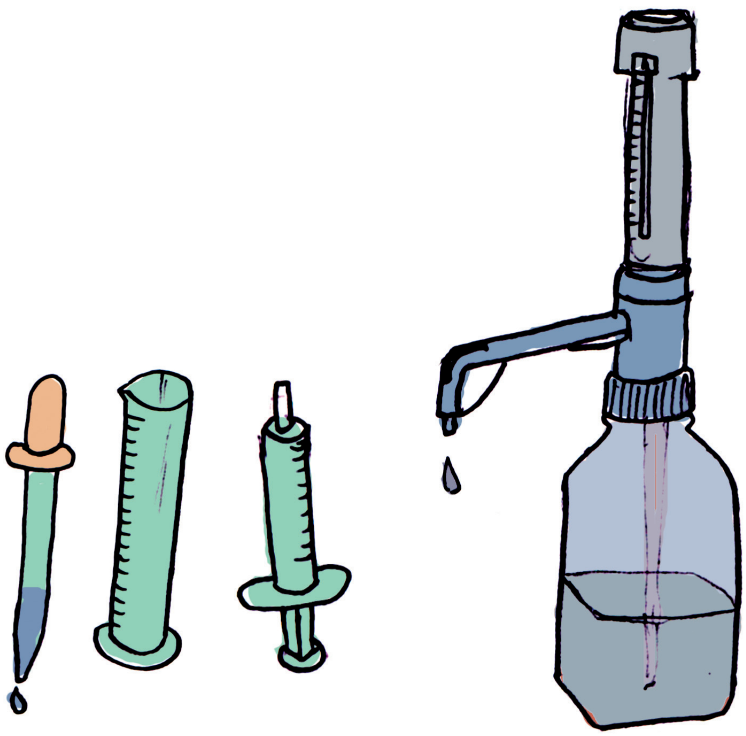
ZOOPLANKTON MANUAL

SAMPLING MANUAL
WORK AT THE STATION



MINISTRY OF FISHERIES AND MARINE RESOURCES

GOBIERNO DE ESPAÑA · MINISTERIO DE ASUNTOS EXTERIORES Y DE COOPERACIÓN · aecid

CETMAR · CENTRO TECNOLÓGICO DEL MAR

This material has been developed within the framework of the Project *SUPPORT FOR THE RESEARCH PROGRAMME OF THE NATIONAL MARINE INFORMATION AND RESEARCH CENTRE (NatMIRC),* funded by the Spanish Agency for International Development Cooperation (AECID) and executed by the National Marine Information and Research Centre (NatMIRC) in collaboration with the Technological Centre of the Sea (CETMAR Foundation), the Spanish National Research Council (CSIC) and the Spanish institute of oceanography (IEO).

It is presented as a working material to support the activities of the environmental staff.

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PREAMBLE

This material has been developed within the framework of the NatMIRC project[1].

The current version of this manual is a draft for adaptation to the needs of the National Marine Information and Research Centre (NatMIRC).

INTRODUCTION

The fundamental part of any scientific study is sample taking and handling. The sampling methodology must be defined in WORK PROTOCOLS. This provides a standardised system to ensure samples are taken in a systematic and consistent manner, irrespective of who takes the samples. Carefully following the instructions set out in Protocols will ensure that samples taken in accordance with those Protocols provide results of great scientific value. However, in the event of carelessness, the work carried out when taking samples will be almost worthless since the quality of the data obtained will be uncertain. Samples do not only have high economic value (daily cost of personnel, the costs of the vessel and equipment, etc.), they have another incalculable value: they are unique. **The information supplied by each sample is unique**: it provides data on the condition of the studied resource, the result of specific oceanographic conditions at a given point in time and in a specific location.

Sampling equipment (nets, flow-meters, laboratory equipment, etc.) is used to collect and handle samples. Therefore, the state of maintenance of this equipment will directly influence sampling results.

A final aspect that must be borne in mind concerns Occupational Safety. The safety of personnel performing different sample collection and handling operations is VERY IMPORTANT. Staff working at any research centre is one of the main assets of such centres. Therefore, special attention must be given to guarantee their physical integrity.

The purpose of the technical support visit by the technical team from the IEO (Spanish Institute of Oceanography) to the NatMIRC has been training for technical personnel on different aspects of their work, as well as the preparation of this Reference Manual.

Presented below are two manuals outlining different WORK PROTOCOLS, which will serve as a basis for the preparation of in-house manuals, in which both research and technical staff may indicate their experiences and requirements in order to improve and adapt these protocols to their needs.

1. SUPPORT FOR THE RESEARCH PROGRAMME OF THE NATIONAL MARINE INFORMATION AND RESEARCH CENTRE (NatMIRC), funded by the Spanish Agency for International Development Cooperation (AECID) and executed by the National Marine Information and Research Centre (NatMIRC) in collaboration with the Technological Centre of the Sea (CETMAR Foundation), the Spanish National Research Council (CSIC) and the Spanish institute of oceanography (IEO).

SAMPLING MANUAL – WORK AT THE STATION

- **PROTOCOL 1: PREPARATION OF SURVEY MATERIAL**
- **PROTOCOL 2: POST-SURVEY TREATMENT OF MATERIAL**
- **PROTOCOL 3: SAFETY PROTOCOLS**

This manual is complemented by the SAMPLING MANUAL – WORK ON BOARD, which contains the following protocols:

- **PROTOCOL 3: SAFETY PROTOCOLS**
- **PROTOCOL 4: METHODOLOGY FOR SAMPLING ON BOARD**

PROTOCOL 1: PREPARATION OF SURVEY MATERIAL

- SUPPLY OF MATERIAL

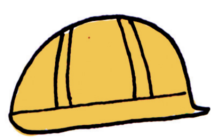
To prepare material for any survey a *Checking List* should be drawn up. This is not a closed list; it will be modified in response to changes in methodologies, available means, etc. It will indicate the material that needs to be used and will take into account the following:

- Sampling to be performed.
- Samples to be taken.
- Participants.

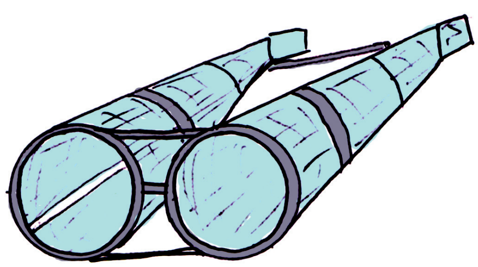
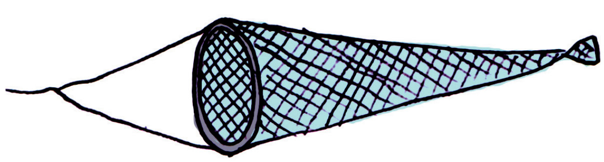
This will allow you to estimate the number of elements required. It is very important to take two of each piece of sampling equipment (nets, mesh-walled buckets, flowmeter) in order to be able to continue sampling in the event of a fault, damage or loss. A *Basic Check List* would include the following:

- *Personal protective and safety equipment*
  - *Working gloves.*
  - *Safety boots.*
  - *Helmet.*
  - *Protective gloves.*
  - *Reagent Safety Data Sheet*

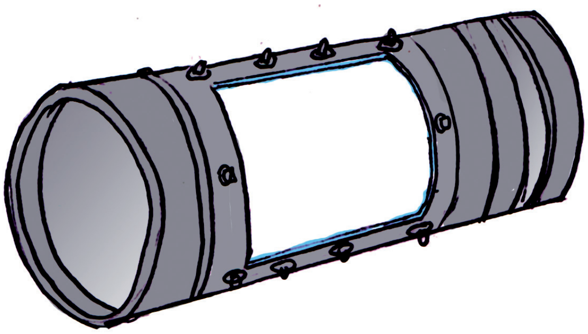


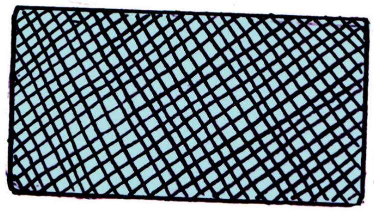


- *Sampling material*
  - *Bongo, WP2, CalVet and other plankton nets.*

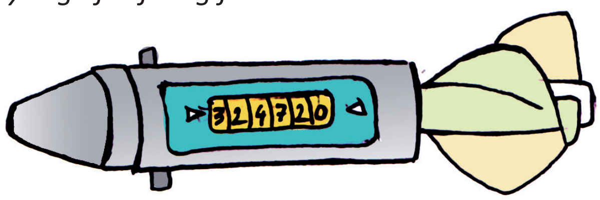



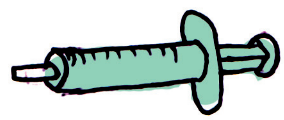
- *Mesh-walled buckets.*
- *Spare mesh-walled buckets.*



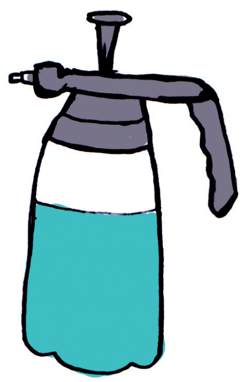


- *Flow-meters.*
- *Syringe for filling flow-meters.*

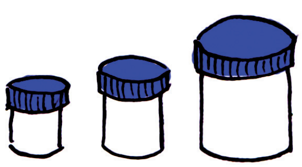


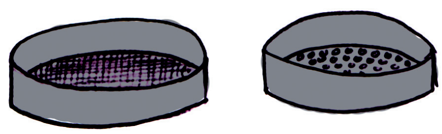


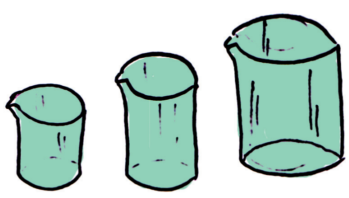
- *Distilled water.*
- *Pressurised water sprayer.*



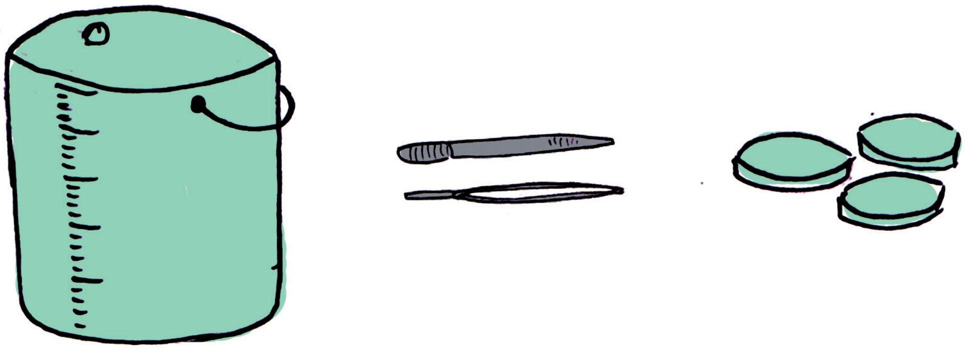
- *Sample flasks/bottles of various sizes (250, 500, 1000, 2000 ml).*
- *Screens/filters (small - 100 µm - to filter samples and sampling seawater and 2000 µm to separate salps, jelly fish), other filters according to requirements.*
- *Beakers (different volumes).*



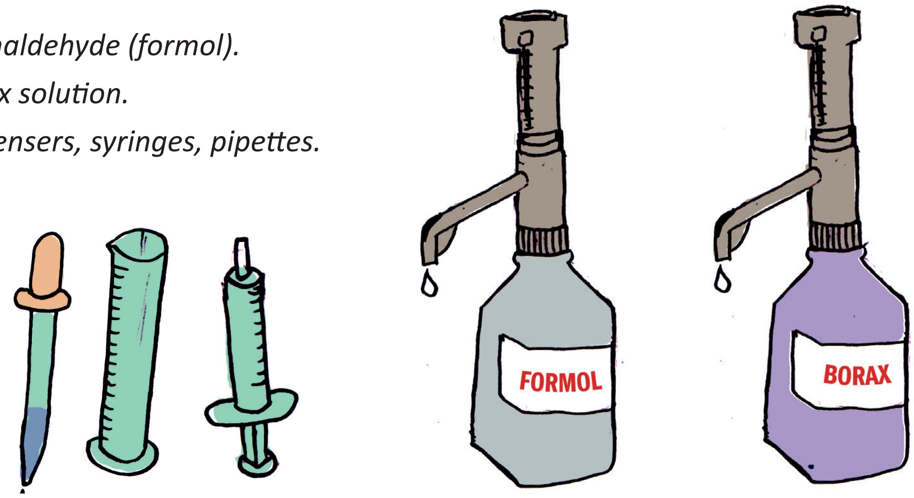




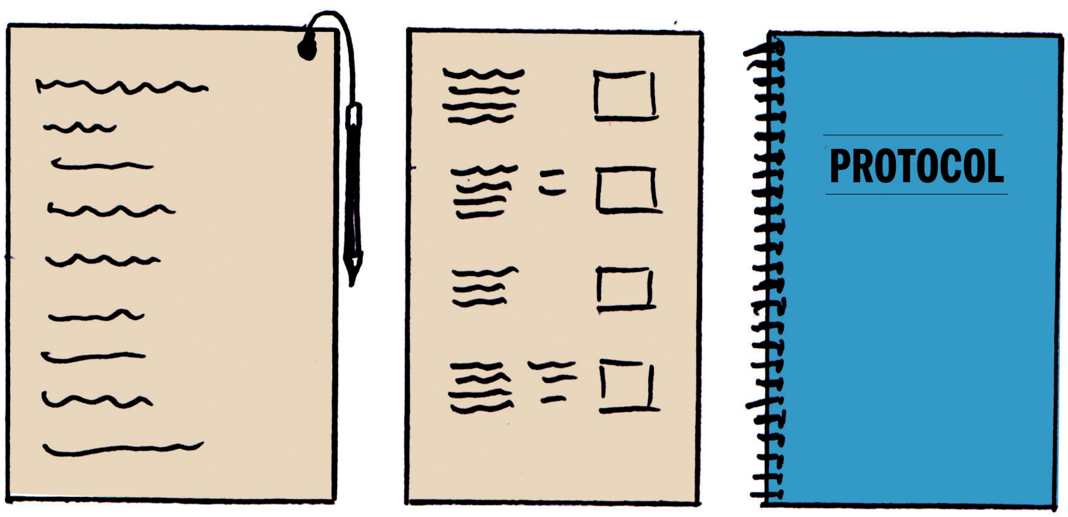
- *Bucket (with sloping sides).*
- *Thin-tipped tweezers.*
- *Petri dishes.*



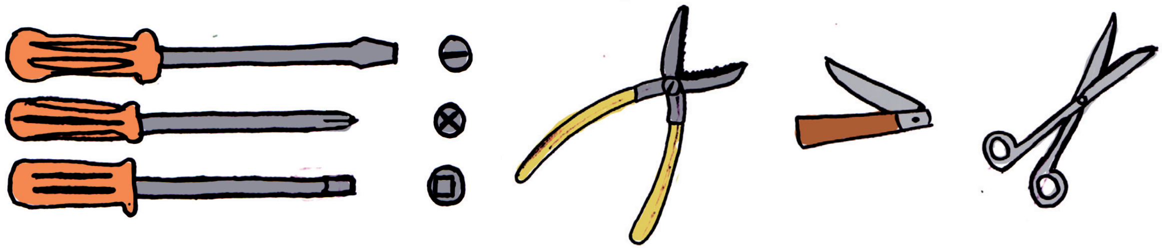
- *Formaldehyde (formol).*
- *Borax solution.*
- *Dispensers, syringes, pipettes.*



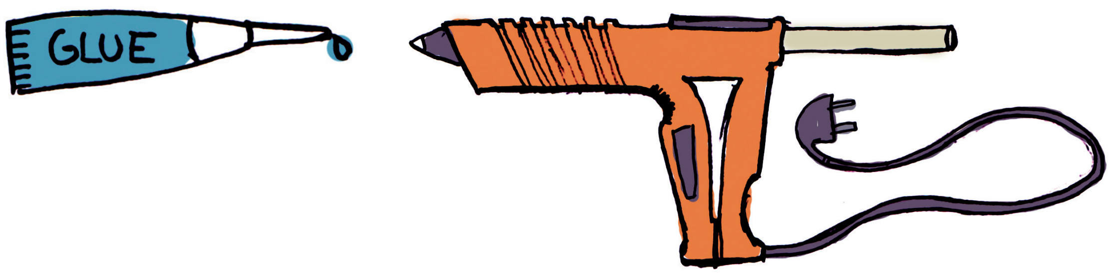
- *Sampling point datasheet.*
- *List of materials by boxes.*
- *Sampling protocols.*



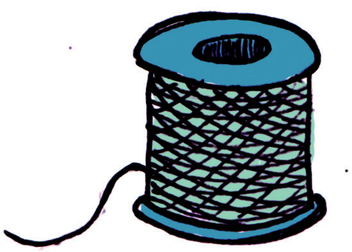
- *Tools:*
  - *Screwdrivers (flat, cross-shaped, square-tipped).*
  - *Pliers.*
  - *Knife.*
  - *Scissors.*



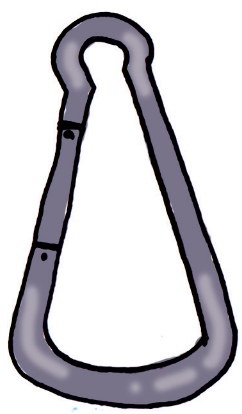
  - *Glue (superglue, contact adhesive).*
  - *Hot melt adhesive or hot glue gun.*

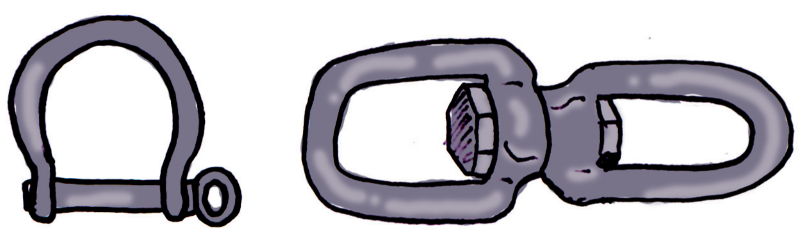


- *Roll out nylon (for lashing items, sample boxes, etc.).*

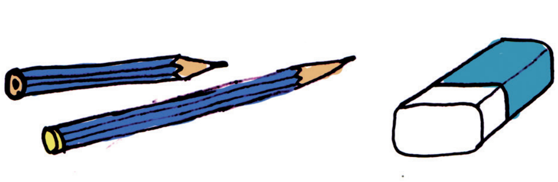


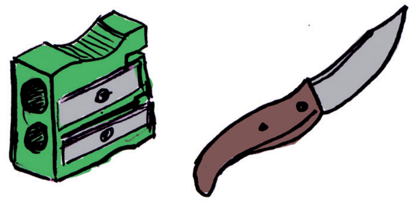
- *Shackles.*
- *Swivels.*
- *Carbine hooks, karabiners.*



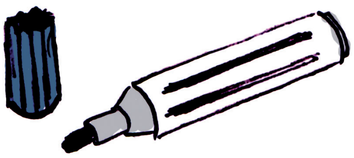


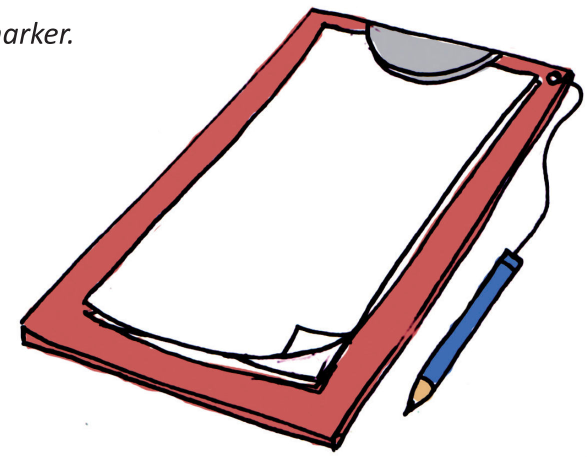
- *Office equipment:*
  - *Pencils.*
  - *Erasers.*
  - *Pencil sharpeners.*



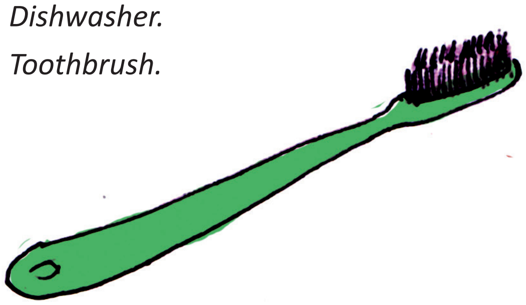


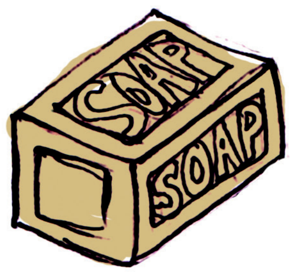
  - *Thick-tipped permanent marker.*
  - *Clipboard.*





- *Cleaning material:*
  - *Dishwasher.*
  - *Toothbrush.*





It is very important to know the total number of samples to be taken and the volume of the flasks/bottles used so that you can calculate the total volume of reagents needed. It is advisable to take reagents and bottles in quantities greater than the quantity of samples you expect to take during the survey (extra samples, macro-zooplankton samples, losses or accidents, etc.).

The following reagents will be used to fix and preserve samples:

- Formaldehyde. Formaldehyde (35-40%) will be used as the base fixing agent (number CASS 50-00-0.). To preserve samples at 4%, add 100 ml of formaldehyde per litre of sample. For example, for 500 ml bottles, add 50 ml of formaldehyde.

- Borax (sodium tetraborate). Borax is used to buffer formol (this prevents the samples from acidifying and increases their shelf-life (CAS 1330-43-4).
  Sodium tetraborate solution is prepared as follows. Add distilled water to a 1-2 l glass bottle (do not fill completely), then slowly add a little borax using a spatula or spoon and shake strongly (approximately 2 g per litre). Stop adding borax when crystals appear on the bottom of the bottle (due to precipitation). Use 40 ml of borax solution per litre of sample. For example, for 500 ml bottles use 20 ml of borax solution.

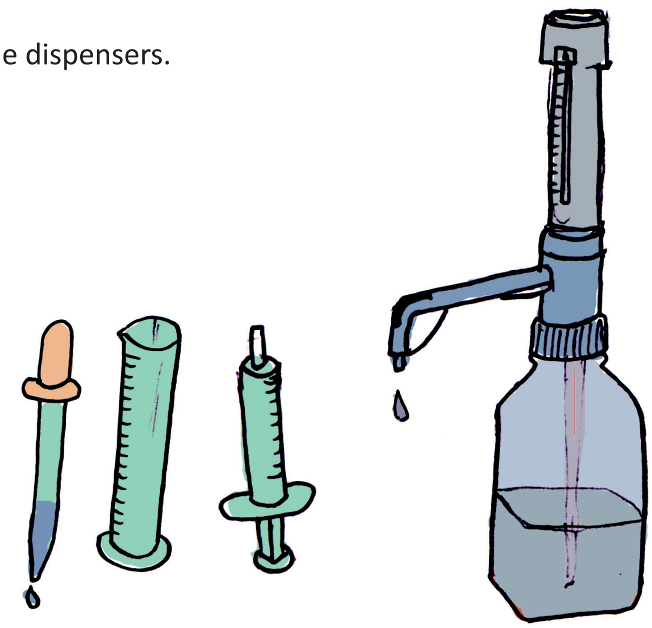
  NOTE: It is advisable to calculate the amount of fixing agent that has to be added to each sampling bottle BEFORE the survey and inform personnel accordingly.

During the survey, it is advisable to use plastic bottles to carry the reagents to be used (formol, borax). This will not only protect the reagent but also prevent injuries such as cuts if glass bottles are used and broken.

Whenever using reagents, it is advisable to have the ***Safety Data Sheet*** handy. This explains how reagents are to be handled and stored and contains details of their physical-chemical properties, as well as the **personal protection measures that must be taken when handling each reagent**, and also **what has to be done in the event of accidental exposure to the reagent in question** (VERY IMPORTANT).

The following instruments can be used to add amounts of reagents to the samples:

- Adjustable dispensers.
- Syringes.
- Pipettes.



NOTE: If there is no suitable place for handling fixing agents on board the vessel, and to simplify sample taking, reagents can be introduced BEFOREHAND in the sampling bottles, in the amount calculated for the total volume of sample and seawater.

• PACKAGING AND TRANSPORT OF MATERIAL

To prevent damage, breakage or loss of material and equipment, the following instructions must be taken into account.

- Use plastic boxes to transport material (avoid using cardboard boxes; they are not resistant to knocks and can also break easily when wet). If possible, use lidded boxes that can be closed using nylon rope or cable ties.

- Be especially careful with reagent containers: if possible, replace glass bottles with plastic bottles. If this is not possible, wrap the bottles in old rags or cardboard to protect them from knocks (used items can be recycled for this purpose).
- Delicate equipment: glassware, special equipment (automatic dispensers, flowmeter, etc.): these must be transported as safely as possible to prevent damage or breakage. For this purpose, use original packaging or small cardboard boxes, if available, and fill the spaces in these boxes with foam, crumpled paper, etc.

Boxes should be numbered and labelled properly (indicating the laboratory of origin, the survey for which the material is intended, the vessel on which the material is loaded, the person responsible for the material, the address and contact telephone number) and draw up a list of material that will be placed in each box. This will allow you to:

- Know the exact number of boxes or packages to be transported from the laboratory to the vessel and vice-versa. Avoid losing equipment.
- Facilitate their transportation if the people participating in the survey are different to those transporting the materials.

- Quickly locate the box in which a specific item is stored whenever this is needed.
- At the end of the survey, this will allow you to identify the location of each item.

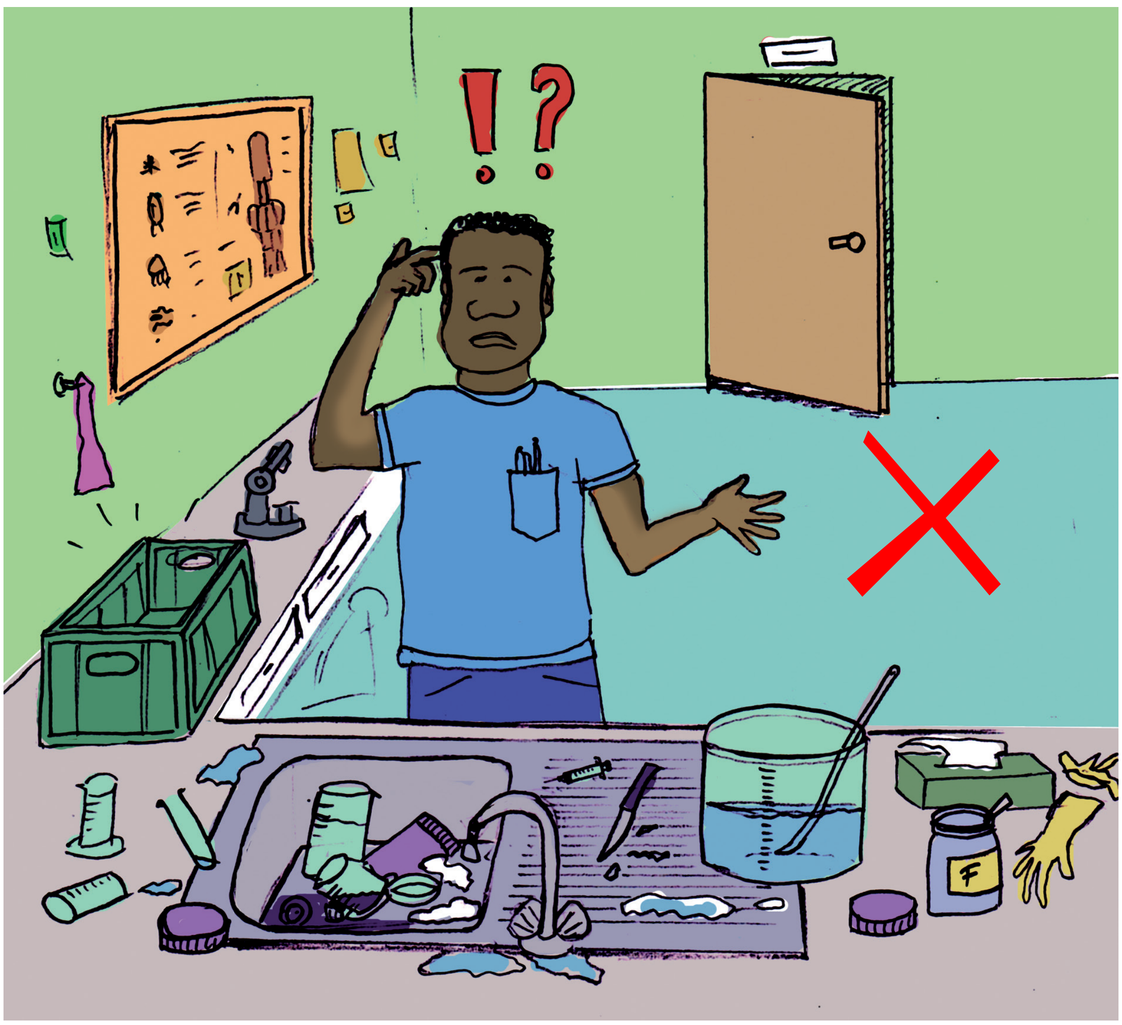
A standard label would be:

National Marine Information & Research Centre (NatMIRC)
Contact (name, address, telephone number)

CRUISE: November/December 2012 Monthly Oceanographic Monitoring
R/V MIRABILIS

box /packaged number

It is IMPORTANT to check that all the equipment to be used in the survey HAS BEEN WASHED, CHECKED AND REPAIRED BEFOREHAND. See the *Post-Survey Treatment of Material Protocol*.



PROTOCOL 2: POST-SURVEY TREATMENT OF MATERIAL

This protocol will be divided into the following sections:

- CLEANING, REPLACEMENT/REPAIR OF MATERIAL

Once the survey has finished, the different equipment must be checked. The materials must be properly cleaned. This will not only keep the equipment in optimum condition, thus guaranteeing good results, it will also increase the useful life of the equipment.

The aim is to prevent the oxidation of metal parts of the equipment (they can still rust even though they are made of stainless steel!). Wash ALL MATERIAL with fresh water, including tools; dry thoroughly and store in a dry and safe place to prevent damage.

If the area is very wet and the equipment is not going to be used for a long time (as may occur with material for seasonal surveys), small screws can be stored in jars with oil.

- Plankton nets

It is important to prevent sample remains (i.e. detritus,debris, dirt etc.) from accumulating in nets because if these remains dry they can block pores, thus undermining the filtration capacity of the nets and also impair the removal of samples from the mesh-walled buckets.

- Cleaning and storage

  If the vessel has pressurised water equipment like that on the R/V Mirabilis, at the end of the season cleaning operations can be performed to remove organic remains from the nets. Nets should not be left on the vessel for several reasons. For example, you do not know how they will be treated by the crew or personnel performing repairs in the laboratory. Another element to consider is the humid environment, which is not conducive to proper maintenance of equipment. Finally, in the laboratory you will have more time and better means for cleaning and repairing equipment: remove rings/support, turn them around and

place them in a recipient with fresh water and in a dish-washer, and leave for at least 24 h. Using a pressurised water hose, remove the soap and sample debris. If necessary, rub the nets together to eliminate sample remains. Nets must be completely dry before storing. Limit their exposure to direct sunlight as much as possible because this can damage them. Fold the nets and store them in a dry place.

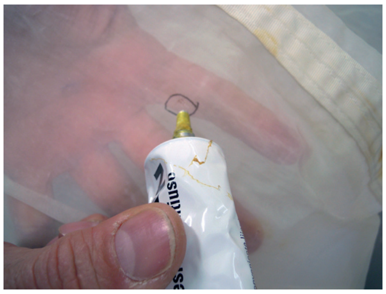
Nets (depending on their condition and use) can be washed every 3-4 years in a washing machine using a "Delicate" wash programme with cold water and using the same amounts of detergent and bleach as with normal laundry.

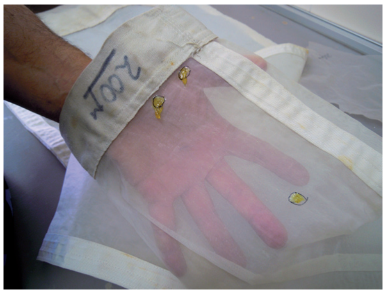
- Repairs

Check nets for holes (especially the ends where the mesh-walled buckets and trawl clamp are fitted).

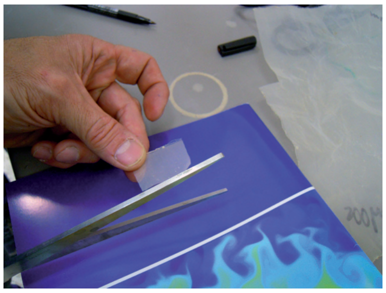
Use a thin-tipped marker pen to indicate the location of damage.

Small holes can be fixed using contact adhesive, which can be applied directly to the damaged area using your fingers (avoid contact with other parts of the net).





In the case of larger holes, a patch can be made using two pieces from the same net: place one piece of net on the inside and the other on the outside.

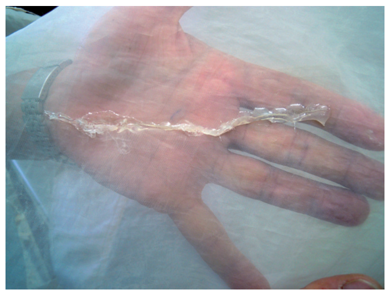


Depending on the size of the hole, apply contact adhesive to the entire surface (small holes), or to the edges of patches in order not to reduce the filtering surface.


A hot melt adhesive (HMA) or hot glue gun can also be used**.** This is a delicate operation. It is best to practice with old nets first to avoid burning equipment. This system can be used with wet nets, but should only be used if you have expertise in the use of such equipment and adhesive in order to avoid damaging equipment in operation.





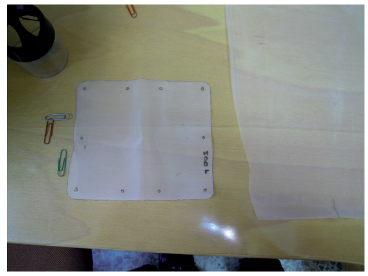


Another way to repair large holes is by sewing the net along the broken area.
Finally, check the seams where the nets may have started to dry.

- Mesh-walled buckets

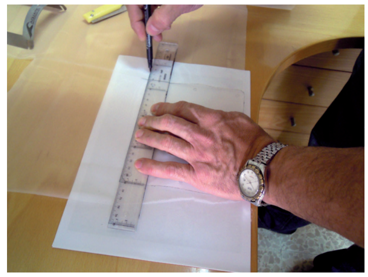
Like nets, the mesh of mesh-walled buckets (for WP2, CUFES and other nets) must be cleaned thoroughly to guarantee their correct operation. *PROTOCOL 2: METHODOLOGY FOR SAMPLING ON BOARD* specifies this requirement.

WP2 bucket mesh can be cleaned using a small electrical appliance normally employed to seal plastic bags (look into the possibility of purchasing one in Namibia or ordering one from Spain).

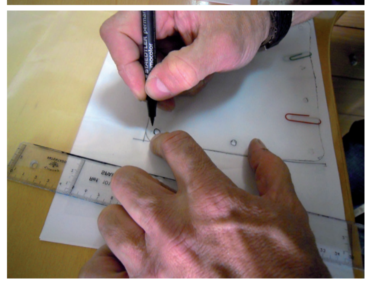


Take a mesh in good condition from the bucket mesh that needs to be repaired and use this as a model piece.

Place the piece on the large piece of net from which the replacement mesh is going to be cut and fix it to the model using clips (to do so, place it along one edge of the large piece of net).

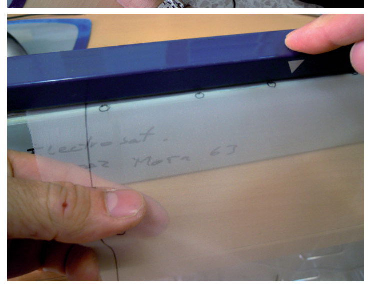


Mark the edges using a ruler and marker pen (using the model as a template).



Mark the position of the holes where the screws will go. Be careful not to move the model to make sure all the holes match.

Cut the piece to be used as a replacement from the large piece of net (try to make maximum use of the mesh surface; to do so, leave a few centimetres at the edges, which can later be cut). The device for sealing plastic bags has a metal wire that is heated by a resistor, allowing the bucket mesh to be cut and its edges sealed at the same time (thus preventing them from fraying). To cut, align the edge of the replacement net with the cutting and sealing wire.

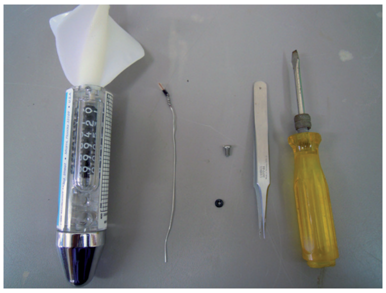


The device is simple to use and works like a press. Pressing the upper part against the lower part activates the resistor, heating the wire and in a few seconds the edges of the replacement net are cut and sealed. The same operation must be repeated on the four edges of the replacement net.

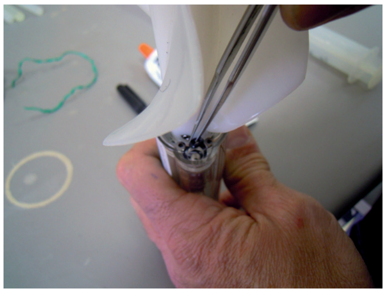
Finally, make the holes in the bucket mesh (through which the fixing screws are inserted into the bucket mesh). To do this, use the punch pliers and select the appropriate head according to the diameter of the holes.

- Flowmeters

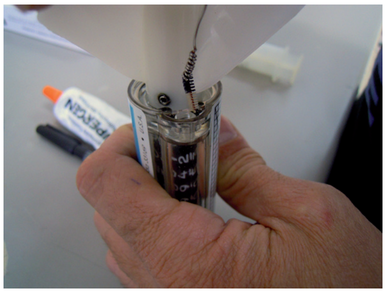
Flowmeter must be rinsed thoroughly with fresh water to eliminate any remains of salt. Avoid oxidation of parts to increase their useful life and proper operation. With use, salt and rust can accumulate on the inner walls of the flow-metre, making it difficult to take readings on the metre. To prevent this from happening, unscrew the flow-metre water tank cover using a screwdriver (this cover is on the base of the rotor).



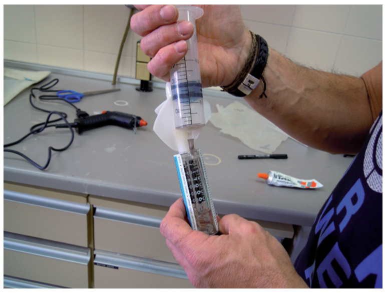
If the rubber O-ring (which keeps the tank watertight) cannot be removed together with the screw, remove it using thin-tipped pliers.



The inner walls of the flow-metre tank can be cleaned using a small brush (a toothbrush and a thin steel wire like dental floss used to clean between teeth).



Flowmeter must be emptied and rinsed several times using a syringe to remove salt and oxide deposits. **ALWAYS use distilled water** to fill flowmeter, and avoid using water in the presence of mineral salts that may accumulate in the moving parts of the flow-metre.



After completing this process, re-insert the O-ring and screw and tighten carefully to avoid forcing the thread of the tank (it is made of plastic and the steel screw can damage it if tightened excessively).

They must be completely dry (on the outside) and properly packaged (in the original box or in adapted boxes) before storage.

The rotor should be removed regularly from the equipment (in models in which this is possible, e.g. General Oceanics flowmeter). To do so, loosen the Allen screw on the side. NEVER FORCE THE SCREW. Apply a little anti-rust lubricant. Wash the flow-metre axis, rotor and Allen screw with fresh water. Once completely dry, re-assemble the equipment carefully. The axis has a specific shape. There is a flat area where the Allen screw must be inserted and tightened.

To check the calibration of General Oceanics flowmeter, special equipment can be used to determine whether flowmeter are correctly calibrated (see the information attached in the *NEW EQUIPMENT* folder on the CD, information on *General Oceanics Model 2030CF Calibration Frame).* If flowmeter are not calibrated correctly, they must be sent to the manufacturer for calibration.

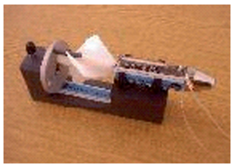
- CALIBRATION OF FLOWMETERS

When considering flowmeter calibration, we have to be quite clear about one thing: if they have not been adequately maintained, it is not conceivable to try to calibrate them. Properly maintained equipment may only need calibration if it has been damaged (for example if one of its parts breaks and has to be replaced), or if a variation in the readings at certain points of the periodic samplings in the same station is observed.

The calibration of equipment must follow a standardized process and present a methodology that will enable us to obtain reliable data. In the case of flowmeters there is literature available that presents various methods of calibration, in many cases they are very complex systems or with highly variable results.

What is most recommendable, in the case of adequately maintained equipment, is to find out quickly and easily if the machines are calibrated. GENERAL OCEANICS provides equipment for this purpose:

MODEL 2030CF CALIBRATION FRAME



If we detect that the flowmeters are not adequately calibrated, it is best to hire an external agent for its calibration to be sure of the measurements it takes.

If the flowmeters need servicing, repairs or calibration, GENERAL OCEANICS has a CALIBRATION SERVICE . Refer to the following web:
http://www.generaloceanics.com/product.php?productid=4153&cat=43&page=1
(Consult the different manuals and information provided on this subject in the CALIBRATION OF FLOWMETERS on the CD).

- PERFORMING THE CALIBRATION ON YOUR OWN

Procedure for calibration in pool:

At the Spanish Institute of Oceanography (IEO) calibration of this equipment was performed based on the methodology provided by various United States researchers at the NOAA in the 80s, resulting from an international cooperation agreement.

- The calibration was performed in a pool where a linear distance of 9 meters was set.

- The count (there and back) at a constant speed was timed to the tenth of a second, with the flowmeters attached to the end of a rod supplied by GENERAL OCEANICS: 2030W – WADING ROD (Figure 1).

- The count conformed to the following times: 8, 10, 12, 14, 16, 18, 20 and 22 seconds.

- Each count (there and back) was performed twice.

- The presence of two people was required. One was in charge of timing the counts and recording the data (times and revolutions). The second person dragged the flowmeter (attached to the rod) at a more or less fixed depth (30-50 cm) from the surface. They also performed the counts (measuring 9 meters) trying to adjust to the times mentioned above. This is the weakest point of the method, because it requires great practice, and still it presents a high level of variability, which affects the calibration itself. **This calibration method only gave approximate results and was only justified because it was not possible to bear the high cost of external calibration services at that moment.**

- The initial and final revolutions of the flowmeters in each count (both there and back) were recorded.

- All the data was recorded in an inventory shown below and that is attached as an Excel sheet, which includes the formulas to calculate the values that are required:

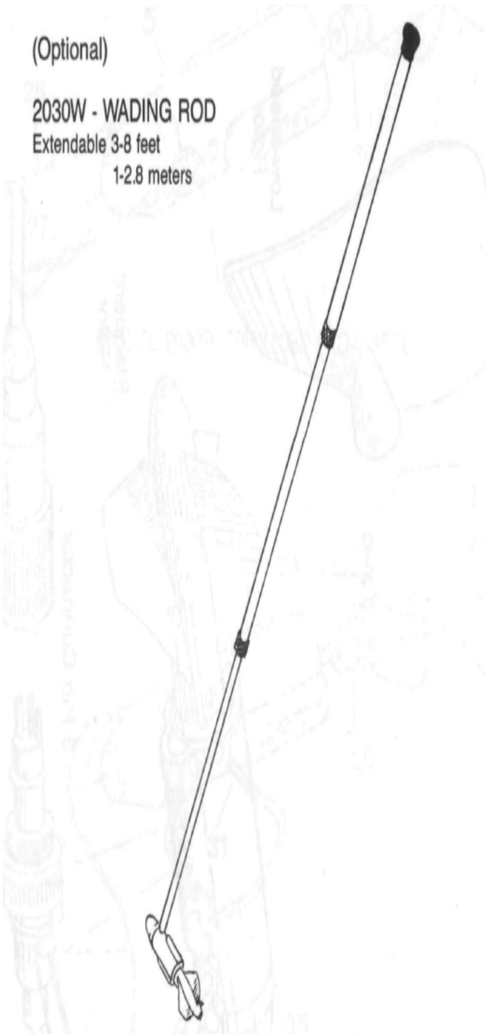


Figure 1: Wading rod

FLOWMETER Nº	HORIZONTAL DISTANCE (m)	LOCATION	DATE yr. mo. day	OBSERVER
2556	9	VIGO	87 04 09	WCF /AMV

SUG. SPD.		FORWARD REVOLUTION	FORWARD TIME- SEC	BACKWARD REVOLUTION	BACKWARD TIME- SEC	AVERAGE DIFFERENCE	AVERAGE TIME	REVS /SEC	METERS / REV
8	FINAL	8816	8.3	9161	8.4	36.30	8.35	4.35	0.248
	INITIAL	8435		8816					
	DIFFERENCE	38.1		34.5					
8	FINAL	9535	8.2	9909	8.9	37.40	8.55	4.37	0.241
	INITIAL	9161		9535					
	DIFFERENCE	37.4		37.4					
10	FINAL	10234	10.1	668	10.9	35.05	10.50	3.34	0.257
	INITIAL	9877		324					
	DIFFERENCE	35.7		34.4					
10	FINAL	7370	10.6	7712	9.4	35.60	10.00	3.56	0.253
	INITIAL	7000		7370					
	DIFFERENCE	37		34.2					
12	FINAL	1051	12.3	1393	12.3	36.25	12.30	2.95	0.248
	INITIAL	668		1051					
	DIFFERENCE	38.3		34.2					
12	FINAL	1777	12.5	2121	11.7	36.40	12.10	3.01	0.247
	INITIAL	1393		1777					
	DIFFERENCE	38.4		34.4					
14	FINAL	2478	14.3	2825	13.8	35.20	14.05	2.51	0.256
	INITIAL	2121		2478					
	DIFFERENCE	35.7		34.7					
14	FINAL	8913	14.2	9245	14.7	34.30	14.45	2.37	0.262
	INITIAL	8559		8913					
	DIFFERENCE	35.4		33.2					
16	FINAL	3215	16.0	3539	16.0	35.70	16.00	2.23	0.252
	INITIAL	2825		3215					
	DIFFERENCE	39.0		32.4					
16	FINAL	3910	16.1	4228	16.0	34.45	16.05	2.15	0.261
	INITIAL	3539		3910					
	DIFFERENCE	37.1		31.8					
18	FINAL	4604	18.4	4909	18.4	34.05	18.40	1.85	0.264
	INITIAL	4228		4604					
	DIFFERENCE	37.6		30.5					
18	FINAL	5286	18.5	5595	18.2	34.30	18.35	1.87	0.262
	INITIAL	4909		5286					
	DIFFERENCE	37.7		30.9					

SUG. SPD.		FORWARD		BACKWARD		AVERAGE		REVS /SEC	METERS / REV
		REVOLUTION	TIME- SEC	REVOLUTION	TIME- SEC	DIFFERENCE	TIME		
20	FINAL	5975	20.5	6289	19.0	34.70	19.75	1.76	0.259
	INITIAL	5595		5975					
	DIFFERENCE	38.0		31.4					
20	FINAL	6625	20.2	6908	20.6	31.10	20.40	1.52	0.289
	INITIAL	6286		6625					
	DIFFERENCE	33.9		28.3					
22	FINAL	7932	22.4	8260	22.0	30.35	22.20	1.37	0.297
	INITIAL	7653		7932					
	DIFFERENCE	27.9		32.8					
22	FINAL	8559	22.6	9563	22.0	30.85	22.30	1.38	0.292
	INITIAL	8260		9245					
	DIFFERENCE	29.9		31.8					

FINAL / REVOLUTION: final reading of flowmeter.
INITIAL /REVOLUTION: initial reading of flowmeter.
DIFFERENCE: final reading - initial reading.
TIME-SEC: time in seconds and tenths of seconds of the count.
AVERAGE DIFFERENCE: average of DIFFERENCE between FORWARD/BACKWARD (there and back).
AVERAGE TIME: average TIME of FORWARD / BACKWARD (there and back).
REV/SEC: revolutions per second.
METERS/REV: meters per revolution.

As we can see, the average of the counts, both for DIFFERENCE and TIME were carried out.

- Then the average of all the counts with similar times was performed. To calculate the quotient the values of those counts whose AVERAGE DIFFERENCE was equal to or higher than 35 (rounding the decimal up, for example, 34.5 = 35). Our example would include the counts for 8, 10, 12, 14, 18,20 and 22.

	AVERAGE DIFFERENCE	AVERAGE DIFFERENCE	AVERAGE DIFFERENCE	METERS / REV	TIME	TIME
8	36.30	36.85	35.67	0.252	8.4	8.45
8	37.40				8.6	
10	35.60	35.33			10.5	10.25
10	35.05				10.0	
12	36.25	36.33			12.3	12.20
12	36.40				12.1	
14	35.20	34.75			14.2	14.30
14	34.30				14.5	
16	35.70	35.08			16.0	16.03
16	34.45				16.1	

	AVERAGE DIFFERENCE	AVERAGE DIFFERENCE	AVERAGE DIFFERENCE	METERS / REV	TIME	TIME
18	34.05	34.18	35.67	0.252	18.4	18.38
18	34.30				18.4	
20	34.70	32.83			19.8	20.08
20	30.95				20.4	
22	30.35	30.60			22.2	22.25
22	30.85				22.3	

- As per our example we would have an AVERAGE DIFFERENCE value of 35.65 that would give us a value of 0.252 (0.25231 , rounded value that results from dividing the distance travelled of 9 meters by the average value of DIFFERENCE = 9/35.65) METERS/REV.

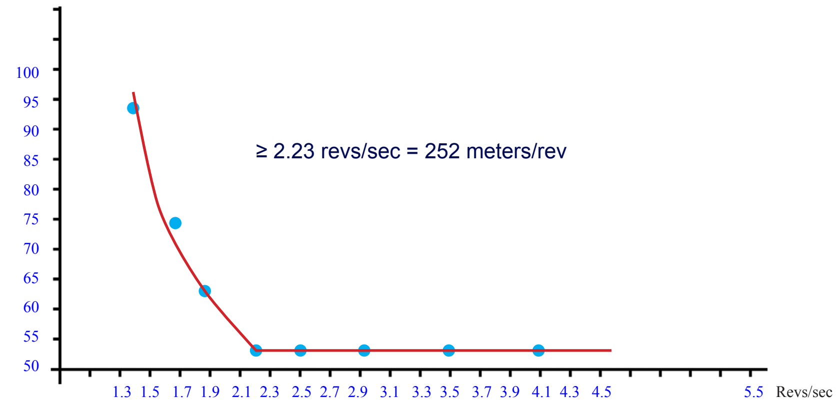
- The final result per count would be:

FLOWMETER Nº	HORIZONTAL DISTANCE (m)	LOCATION	DATE yr. mo. day	OBSERVER
2556	9	VIGO	87 05 21	1MV /CH

SUG. SPD.		FORWARD		BACKWARD		AVERAGE		REVS /SEC	METERS / REV
		REVOLUTION	TIME- SEC	REVOLUTION	TIME- SEC	DIFFERENCE	TIME		
8	FINAL					35.67	8.45	4.22	0.252
	INITIAL								
	DIFFERENCE	0		0					
10	FINAL					35.67	10.25	3.48	0.252
	INITIAL								
	DIFFERENCE	0		0					
12	FINAL					35.67	12.20	2.92	0.252
	INITIAL								
	DIFFERENCE	0		0					
14	FINAL					35.67	14.30	2.49	0.252
	INITIAL								
	DIFFERENCE	0		0					
16	FINAL					35.67	16.03	2.23	0.252
	INITIAL								
	DIFFERENCE	0		0					
18	FINAL					34.18	18.38	1.86	0.263
	INITIAL								
	DIFFERENCE	0		0					
20	FINAL					32.80	20.08	1.63	0.274
	INITIAL								
	DIFFERENCE	0		0					

SUG. SPD.		FORWARD		BACKWARD		AVERAGE		REVS /SEC	METERS / REV
		REVOLUTION	TIME- SEC	REVOLUTION	TIME- SEC	DIFFERENCE	TIME		
22	FINAL					30.60	22.25	1.38	0.294
	INITIAL								
	DIFFERENCE	0		0					

Graphing these results would give the following curve:



The flowmeter handbook provides the values of the constants and the calculations necessary to obtain the volume of cubic meters filtered.

- Calculations

10 counts are equal to 1 rotor revolution on the graphic labels on all flowmeters. The cts/sec is "counts per second" and must not be used as revolutions per second for calculations.

ROTOR CONSTANTS:

Standard Speed Rotor Constant = 26,873

Low Speed Rotor Constant R6 = 57,560

(R2) Low Speed Rotor Constant = 51,020

A. DISTANCE in meters = Difference in COUNTS x Rotor Constant

999999

(Example: Where the graph may indicate 100 cts/sec this is also equal to 10 revolutions/sec). Therefore please ensure the correct units are being used when measuring and calculating.

B. SPEED in cm/sec = Distance in meters x 100

Time in seconds

C. VOLUME cubic meters = 3.14 x Net Diameter^2 x Distance

Therefore we would have to use a New STANDARD SPEED ROTOR CONSTANT, that in our example would be **25,231** (the constant is calculated in centimeters/second and we performed the calculations in meters/second).

- STORAGE OF SAMPLES

General storage guidelines:

- Optimise available space.
- Store equipment and materials in optimum storage conditions. Avoid damage (snagging of nets, accumulation of dust, moisture, etc.). For this purpose, place material and equipment in correctly labelled boxes, as required.
- Ensure quick location: group material together by areas (sampling equipment, containers, spare parts, tools, etc.).
- Eliminate damaged, old or obsolete equipment and materials.
- Make an inventory of materials and equipment available by department.
- Plan equipment and material to be purchased, depending on stocks and consumption or the requirements of surveys or projects to be performed.

Therefore, samples obtained during surveys must be stored in a safe and dark place. Direct and continuous exposure to sunlight alters their preservation.

Samples from each survey should be stored in boxes of a manageable size (avoiding excessive weight). Each box must be properly labelled (name of survey, date, number of samples in the box) and labels must be placed in a visible place (once placed on the shelf where it will be stored). It is also advisable to keep an inventory of the samples in the laboratory and the place where they are stored, number of boxes per survey, etc. This will not only allow samples to be located quickly, it will also provide information on their “age” and thus making make it easier to eliminate those samples that do not have to be kept in the storage area for longer than necessary.

PROTOCOL 3: SAFETY PROTOCOLS

It is important to establish SAFETY PROTOCOLS outlining the main safety rules that must be followed by technical staff and researchers in both the laboratory and on board vessels, in order to reduce their exposure to reagents used and reduce or eliminate possible risks to which they may be exposed.

When working with any chemical product, it is important to have ***Reagent Safety Data Sheets***. These contain details of all the protection measures, handling and storage procedures, as well as other information of interest that personnel must know in order to use a given reagent.

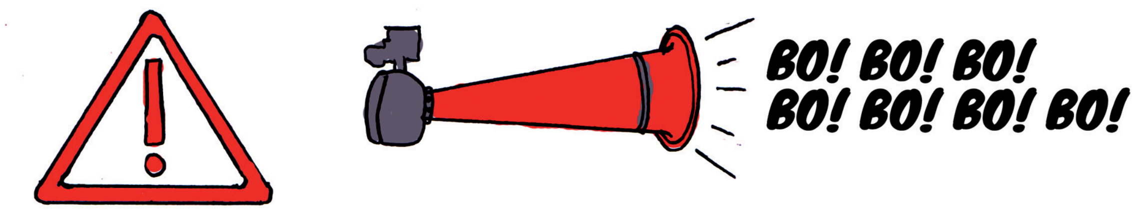
- SAFETY ON VESSELS

It is recommended to organise a security meeting in the first 24 hours on board, especially if any member of the team is embarking for first time or if you are on your first cruise in a particular vessel. During the cruise it recommended that security exercises/drills be conducted on different emergency situations.

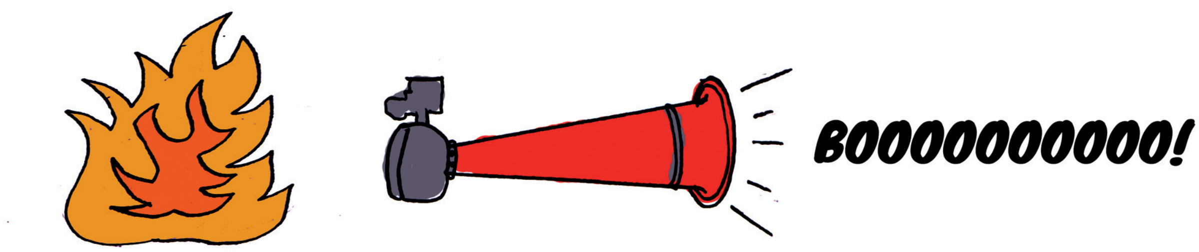
- **Alarm codes**

It is very important for all staff on-board to know and familiarize themselves with the warning system used in different emergency situations, especially:

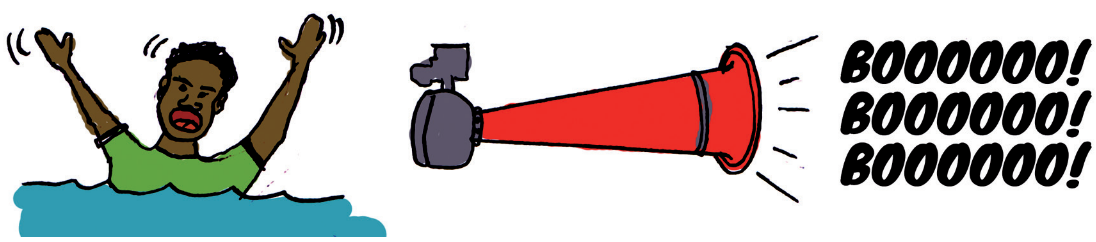
- **General Alarm Signal**: seven or more short blasts followed by one long



- **Fire Signal**: continuous sound alarm bell.



- **Man Overboard**: three blasts long.



According to the International Regulations for Preventing Collisions at Sea[1]:

- **Short blast**: From approximately 1 second.
- **Prolonged blast**: From last approximately 6 seconds.

In case of hearing one of these alarms, suspend all activities and go to the place indicated at the initial meeting.

International regulations applicable to safety at work on board is governed by the IMO, through the ISM code[2]. You must consider whether there is any risk prevention regulations at work on the national level in the State of the flag of the vessel.

- **Mandatory Safety Equipment**

Generally, you should keep in mind:

- The use of safety footwear should be mandatory when working on board.
- During the performance of work on deck you must wear helmet.
- All tasks of shooting and hauling equipment astern, and works implying any risk of falling into the sea, should be performed with a safety harness asserted to a lifeline.
- All tasks of shooting and hauling equipment astern, and works implying any risk of falling into the sea, should be performed with a lifejacket.

1. International Maritime Organization, Convention on the International Regulations for Preventing Collisions at Sea, COLREG, 1972 http://www.imo.org/OurWork/Safety/Navigation/Pages/Preventing-Collisions.aspx
2. International Safety Management Code, Resolution A.741(18) as ammended by MSC.104(73), MSC.179(79), MSC.195(80) and MSC.273(85) http://www.imo.org/OurWork/HumanElement/SafetyManagement/Pages/ISMCode.aspx

• SAFETY ON VESSELS / IN THE LABORATORY

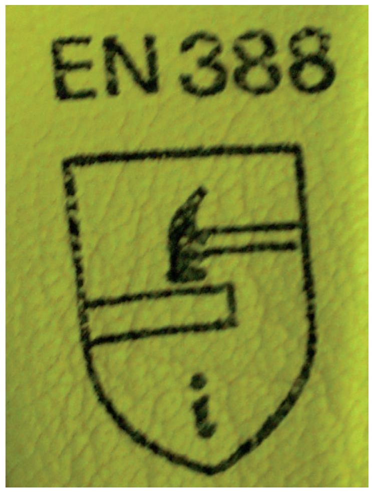
The use of individual protective equipment for work on deck is regulated within the requirements to be met by the crew, and may vary according to the length of the ship, its classification (research / fishing / Merchant), or the existence stern ramp. At least we recommend using the following equipment during manoeuvres on deck: hard hat, safety shoes, and life vest.

• Hand / skin protection

The following protective equipment should be used:

- **Working gloves**

  Working gloves must be used to avoid injuries or cuts when handling equipment. This is especially important when doing tasks with cranes. Gloves must have the pictogram shown in the image.

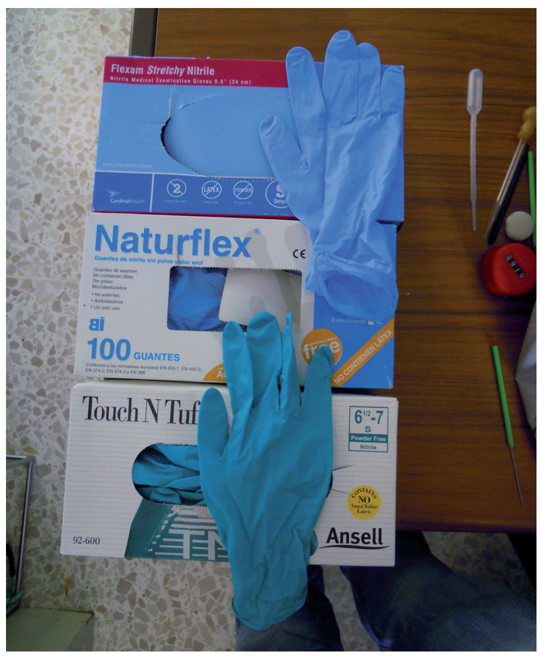
- **Nitrile gloves**

  Protective gloves must be used when working with formaldehyde. Latex gloves ARE NOT SAFE. Nitrile gloves must be worn (similar to those used in health care/surgery).

  Examples:

  http://www.ansell.com/brand/touchntuff

  http://www.cardinal.com/us/en/Gloves/ExamGloves



- **Respiratory protection equipment**

It is ALWAYS preferable to use extraction hoods (or fume cupboards) when handling reagents. If these are not available or in good working order, individual respiratory equipment with special air filters must be used. Some are specific for formaldehyde:

http://goo.gl/sDure

Safety precautions must be taken when working with large concentrations of formaldehyde, or small concentrations for longer periods.

Respiratory protection masks with special filters must be used. For images of this type of mask, consult the following website:

http://goo.gl/tcJB3

The type of filter to be used must be similar to the 3M™ Formaldehyde/Organic Vapor Cartridge 6005, Respiratory Protection 60/ Case:

http://goo.gl/FVTvC

For information on 3M products available in Namibia, consult the following website:

http://solutions.3m.com.na/wps/portal/3M/en_NA/World/Wide/

- **Eye protection**

Eye protection equipment covering the entire eye area should be used to prevent injuries caused by splashing when handling reagents.

As an example see: http://goo.gl/X12lP

(See the different manuals and information on this subject in the *SAFETY EQUIPMENT* folder on the CD).

- BIBLIOGRAPHY ON CALIBRATION OF FLOWMETERS

There is outdated bibliography on how to perform the calibration without resorting to external services. To do so we can refer to the publications listed below:

• Standard Techniques for pelagic fish egg and larva surveys. Paul E. Smith, and Sally L. Richarson. FAO Fisheries Technical Paper nº 175 (1977). Pag. 32 - 36.

• A Circular Towing Tank for Calibratinting Plankton-net Flowmeters. David C. Potter. Laboratory reference nº 78-32, National Marine Fisheries Service. Northeast Fisheries Center (1978).

• Collecting and Processing Data on Fish Eggs and Larvae in the California Current Region. David Kramer, Mary J. Kalin, Elizabeth. Stevens, James R. Tharailkill, and James R. Zweifel. NOAA Technical Report NMFS CIRC-370 (1972). Pag. 32 a 38.

• Manual for estuarine environmental and zooplankton studies. Edited by William J. Hauser, Compiled by Norma Dubiak. Alaska Department of Fish and Game Division of Fisheries Rehabilitation Enhancement and Development. (1981). Pag. 16 - 18.

• A rotary system for calibrating flowmeters used for plankton sampling. Stanley M. Harrison, and W. H. Bé Allan. Limnology and Oceanography 17-1 (1972). Pag. 152 - 156.

MINISTRY OF FISHERIES
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