Cruise report – Pickled Protists

R/V OCEANUS, 04 August 2009

K. Kormas, M. Pachiadaki, P. Sigala

Collected samples

	Discovery	Urania
Water column	Reference (CTD4) Interface (CTD11, 3, 1) Brine (CTD2)	Reference (CTD6) Interface (CTD8) Brine (CTD5)
Sediment	Reference (DMC2) Brine (DMC1)	Brine? (UMC3) Reference (UMC1) Carbonate crust (UMC4)

Water column

- X4 GFF filters for SPM, SPOM, POC (and possibly PON)
- X1 0.2 μm pore size filter for prokaryotes
- X1 50ml Falcon for DAPI counts

Sediment

- X2 50ml Falcon tubes for DNA extraction and back up.
- 1 Corning in 2% formaldehyde for DAPI counts (+ overlying water)

DMC1 = Oc454-2-E6-MC800-1
DMC2 = Oc454-2-E7-MC800-2
UMC1 = Oc454-2-E18-MC800-5
UMC3 = Oc454-2-E20-MC800-7
UMC4 = Oc454-2-E25-MC800-9

Work plan

1. Analyses of particulates will be completed by December 2009. We have to see whether PON is feasible
2. Bacterial DAPI counts in water and sediment samples will be completed by March 2010.
3. We want to do prokaryotic diversity in selected water column samples but we do not have funding, yet (?), for this (i.e. mostly sequencing). To eliminate redundant analysis and cost, we suggest to make a wish list of samples to be analysed first based on the findings of eukaryotic analysis.
4. Regarding sediments, after DAPI counts, and Joan's findings, we might be able to analyse 16S rDNA diversity, as well. What about 18S rDNA???
5. Rita Miroshnichenko, Russian Academy of Sciences, Moscow, is interested in halophiles isolation. If it is alright with the project, and after arrangements with Ginny, I can send her "live" sediments (4^0C).

Lab protocols to be used

SUSPENDED PARTICULATE MATTER (SPM)
SUSPENDED PARTICULATE ORGANIC MATTER (SPOM)

I. Apparatus - Reagents

1. Furnace (at 500^{0}C).
2. Glass fiber filters (Whatman GF/F 47 mm).
3. Vacuum pump and filtering apparatus
4. Plastic volumetric cylinder, pre filter (optional), forceps, aluminium foil.

II. Field

1. Sea water is collected in polyethylene containers. The water volume is defined from the season and the sampling area (0.5-5 l).
2. The sample is filtered immediately upon the return to the laboratory and no more than 6 hours. It can be frozen for as much as one night.

III. Laboratory

1. A defined volume of sea water is filtered on a pre-weighted and pre-combusted (5000C , 4 hours) filter of glass fibres.
2. At the end of the filtering, the filter is washed with 20 ml of sterilized water to remove the salts (Wassmann 1984).
3. The filter is removed, folded in half with the use of a forceps and is kept in foil at -20^{0}C until moving to step III.4.
4. The filter is dried at 60^{0}C until the weight remains stable (usually 2-3 hrs).
5. The filter is weighted and placed at 500^{0}C for 4 hours. After cooling down in a desicator, it is weighted again.

IV. Calculations

1. SPM is calculated by the difference of the dry filter and the tare of the same filter (g or mg) and is converted per 1 l of filtered sea water (g/l or mg/l)
2. SPOM is calculated by the difference of the burn filter and the dry one (g or mg) and is converted per 1 l of filtered sea water.

V. Notes

The most commonly used filters are WHATMAN GF/C (nominal pore size 1.2 µm). Any other filter can be used, depending on the targeted research. The filters must always be of the same diameter to the filter apparatus.

The pre-filter is usually a plastic net of 200 or 63 µm and it is used for the exclusion of big matter and large mesozooplanktic organisms.

V. References

Strickland, J.D.H., Parsons, T. R. (1972). A practical handbook of seawater analysis. Bulletin 167, Fisheries Research Board of Canada, Ottawa.

Wasmmann P (1984) Sedimentation and benthic mineralization of organic detritus in a Norwegian fjord. Mar. Biol. 83: 83-94

(PARTICULATE ORGANIC CARBON, POC)

I. Apparatus - Reagents

1. Furnace (at 500^{0}C).
2. Glass fiber filters (Whatman GF/F 47 mm).
3. Vacuum pump and filtering apparatus.
4. Plastic volumetric cylinder, pre filter (optional), forceps, aluminium foil.
5. 50 ml glass volumetric cylinder with plug of, pipette.
6. 125 ml conical flasks and 25 ml beakers.
7. Hot plate.
8. Bichromate acid (4.84 g $K_2Cr_2O_7$ is dissolved in 20 ml distilled water). Then this solution is added with extra attention to approximately 500 ml of dense (sw 1,82) H_2SO_4. After it cools down, it is added to 1000 ml dense (sw 1,82) H_2SO_4 and it is kept in a glass bottle.
9. Phosphoric acid 70%.
10. Na_2SO_4 (45 g are diluted in 1000 ml sterile water) (optional).
11. α-D-glucose (7,5 g and some crystals of $HgCl_2$ are diluted in 100 ml sterile water). It remains stable for several months. Discard when turbidity develops. If no $HgCl_2$ is added, the solution is stable for a couple of weeks, kept at 4^{0}C.

II. Field

3. Sea water is collected in polyethylene containers. The water volume is defined from the season and the sampling area (0.5-5 l).
4. The sample is filtered immediately upon the return to the laboratory and no more than 6 hours. It can be frozen for as much as one night.

III. Laboratory

1. A defined volume of sea water is filtered on a pre-weighted and pre-combusted (5000C , 4 hours) filter of glass fibres.
2. At the end of the filtering, the filter is washed with 20 ml of sterilized water to remove the salts (Wassmann 1984).
3. Add 2 ml of Na_2SO_4 solutionto a dry filter and it is filtered until is dried again (optional).
4. The filter is removed, folded in half with the use of a forceps and is kept in foil at -20^{0}C until moving to step III.5.
5. The filter, with the use of a forceps, is placed in the conical flask and add 1 ml of phosphoric acid 70% and 1 ml of sterile water.
6. The conical flasks are covered with the 25 ml beakers and they are placed on the hot plate at 100^{0}C for ½ hour.
7. After removing them from the plate and are cooled down, add in every conical flask the above mentioned quantities of bichromic acid – sterile water (it depends from the expected concentrations[(3)]:

Expected concentration (μg)	Oxidant (ml)	Water (ml)	Final volume (ml)	Cell length (cm)
< 300	2,0	0,8	100	10
300-700	4,0	1,6	50	2,5
700-2000	10,0	4,0	50	1

8. The samples are placed on the hot plate at 100^0C for 1 hour.
9. The content of each conical flask is transferred in a 50ml volumetric cylinder with a stopper. The conical flask is washed 2-3 times with sterilised water. We transfer the water of every wash in the cylinder. Finally, sterile water is added water to 50 ml.
10. After the samples are cooled down, a little portion is transferred in a 10/15 ml centrifuge tube and the samples are centrifuged at 2000 rpm/min for 10 minutes.
11. The absorption of the supernatant is measured at 440 nm against the blank, but the photometer is calibrated with the sample and the measurement is taken from the absorption of the blank.
12. The blank contains sterilized water instead of a sample and it is measured against sterilized water. The analysis is done twice. The measurements must not differ significantly
13. The standard solution is prepared each time from the stock glucose solution by dilution 1 ml to 100 ml sterile water. The analysis is done three times. The third quantity of bichromic acid is used. Two blanks are prepared to use as standard solutions in the photometry.

IV. Calculations

1. The factor F is calculated according the next equation:

$$F = 120 / E_s$$

Where, E_s = the average of the absorption of the standard solutions x 1,1

2. The concentration of the phosphoric salts is calculated by the next equation :

$$\mu g\ C\ l^{-1} = F \cdot E \cdot 1{,}1 \cdot v / V$$

where, E = the sample's absorption
F = the factor F
v = the volume of bichromate acid
V = the volume of the sea water that was filtered

V. Notes

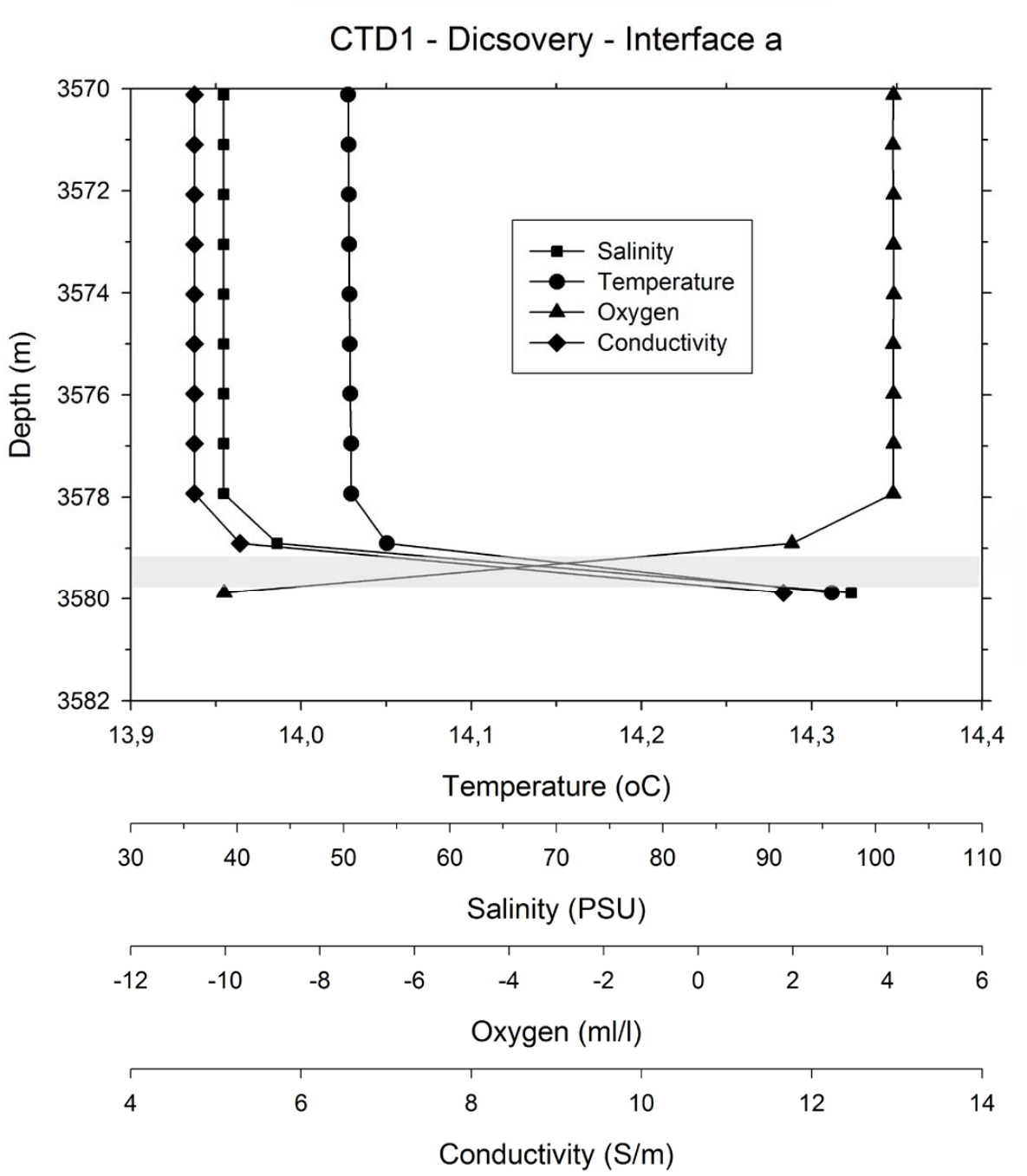
1. The most commonly used filters are WHATMAN GF/C (nominal pore size 1.2 μm). Any other filter can be used, depending on the targeted research. The filters must always be of the same diameter to the filter apparatus.
2. The pre-filter is usually a plastic net of 200 or 63 μm and it is used for the exclusion of big matter and large mesozooplanktic organisms.
3. Usually the second quantity is used in a 1cm cell length.

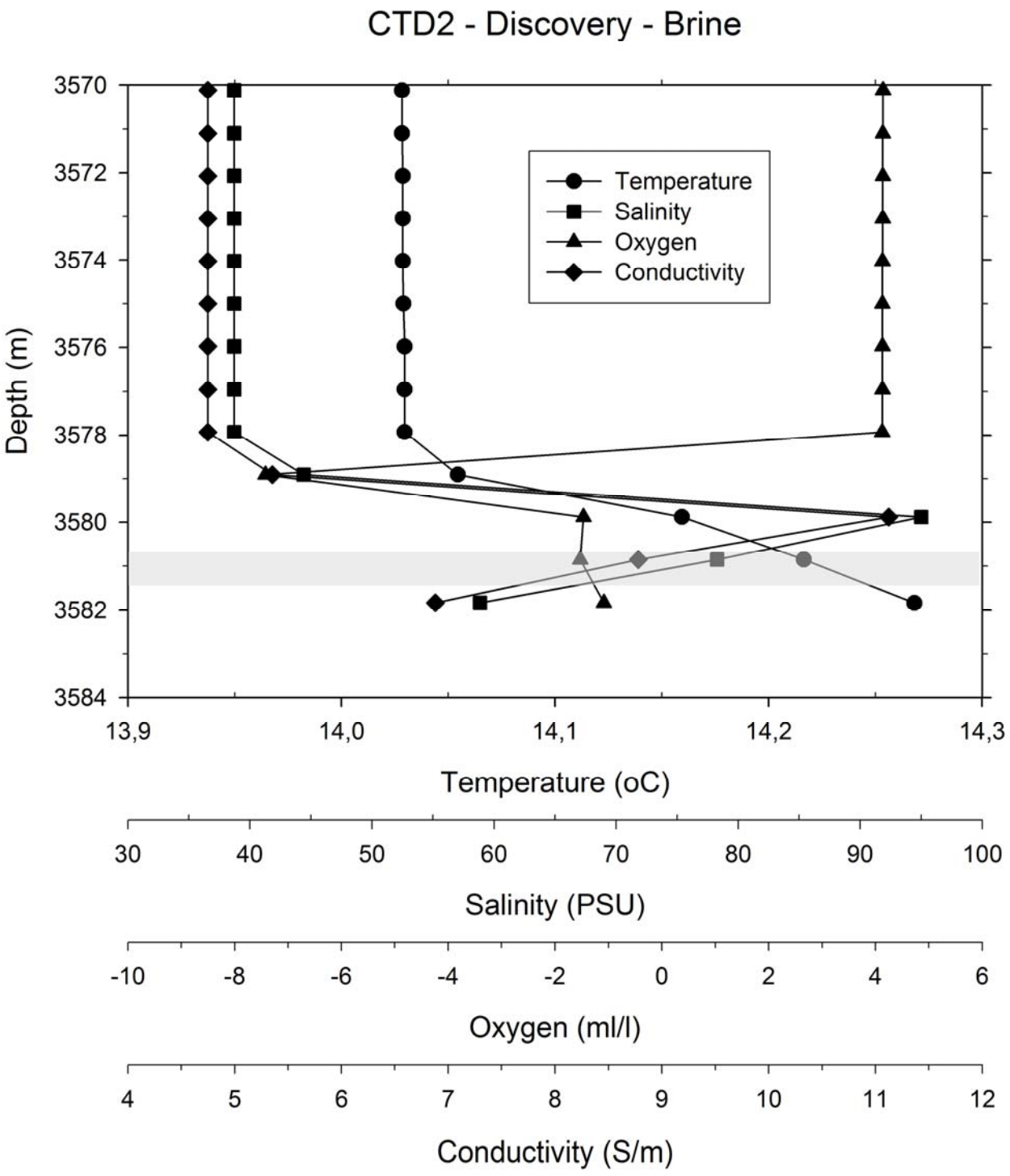
V. References

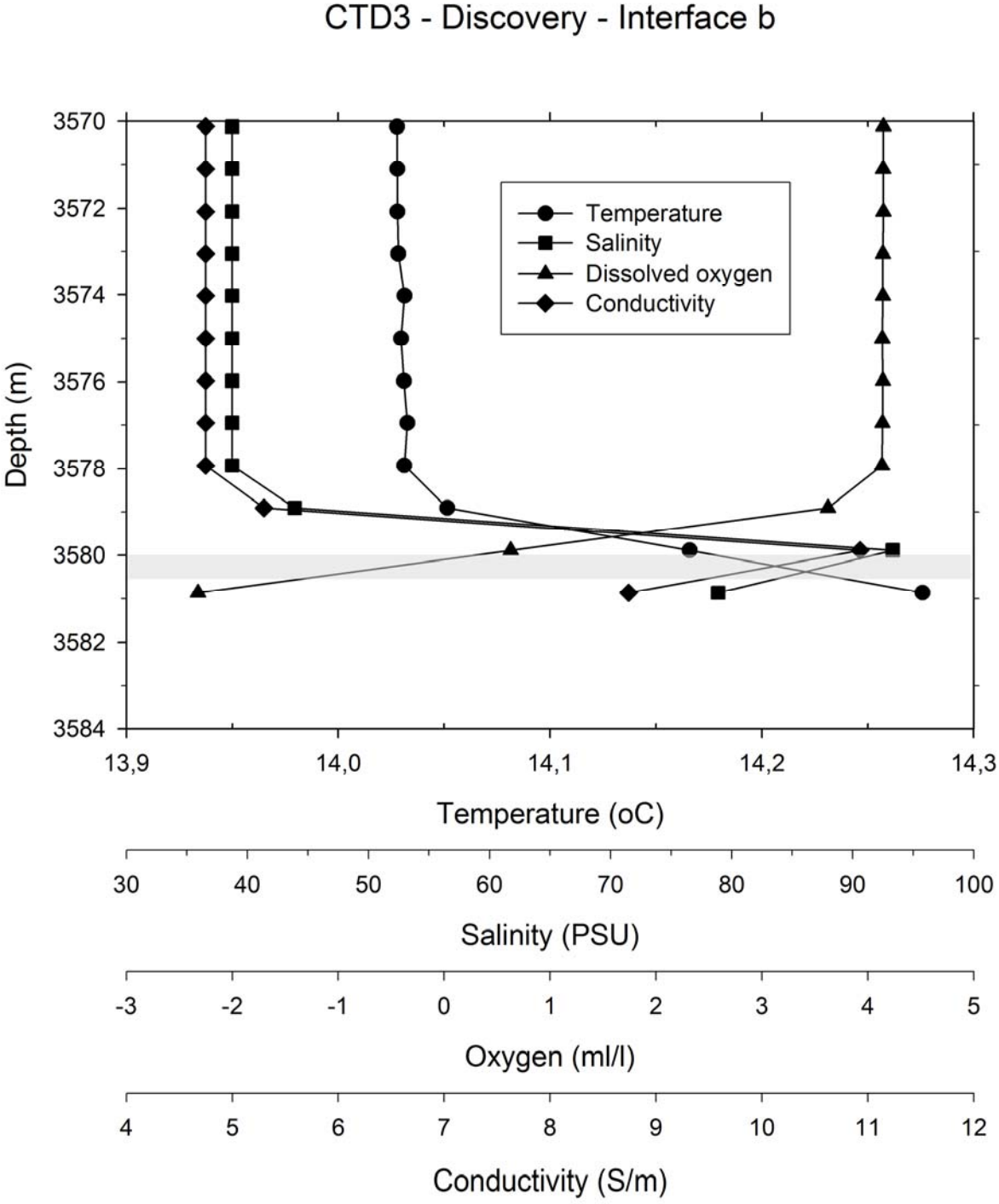
Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford.

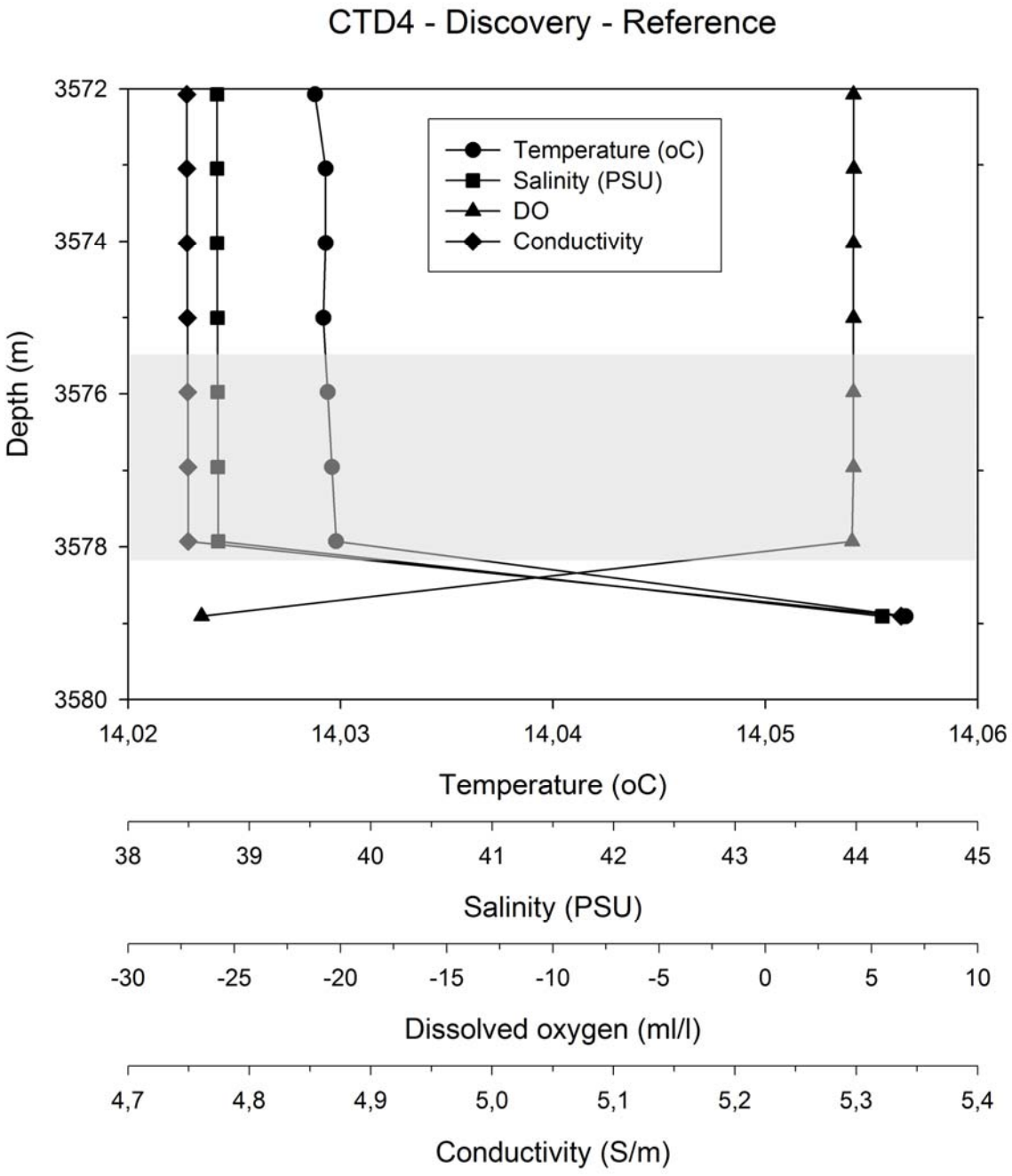
CTD casts

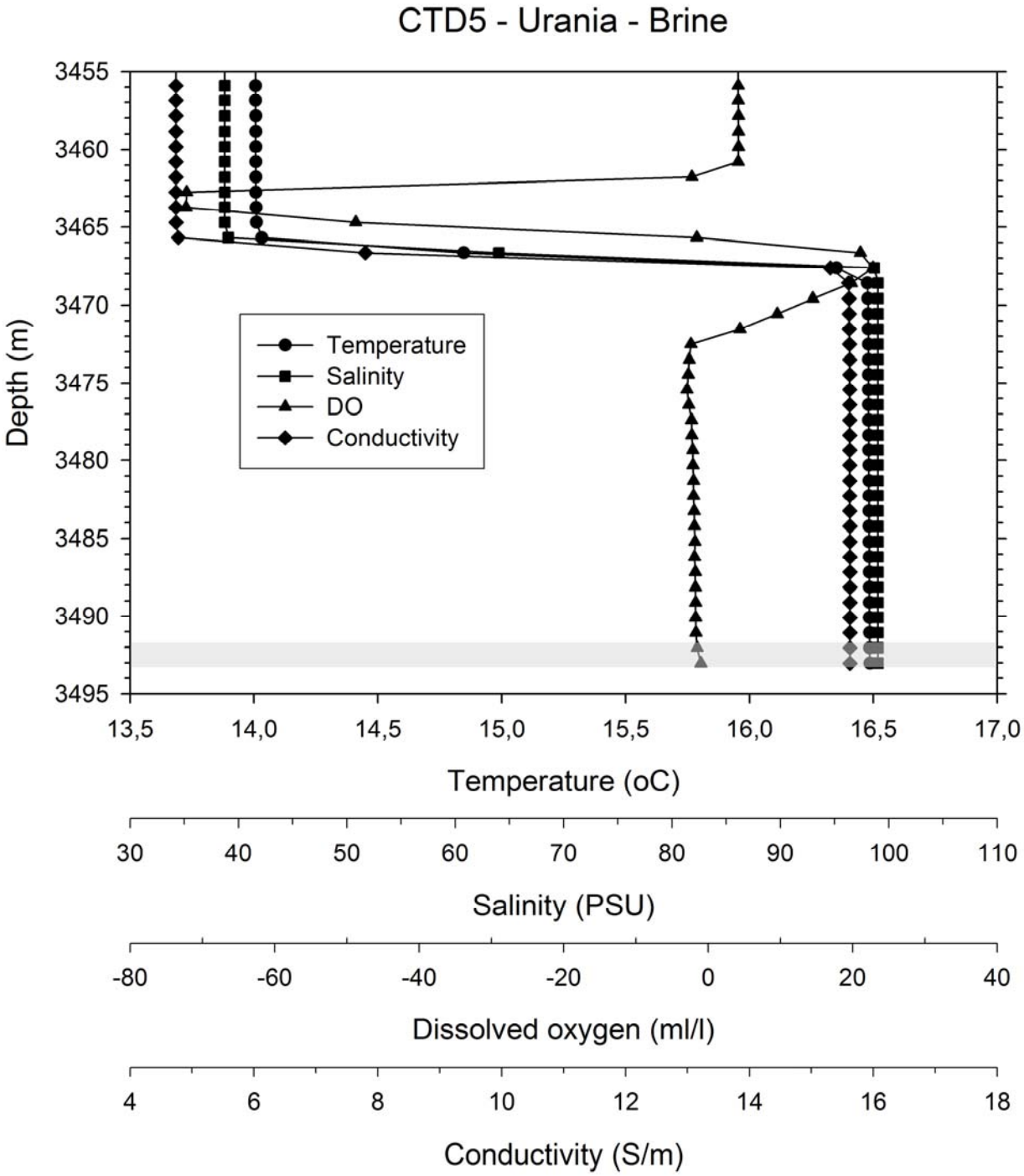
Grey zone indicates depth from where the Niskin bottles were retrieved.

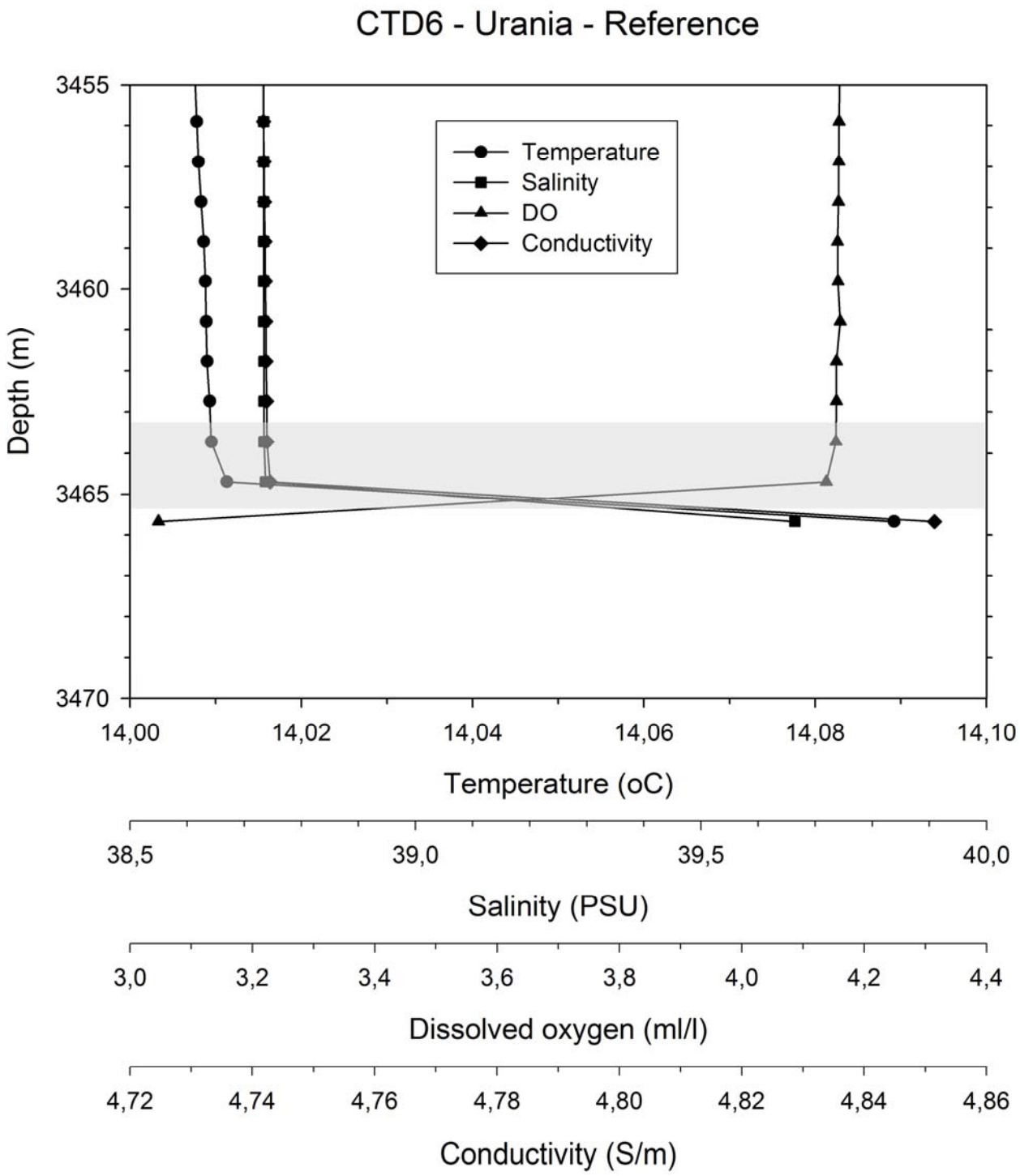


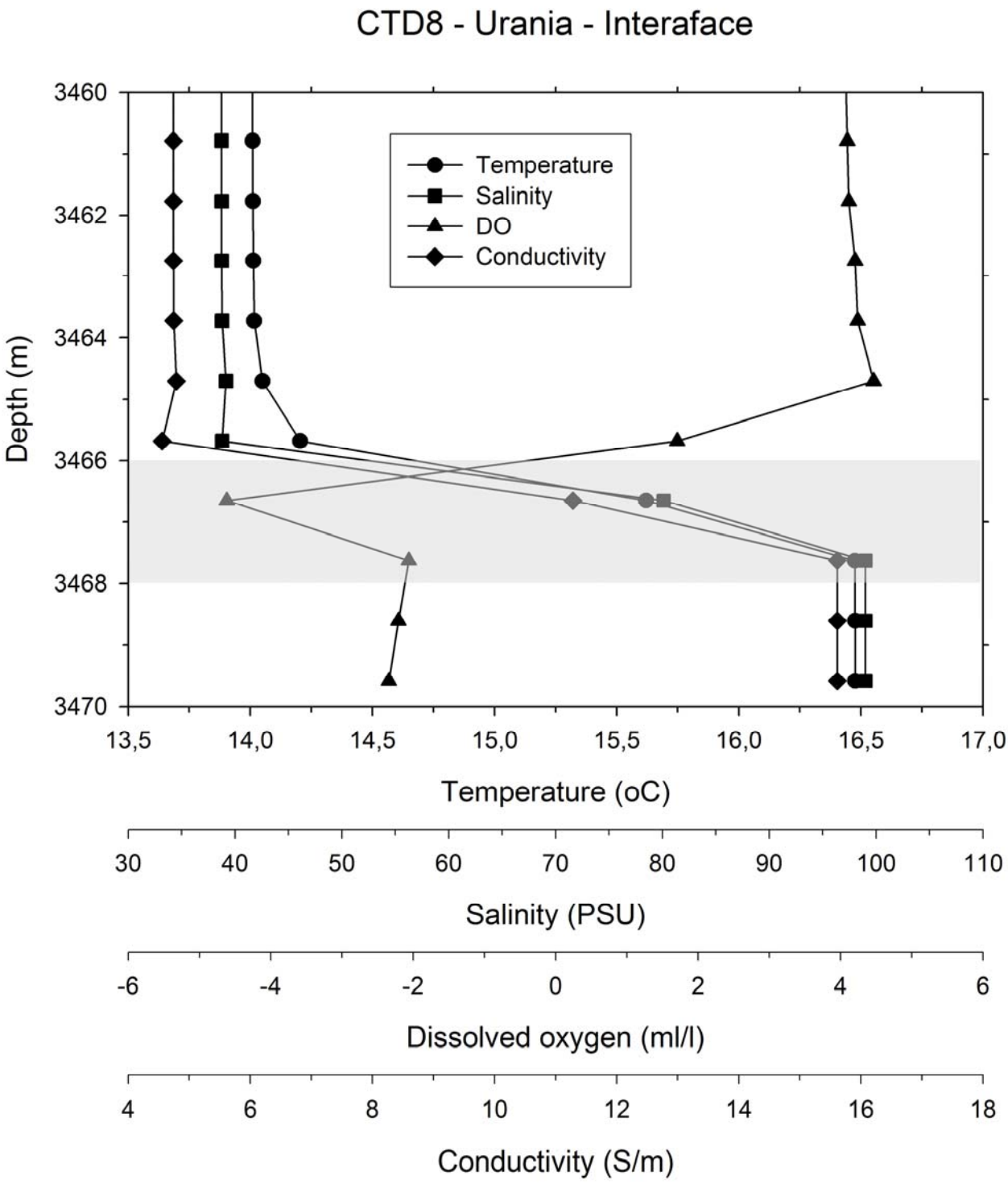

CTD2 - Discovery - Brine
Depth (m)
3570
3572
3574
3576
3578
3580
3582
3584
Temperature
Salinity
Oxygen
Conductivity
13,9
14,0
14,1
14,2
14,3
Temperature (oC)
30
40
50
60
70
80
90
100
Salinity (PSU)
-10
-8
-6
-4
-2
0
2
4
6
Oxygen (ml/l)
4
5
6
7
8
9
10
11
12
Conductivity (S/m)

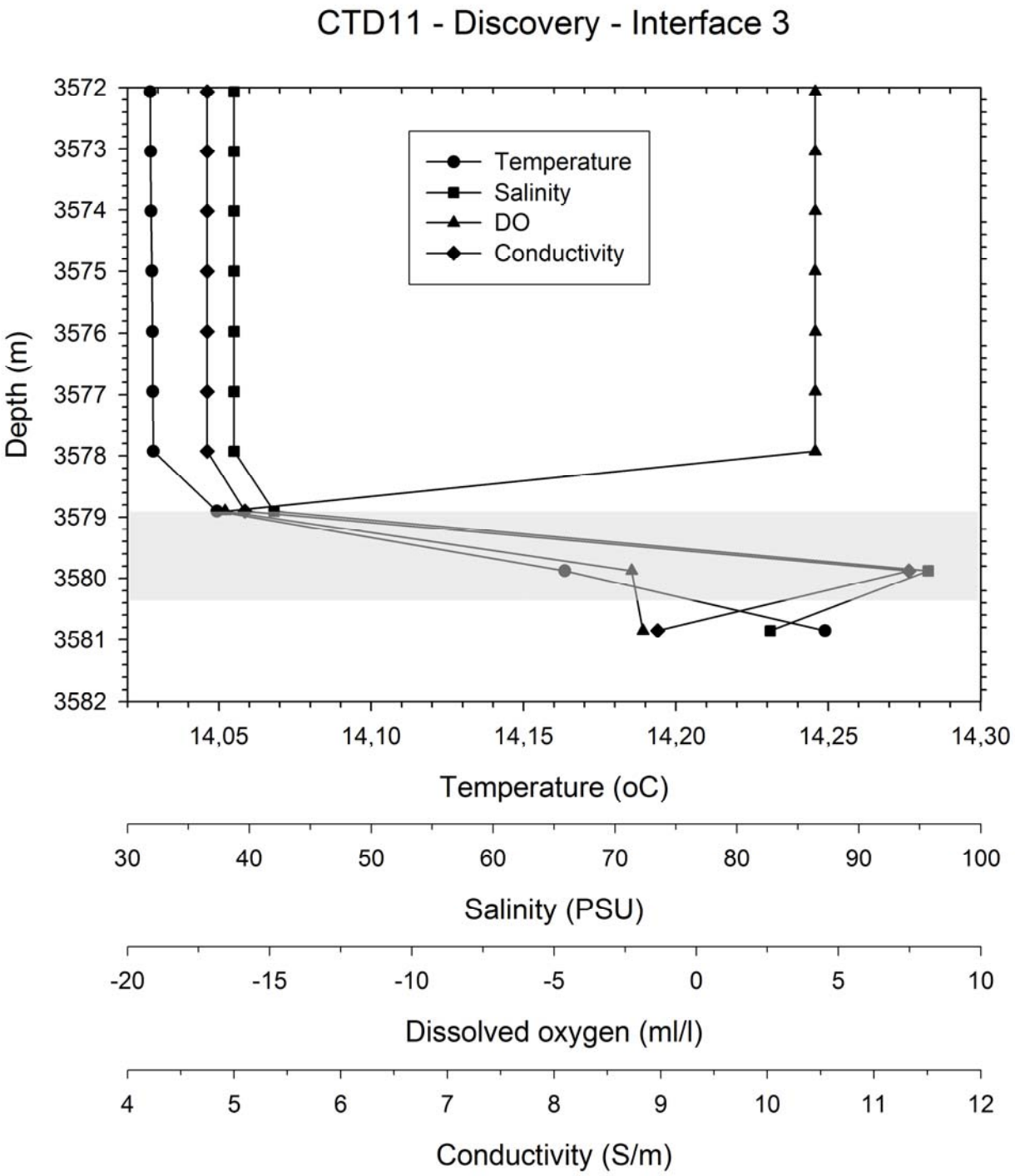

CTD3 - Discovery - Interface b
Depth (m)
3570
3572
3574
3576
3578
3580
3582
3584
Temperature
Salinity
Dissolved oxygen
Conductivity
13,9
14,0
14,1
14,2
14,3
Temperature (oC)
30
40
50
60
70
80
90
100
Salinity (PSU)
-3
-2
-1
0
1
2
3
4
5
Oxygen (ml/l)
4
5
6
7
8
9
10
11
12
Conductivity (S/m)


CTD4 - Discovery - Reference
Depth (m)
3572
3574
3576
3578
3580
Temperature (oC)
Salinity (PSU)
DO
Conductivity
14,02
14,03
14,04
14,05
14,06
Temperature (oC)
38
39
40
41
42
43
44
45
Salinity (PSU)
-30
-25
-20
-15
-10
-5
0
5
10
Dissolved oxygen (ml/l)
4,7
4,8
4,9
5,0
5,1
5,2
5,3
5,4
Conductivity (S/m)


CTD5 - Urania - Brine
Depth (m)
3455
3460
3465
3470
3475
3480
3485
3490
3495
Temperature
Salinity
DO
Conductivity
13,5
14,0
14,5
15,0
15,5
16,0
16,5
17,0
Temperature (oC)
30
40
50
60
70
80
90
100
110
Salinity (PSU)
-80
-60
-40
-20
0
20
40
Dissolved oxygen (ml/l)
4
6
8
10
12
14
16
18
Conductivity (S/m)


CTD6 - Urania - Reference
Depth (m)
3455
3460
3465
3470
Temperature
Salinity
DO
Conductivity
14,00
14,02
14,04
14,06
14,08
14,10
Temperature (oC)
38,5
39,0
39,5
40,0
Salinity (PSU)
3,0
3,2
3,4
3,6
3,8
4,0
4,2
4,4
Dissolved oxygen (ml/l)
4,72
4,74
4,76
4,78
4,80
4,82
4,84
4,86
Conductivity (S/m)


CTD8 - Urania - Interaface
Depth (m)
3460
3462
3464
3466
3468
3470
Temperature
Salinity
DO
Conductivity
13,5
14,0
14,5
15,0
15,5
16,0
16,5
17,0
Temperature (oC)
30
40
50
60
70
80
90
100
110
Salinity (PSU)
-6
-4
-2
0
2
4
6
Dissolved oxygen (ml/l)
4
6
8
10
12
14
16
18
Conductivity (S/m)


CTD11 - Discovery - Interface 3
Depth (m)
3572
3573
3574
3575
3576
3577
3578
3579
3580
3581
3582
Temperature
Salinity
DO
Conductivity
14,05
14,10
14,15
14,20
14,25
14,30
Temperature (oC)
30
40
50
60
70
80
90
100
Salinity (PSU)
-20
-15
-10
-5
0
5
10
Dissolved oxygen (ml/l)
4
5
6
7
8
9
10
11
12
Conductivity (S/m)