89	1047499	235830.3	14	18	17	235919.3	27.206398		
[Tue Jul 26 14:23:19.868 2011]	198.77	1047499	235832.0	11	16	13	235920.2	27.206301		
[Tue Jul 26 14:23:31.115 2011]	198.33	1047500	235832.0	12	17	15	235920.4	27.206271		
[Tue Jul 26 14:23:42.362 2011]	197.55	1047500	235835.6	15	19	12	235924.7	27.205801		
[Tue Jul 26 14:23:53.608 2011]	197.78	1047499	235835.9	16	20	17	235924.9	27.205777		
[Tue Jul 26 14:24:04.852 2011]	198.11	1047500	235838.4	15	19	12	235927.0	27.205541		
[Tue Jul 26 14:24:16.098 2011]	198.16	1047499	235838.3	13	18	17	235927.1	27.205528		
[Tue Jul 26 14:24:27.344 2011]	198.07	1047500	235841.3	14	20	22	235929.9	27.205227		
[Tue Jul 26 14:24:38.591 2011]	198.35	1047501	235838.3	15	19	19	235926.5	27.205595		
[Tue Jul 26 14:24:49.838 2011]	197.75	1047500	235842.0	13	19	16	235930.8	27.205121		
[Tue Jul 26 14:25:01.081 2011]	198.37	1047500	235846.8	15	16	19	235935.2	27.204632		
[Tue Jul 26 14:25:12.328 2011]	198.17	1047500	235847.9	12	18	12	235936.6	27.204486		
[Tue Jul 26 14:25:23.574 2011]	198.15	1047500	235846.8	14	19	15	235935.3	27.204630		
[Tue Jul 26 14:25:34.821 2011]	197.43	1047499	235849.1	16	14	20	235938.4	27.204279		
[Tue Jul 26 14:25:46.068 2011]	198.34	1047500	235850.4	12	21	16	235938.9	27.204226		
[Tue Jul 26 14:25:57.311 2011]	197.68	1047500	235846.9	13	18	13	235935.8	27.204572		
[Tue Jul 26 14:26:08.558 2011]	198.37	1047499	235849.8	12	17	14	235938.5	27.204273		
[Tue Jul 26 14:26:19.805 2011]	197.98	1047499	235850.2	13	17	16	235939.1	27.204209		
[Tue Jul 26 14:26:31.051 2011]	198.27	1047500	235851.7	12	17	12	235940.2	27.204081		
[Tue Jul 26 14:26:42.298 2011]	197.91	1047499	235855.3	13	18	15	235944.2	27.203638		
[Tue Jul 26 14:26:53.542 2011]	197.66	1047500	235857.5	14	18	20	235946.5	27.203383		
[Tue Jul 26 14:27:04.788 2011]	198.05	1047500	235857.9	17	22	18	235946.6	27.203379		
[Tue Jul 26 14:27:16.036 2011]	197.95	1047500	235862.2	16	19	16	235950.9	27.202895		
[Tue Jul 26 14:27:27.281 2011]	198.09	1047499	235865.7	14	18	16	235954.6	27.202491		
[Tue Jul 26 14:27:38.525 2011]	198.30	1047501	235865.4	11	17	18	235953.7	27.202584		
[Tue Jul 26 14:27:49.772 2011]	198.73	1047500	235863.9	14	17	17	235952.0	27.202782		
[Tue Jul 26 14:28:01.018 2011]	198.20	1047500	235889.1	17	16	114	235977.7	27.199930		
[Tue Jul 26 14:28:12.265 2011]	198.27	1047500	236129.7	13	17	542	236218.5	27.173305		
[Tue Jul 26 14:28:23.512 2011]	197.74	1047500	238014.8	14	20	5488	238106.2	26.965657		
[Tue Jul 26 14:28:34.754 2011]	197.78	1047500	240397.1	16	18	3036	240491.4	26.705952		
[Tue Jul 26 14:28:46.001 2011]	198.01	1047500	239822.3	15	16	2149	239915.9	26.768352		

The SBE-35 time series the last 5 readings occurred after adding ice in the tank.

## Data Analysis

Generally the calibration data analysis involves defining the drift and trying to minimize it. Special care should be given to data logging during the calibration procedure. At the end of the experiment we have to deal with almost 8 hours long time series with sampling intervals of 15 seconds. The first step is to sort all the available data using as a guide the log book of the experiment. The goal is to discriminate the 5 minutes long time series of each calibration point and average the measurements of this interval. The graph above presents the whole time series of the SBE 35 reference thermometer during an experiment and focus on a calibration point.



After doing that for all reference and to be calibrated instruments (the salinometer measurements are performed the next day and are already sorted) we can process the data.

There are two approaches

**Approach A:** Linear fit between reference values and sensor measurements (most cases applied to the data).  $T_{ref} = a T_{sens} + b$

**Approach B:** Recalculating the calibration coefficients of the sensor itself (applied to the sensor e.g SBE CTs).

Seabird Temperature equation  
 $f_o$ ,  $f$ : sensor frequencies

$$ITS-90 = 1 / \{a_0 + a_1 [\ln(f_o/f)] + a_2 [\ln^2(f_o/f)] + a_3 [\ln^3(f_o/f)]\} - 273.15(^{\circ}C)$$

The second approach involves **raw** sensor data that permit the recalculation of the calibration coefficients of the sensor. Usually we can log the raw and the engineering units of the sensor but in some cases we need to convert engineering units to raw with a small price to accuracy because we are processing mean values.

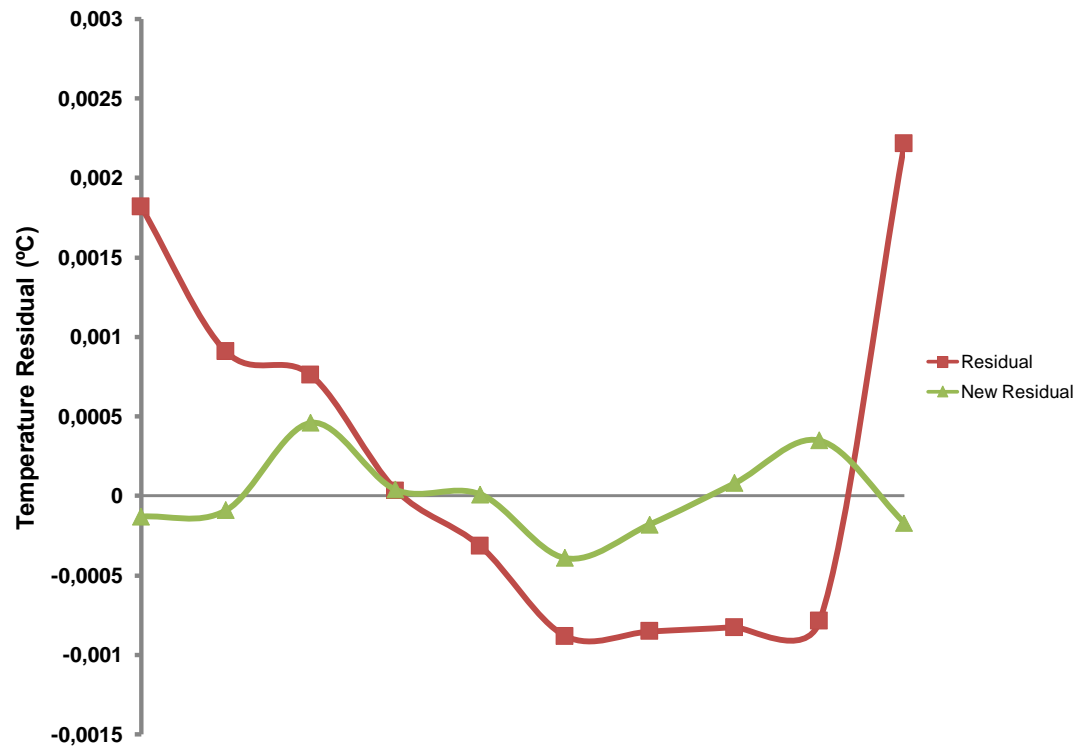
Example of a SBE 16 plus CTD

Calibration points	Reference Temperature	Temp	Pre calibration residual	Instrument Raw Output	New Temp	Post calibration residual	
1	30,047828	30,046010	0,001818	192108,25	30,04795	-0,00013	
2	28,432608	28,431700	0,000908	205278,40	28,43270	-0,00009	
3	27,206936	27,206175	0,000761	215795,55	27,20648	0,00046	
4	25,755863	25,755825	0,000038	228843,35	25,75582	0,00004	
5	24,616346	24,616663	-0,000317	239571,35	24,61634	0,00001	a0= 1.10941082767564e-03
6	23,303023	23,303905	-0,000882	252476,10	23,30341	-0,00039	a1= 3.31638473667286e-04
7	22,045147	22,046000	-0,000853	265408,70	22,04532	-0,00018	a2= -9.04538875861129e-06
8	20,077342	20,078170	-0,000828	286801,80	20,07726	0,00008	a3= 5.35100469170114e-07
9	18,281341	18,282130	-0,000789	307624,05	18,28099	0,00035	
10	16,073931	16,071715	0,002216	334990,55	16,07410	-0,00017	

And the plots of the sensor residual before and after the calibration



Temperature Residuals



# **Calibration Protocol- Dissolved Oxygen**

## **Background**

Two types of oxygen sensors are used. The optode (manufactured by Aanderaa), and the Clark Type sensor (manufactured by Sea Bird). These operate on a different principal. The optode sensor optically measures a specific substance using a chemical transducer. A chemical foil is glued to the tip of an optical cable, the fluorescence properties of this film depend on the oxygen concentration. The optode can work in the whole range of oxygen saturation conditions and no oxygen is consumed during the measurement therefore the sensor is insensitive to stirring.

The clark type sensor works on the principle of a cathode and an anode being submerged in an electrolyte, oxygen enters the sensor through a permeable membrane by diffusion and is reduced by the cathode creating a measurable current. During this measurement oxygen is consumed therefore the sensor needs to be stirred in order to achieve accurate measurements. However, the smaller the sensor the less the oxygen consumption.

The calibration of the sensors is done by comparing their readings with the Winkler method which is the standard reference method for oxygen measurements. In essence, the dissolved oxygen present within a seawater sample is coerced under alkaline conditions to quantitatively oxidize divalent manganese to a trivalent state. The solution is then acidified, which converts iodide ion to iodine in an amount stoichiometrically proportional to the amount of dissolved oxygen contained within the original sample. The amount of iodine is then determined by titration with a thiosulfate solution of known concentration.

## **Sensor calibration**

Optode sensors require a two point calibration, as recommended by the manufacturer. Saturated (100%) and zero oxygen seawater is prepared and the sensors are immersed in the two different concentration tanks following the steps provided by the OxyView (Aanderaa) software. The 100% saturated seawater is prepared by bubbling seawater with an aquarium pump over at least 24 hours prior to calibration. The zero oxygen solution is prepared using sodium sulfite ( $\text{Na}_2\text{SO}_3$ ) powder.

The clark type sensors are calibrated by creating a gradient of oxygen concentrations from which sensor readings are cross referenced with winkler measurements.

Changing the  $O_2$  concentration is achieved by changing the seawater temperature, pressure or salinity. In general, changing the temperature is the preferred approach whilst the other two variables remain constant. A 5 point temperature gradient is desirable following observed regional temperature fluctuations.

## **Procedure**

### **Calibration of OPTODE type sensors:**

Seawater collected prior to the experiment is left in the calibration room to acclimatize, whilst the temperature is monitored. Water for the optode sensor calibration is placed in two separate 20 lt insulated containers one of which is immediately bubbled at the surface with a common aquarium pump for at least 24hrs until calibration, the second container will be used for the zero oxygen solution.

Each sensor is connected to a pc and the oxyview software is run where the calibration procedure is activated, initially the sensor is left for 5 minutes (or until measurements stabilize) in the 100% saturated solution. When this step is complete the sensor is immersed in the zero oxygen solution. This is prepared by mixing Sodium Sulfite with seawater (100gr  $Na_2SO_3$  per 10lt seawater). Sodium sulfite reacts with dissolved oxygen forming sodium sulfate ( $Na_2SO_4$ ). The use of this chemical inhibits the Winkler measurements so cross calibration isn't possible. However following the two step calibration optode sensors can be additionally tested by following the Clark type sensor calibration procedure.

### **Calibration of Clark type sensors:**

Seawater collected is poured into a 200lt tank and left at ambient temperature, water is placed in incubators to warm and at the same time seawater is frozen, a portion of seawater is reserved at room temperature in a separate tank. Seawater is left to stand for at least 24hrs.

The temperature gradient is decided based on the ambient water temperature using 3 degree increments. Usually, two points above the ambient temperature and two below ambient temperature are used. Higher than ambient temperatures are achieved by replacing tank water with seawater warmed in incubators and lower than ambient are achieved by placing frozen sea water in the tank. At each temperature step, all sensors are placed consecutively in the tank until reading stabilize while the temperature is continuously monitored and Winkler samples are taken at each calibration step, in triplicate where possible. The calibration procedure is complete when Winkler analysis is compared to sensor reading and the new linear coefficients are calculated.