Marc Picheral

Cruise Report

Nice-Bizerte

Bizerte-Naples

2009/10/22

Scientific team onboard and work repartition:

Date	**20091010 Nice-Bizerte**	
	Operation/Protocol	**Operator**
Oceanography gears	Instrumented pump (dcm)	Oceano-engineer
	net deployment	Oceano-engineer
	CTD-carousel	Oceano-engineer
	CTD-alone	Oceano-engineer
Oceanography DryLab	ACS	Oceano-engineer
	TSG	Oceano-engineer
	FRRF	Oceano-engineer
Imagery DryLab	Microscopy	not managed
	FlowCam	not managed
	CytoFlow	Oceano-engineer
Zooplankton	Sample handling	Marc Picheral
Water Sampling CTD/NISKIN	HPLC	Floriane Desprez
	Salinity	Floriane Desprez
	Carbonates	Floriane Desprez
	Nutrients	Floriane Desprez
WET LAB Biology	TANIT	not sampled
	VIRUS	not sampled
	GIRUS	not sampled
	DNA extracell	not sampled
	PROTISTS	not sampled
	BACTERY	not sampled
IMAGERY Zooplankton	Binocular	not managed
	Camera	not managed

Picheral	Marc	marc.picheral@obs-vlfr.fr
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Desprez	Floriane	fdesprez@obs-vlfr.fr
Carmichael	Margaux	carmichael@sb-roscoff.fr
Krzic	Uros	krzic@embl.de

Date	20091017 Bizerte-Naples	
	Operation/Protocol	**Operator**
Oceanography gears	Instrumented pump (dcm)	Oceano-engineer
	net deployment	Oceano-engineer
	CTD-carousel	Oceano-engineer
	CTD-alone	Oceano-engineer
Oceanography DryLab	ACS	Oceano-engineer
	TSG	Oceano-engineer
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Zooplankton	Sample handling	Marc Picheral
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	Salinity	Floriane Desprez
	Carbonates	Floriane Desprez
	Nutrients	Floriane Desprez
WET LAB Biology	TANIT	Floriane Desprez-Fran Cornejo
	VIRUS	Floriane Desprez-Fran Cornejo
	GIRUS	Pascal Hingamp
	DNA extracell	not managed
	PROTISTS	Pascal Hingamp
	BACTERY	Floriane Desprez-Fran Cornejo
IMAGERY Zooplankton	Binocular	not managed
	Camera	not managed

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Station performed

STATION 12

43°21.645 N
7°53.669 E

Operation	Depth	Duration	ordre	0	hours	
CTD+Niskin	1700	60	2	60	1.00	**done**
double 20µm	0	15	7	75	1.25	**done**
Regent	500	40	4	115	1.92	**done**
bongo300µm	500	40	5	155	2.58	**done**
double 20µm	DCM	15	7	170	2.83	**done**
NISKIN	0	5		175	2.92	**done**
CTD+Niskin	240	15	2	190	3.17	**done**
CTD+Niskin	440	30	2	220	3.67	**done**
TOTAL		**220**				

STATION 13

37°59.376 N
8°02.888 E

Operation	Depth	Duration	ordre	
CTD	1000	50	1	**done**

STATION 14

39°53.537 N
12°50.840 E

Operation	Depth	Duration	ordre	0	hours	
Pump	0	10	1	10	0.17	**done**
Pump trial	50	30	3	100	1.67	**done**
CTD+Niskin	1000	60	2	70	1.17	**done**
Regent	500	40	4	140	2.33	**done**
bongo300µm	500	40	5	180	3.00	**done**
WP2 200µm	100	15	6	195	3.25	**done**
double 20µm surface	0	15	7	210	3.50	**done**
TOTAL		**210**				

note : all operations performed in less than 3 hours !

Main works for the project

1. We installed and fully checked the CTD_standalone and the CTD_carousel and wrote deployment protocols.
2. We started to set the process protocols for the CTDs.
3. We set the deep instrumented (eco-triplet) pumping system and tested it down to 45 meters. Both 60m extends are now set and ready to deploy.
4. We set the Oceano-engineer working/storage area in the aft room and in the Library : UVP5 deck Unit, CTD Li-Ion charger, ECOtriplet Deck Unit, RS232 hub, StarOddi reader.
5. We set the depth recorders for the nets ready to use. Protocol done.
6. We installed the navigation NMEA onto the oceano computer allowing properly setting the clocks of the depth recorder and visualizing the ship track on the computer for better cruise management with expert on land.
7. We installed and connected a second laptop for the oceano engineer work
8. We labelled all the water circuit system according to Herve Legoff schematics
9. We constructed a new NiCad spare battery for the CTD-carousel
10. We installed the two CTD battery chargers (NiMh and NiCad) onto the oceanographic bench.
11. We improved the aft platform adding two lateral retainers for the rosette.
12. We added plastic covers on the CTD rails to facilitate the rosette displacement to its storage area
13. We set four gaffes and hooks to secure the CTD-carousel during recovery
14. We improved the log-sheets for all operations performed and linked better the biology operations with sampling gears
15. We bought a binding machine to be able to make logbooks onboard and to allow better adjustment of the content of the sheets.
16. We re-defined the use of the “low level” bar codes stickers for the station 14 and partially for station 12.
17. We started to organize the “Chief Scientist” documentation folder.
18. We started to write a Chief Scientist Vademecum to be validated by project’s PIs.
19. We re-organise the list of tasks to be performed by scientific team onboard (Work_repartition.xls file).
20. We installed a BackUp server in the Dry Lab
21. We installed a Science server (taraserver) in the Communication Lab to facilitate exchange of information through all scientists and crew onboard.
22. We made an inventory of all scientific chemicals onboard
23. We set spare silks for Net Cod ends
24. We re-organise partially the storage of scientific equipments grouping TSG spares with CTD spares and all spares for oceano-engineer equipments.
25. We installed the pre-filter on the MilliQ system
26. We improved and documented protocols for ACS, TSG, FRRF, Zooplankton, Depth Recorders, Protists……..

To do onboard ASAP

1. Set the protocol for CTD processing onboard
2. Set the protocols for CTD data transmission to Lab.
3. Fix the ISUS nitrate sensor problem on the CTD
4. Set the Oceano-PC backup system
5. Write the Oceano-protocols
6. Establish the Oceano data management system

7. Update antivirus database on Toughbook
8. Inventory of all consumable for biology. Improve managing tools.
9. Improve biology protocol management.

To do on land (M. Picheral)

1. Make better sieves for Zooplankton samples
2. Set the protocols for CTD data transmission to Lab.
3. Buy Dummies and Locking sleeves for pump extends

Observations

We left Nice with very few information about the biologic work to do onboard. Floriane had to learn the protocols and find all consumables for it during the first week. Pascal did the same job in Bizerte with the help of Margaux.

Due to bad sea conditions, we could not make more stations than the 3 described above. The initial objectives of checking the CTD-carousel system, Depth recorders for nets, Sensors for pump were completed even with very few trials to test. The logic of the release of the bottles was no simple to establish at the early stages.

As Chief Scientist onboard TARA for the first time, I found a completely disorganised system where the Captain handled most of the information and did partially the job of Chief Scientist. I could not meet the previous Chief Scientist and received opposite recommendations from different PIs of the projects concerning the barcoding for example. Most of protocols onboard were not fully documented when available. The main grey book containing most protocols was not accessible during the first days. I did not know it exists and I could not ask people to search for it !

We decided to send Margaux back in Bizerte because she was too tired and seasick during the storm to be able to handle two more weeks at sea.

I noticed that the wet lab facilities DO NOT allow working safely with the formaldehyde and the gluta except for Zooplankton for what the Borax and Formalehyde dispensers have been evaluated by the Villefranche safety engineer (ACMO). As chief scientist, it is my responsibility to help our young colleagues to work with these dangerous chemical in good conditions that are not yet set onboard TARA. It is thus mandatory that we find safe working condition. We cannot allow people to work inside the wet lab with opened vials containing either Gluta or Formaldehyde. **I suggest that the left side of the Wet Lab should be urgently modified to create a fume hood extracting the air through the lateral aft window of the lab or through a new hole in the roof.**

In addition to the flume, there should be at least 3 efficient lights (above each filtration stand) in the Wet Lab and a 4th one above the aft outside table.

Recommendations

- If the scientists have been replaced, do not set the first station the first day at sea to help scientists to cope with seasick and to allow time to prepare the first station. Allow anyhow one recovery day between two stations for sampling preparation, inventory, lab cleaning... Additional CTD-carousel and nets (WP2 200µm, Regent 670µm, Bongo 300µm) can be easily sets between biology stations.
- Finalize an useful and agreed Vademecum for Chief Scientist and send it to all people having links to chief scientist onboard
- Organise scientist's lists according to the operations to be performed onboard and fill in the Work_repartition.xls file in advance when the scientific teams are composed. Check that participants are aware of the tasks they are supposed to perform onboard.
- Send scientist list to chief scientist onboard at least two weeks in advance

- Do not change both biologists at the same time. Insure at least a one week cross for one of them to share experience.
- Recruit a WetLab technician to stay onboard most of the time and manage the biology works with scientists coming onboard.
- Organise preliminary Chief Scientist meeting with Tara Science manager (who?) to explain the work onboard.
- Send documentation to Chief Scientist (Vademecum) at least two weeks in advance.
- Send protocols of work to be done by scientists (biologists) when they are chosen in order to make them aware of the job to be done and the requested competencies. Share this information on a web server on land.
- Set "station cases" of all consumables to be utilized for one standard station.
- Organize trainings for biologists to board on TARA
- Keep one month onboard the scientists having no experience of work onboard TARA so they can train and be efficient.
- Set rules for scientific rotation during stopover (minimum of one full day to meet and exchange information and even more if people coming are not trained with protocols)
- Fill in the safety doc files for Chief Scientist documentation
- Set the database system using experience of seawork and reports from scientists on board and on land.
- Define clearly when arriving people can sleep on board (Friday?)and when they must leave at the end of their stay (Friday?).
- We should organize the calibration of Oxygene sensor on the CTD.
- Set the flume (extractor) ASAP.

Conclusion

I did enjoy the collaboration with everybody on board. I apologize having so bad sea conditions and having to cancel most sea operations. We did a great job organizing the work onboard and setting all instruments and protocols to work. Even if the quantity of collected data and sample is limited, we feel happy of the completed job.

Bizerte – Naples leg: biology notes

Pascal Hingamp
23rd October 2009

Weather constraints limited sampling to one swift 3h station late in the afternoon on tuesday 20th october. We carried out one surface pumping (TANIT, GIRUS, & VIRUS) and one surface 20µm bongo net (PROTIST). The protocols we followed were the latest versions in our possession of the "short" protocols.

General comments

The consensus being that these "short" protocols, once settled and validated, should become the basis of the routine sampling, we propose that this set of protocols be refered to as the "**core**" protocols (indeed these protocols are not that short...)? After it has been shown that this core set of protocols can be carried routinely (eg 6-12 stations), protocol extensions could be added to the core set by seasoned scientific crews, weather conditions permitting.

It is very useful to have a general diagram type "overview" of each protocol, both for scientific crew training and for handy lab memos of operations during station sampling. Such diagrams are available for TANIT, VIRUS and GIRUS. A set of diagrams were produced for the PROTIST core protocol (available in plastic cover sheets in the protocol binder on board, and in digital form on the taraserver "**Public**" folder). This PROTIST diagram should be verified by the PROTIST group, and these "core" diagrams could be extended to produce a "extended" version of the protocols?

The competence transfer experience during the Bizerte stopover (Margaux briefing Pascal about the PROTIST protocols) shows that 2 days is a **strict minimum** to reasonably brief a novice scientific crew member. This is when the protocols have been studied **in detail** before arriving on board (which means roles on board can't be discovered while embarking, unless all protocols are studied in detail before hand...). Even this is far from ideal, as only a sampling station carried out together could guarantee that most of the accumulated experience is carried forward. It is also not ideal that full scientific crews are rotated together during the same stop over.

In order to help good and timely diffusion of the latest version of protocols to all concerned, it would be very useful to have a **land web server**. This could host a wiki type service, that allows easy versioning and collaborative document production, with easy upload/downloading of protocols in their native formats (.ppt .docs etc.). Eric suggested Uros, Pascal and Stéphane make propositions to put together this service.

Uros set up a fileserver on board with two folders:

- a "**Public**" folder accessible through a web browser on http://192.168.101.7/Public/ (only the Chief Scientist and Oceanography Engineer have write access)
- a "**Temp**" folder that everyone can read and write into (is mounted as a network drive, a howto document is on board for Mac, Windows and Linux users)

The Public server hosts all the protocols, manuals and spreadsheets related to sampling operations. The Temp folder is a shared space for exchanging files between crew members.

Sampling comments

- on the day following sampling, we rinsed all lab equipment (filter holders, benches, cans etc) with ship ordinary fresh water (used the hose located just outside the lab, at the back usually wrapped around the tall gas bottle). It might be very interesting to rinse containers, tubing and filter holders with distilled/MilliQ water, since that could then mean we could do without pre-rinsing with filtered station sea water? This rinsing on the day of sampling takes a lot of time, on a day where time is precious…
- we prepared formol and glutaraldehyde aliquots (for two stations) using the Bizerte Science Faculty chemical fume hood. Unless a fume hood can be installed in the lab (see Marc's report), using a local laboratory fume hood is also a good opportunity to establish local contact (but requires good logistics).
- we chose to store all chemical aliquots in the lab chemical cupboard. If more than 2 station's worth of aliquots are prepared (such as the 5 prepared here at Naples), then they can't fit into the small cupboard. We propose to store the aliquots in cardboard freezer tower boxes in a large watertight plastic box stored inside the lab (not inside in forward hall).
- it's a shame 50ml Falcon's can't be stored in their holder on the lab's chemical cabinet top shelf (missing 5mm! **TODO**?). It would also be really good if the cabinet could be under mechanical air extraction (linked to fume hood? **TODO**).
- tweezers are kept in falcon tubes duck taped to the lab wall, with ethanol. Because tweezers used to lift and fold filters end up caked with ocean goo present on the filters, it would be very wise to keep tweezers separate! Label the tweezers, and keep each tweezer in a separate falcon! Don't mix tweezers or else expect mixed samples...
- A recurring question that is relevant to all protocols: should we always change clogged filters? For instance, if a DNA metagenomic filter clogs up after 50% of planned volume of charged sea water is passed, isn't there enough DNA ? Is there really any point doing a second filter? What more is that second (or third, or fifth) filter going to tell?
- Concerning GIRUS (and TANIT) GF/A 142mm filters: we have had 100% of filter ruptures (>4 filters)! After the first rupture (peristaltic pump at 13 speed), I tried speed 9, but its still ruptured, even though the sea water was quite “oligotrophic”. This could be due to the working filter stock of GF/A having been to exposed to humidity? Or there is a need for a support membrane? Or slower flow rate? Until proper parameters are found that eliminate filter rupture, it seems pointless to filter on 1.6µm, and therefore seems pointless to try GIRUS surface protocols. (**TODO**)
- GPSS arms are damaged (many badly bent or broken slits for hanging onto lab wall grid). Apparently some spare arms are located in forward hall?(**TODO**) The GPSS was not tested during this leg.
- For the PROT-ETHANOL (20-180µm) sample, the volume goes through the 20µm sieve OK until the last few mls (2-20ml?), and then there's no way to get more liquid out. I rinsed what was left in the sieve with ethanol, and then rinsed the material into a Falcon (total 30-40ml?). There's bound to be a small

amount of water left. Could more protocol details be provided? (**TODO**)

Missing equipment:

- 2 large bungs for the bottom outlet of the 100L plastic tanks (PROTIST)(**TODO**)
- 1 insulated cool box (eg polystyrene) to keep samples cool in the lab during sampling (**TODO**)

Annexe:

List of logbooks linked to deployments of instrumented equipments:

This document excludes the instruments connected on the seawater circuit.

We have created or improved 8 logbooks linked to specific instrumented gears that are deployed either for sample collection (nets, sbe9carousel, Niskin, pump/ecotriplet) or in-situ measurements (sbe9carousel, sbealone).

The filtrations have been divided in two separate logbooks that are linked to the two main sources of samples. First is Seawater from pump or Niskin, second is Nets. A new set of datasheet is utilized for each different depth (surface, deep chlorophylle max (DCM)).

As example, a station containing a surface pumping and a surface 20µm bongo net will be recorded in two different logbooks. One is for Seawater and the other for Nets. If both a DCM pumping and a DCM net are added, a new set of datasheets will be filled in both logbooks after the ones from the surface sampling is completed.

The first 6 logbooks are filled by oceano-engineer and kept in the Library. The filtration logbooks are managed by biologists and Gear datashhet reference is used to link the samples with the events and the recorded values from the associated sensors (Flowmeter, Depth recorders, CTD, Ecotriplet)

1. NETS (All nets)
2. SBE9 Carousel (NISKINS)
3. SBE9 standalone
4. PUMPING Surface Chlorophylle Max (DCM)
5. NISKINS (Closed by messengers)
6. UVP5 (On CTD carousel)

7. FILTRATIONS PROTISTS NETS
8. FILTRATIONS BACTERIES-VIRUS DNAext-GIRUS PROTISTS Seawater/Pump Niskin

Bar coding:
We have started again to set the low level barcodes stickers on the different samples. This operation takes much time but it can be performed in advance.