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# Guideline of Ocean Observations

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## Foreword

Measures to mitigate and adapt to climate change are urgently needed; the importance of understanding the state of climate change in the oceans is rising. In monitoring environmental changes at the global scale, it is obviously vital to publish data that guarantees traceability and comparability with appropriate reference materials or certified reference materials and that is clear about its uncertainties.

In recent years we have been building our knowledge of changes within the oceans through international cooperation and collaboration, for example, by re-occupation of World Ocean Circulation Experiment (WOCE); our findings were cited in the Fifth Assessment Report from the IPCC. To implement plans to make all measurement values used in climate change research completely SI-traceable, the General Conference on Weights and Measures has been providing advice to relevant institutions. Through measures such as promulgating the use of standard materials for nutrients, we are making progress in comparability of data, research that depends on this comparability, and R&D on standard materials.

However, the guidelines used for measurement and analysis do not seem to be keeping up with this progress. The Oceanographic Observation Guidelines published by the Japan Meteorological Agency in 1999 are relatively widely used in Japan, but their content is not always completely up-to-date and the Guidelines are now quite hard to obtain. In 2010, the WOCE Manual was revised and published as the GO-SHIP Oceanographic Observation Manual (IOCCP Report No.14, 2010), but this is principally for repeat hydrography in the open ocean; it was not intended to guide a wide range of users. There are a number of other manuals and guidelines available but some of them are only written in Japanese, whereas others are only written in English; moreover, they mix together up-to-date content and less up-to-date content.

In this context, the Oceanographic Society of Japan (JOS) has decided to set up an editorial committee for oceanographic observation guidelines, to review and collate the

various existing guidelines, and to incorporate necessary revisions and fill in any gaps. We will publish Oceanographic Observation Guidelines describing the most up-to-date oceanographic observation methods and analytical techniques, and we will make these new guidelines available through the JOS website.

We intend to continuously update these guidelines so that the most up-to-date methods are always accessible. We hope that these guidelines will be used by many researchers worldwide and will contribute to the advance of oceanography.

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## Table of Contents

Foreword

List of authors

### **Vol. 1      Quality Control and Standard Materials**

Chap. 1	Ocean Variables and the International System of Units (SI)	G101EN:001-006	Michio AOYAMA
Chap. 2	Quality Control with Standard Equipments and Materials	under writing	
Chap. 3	Essential Ocean Variables (EOVs) of GOOS	G103EN:001	Masao ISHII, Toshio SUGA, Sanae CHIBA
Chap. 4	Quality Control by Property-property Characteristics	under writing	
Chap. 5	Data publication and international exchange	G105EN:001-008	Toru SUZUKI, Yutaka MICHIDA
Chap. 6	Calculation of the Thermophysical Properties of Seawater (2010)	G106EN:001	Hiroshi UCHIDA

### **Vol. 2      Physical Observation**

Chap. 1	Water Sampling	under writing	
Chap. 2	Water Temperature	G202EN:001-002	Toshiya NAKANO
Chap. 3	Salinity	G203EN:001	Takeshi KAWANO
Chap. 4	Density of Seawater	G204EN:001-002	Hiroshi UCHIDA
Chap. 5	Transparency	under writing	

### **Vol. 3      Seawater Analysis I (Dissolved Substances)**

Chap. 1	Dissolved Oxygen	under writing	
Chap. 2	Determination of dissolved nutrients (N, P, SI) in seawater with high precision and inter-comparability using gas-segmented continuous flow analysers	G302EN:001-095	Michio AOYAMA
Chap. 3	Trace Metals	G303EN:001-005	Hajime OBATA
Chap. 4	DIC	G304EN:001	
Chap. 5	Determination of total alkalinity in sea water by spectrophotometry	G305EN:001-012	Masao ISHII, Naohiro KOSUGI
Chap. 6	pH	G306EN:001	
Chap. 7	pCO <sub>2</sub>	G307EN:001	
Chap. 8	Chlorofluorocarbons (CFCs) and Sulfur Hexafluoride (SF <sub>6</sub> )	under writing	

Chap. 9	Carbon isotopes in dissolved inorganic carbon	under writing
Chap. 10	DOC/DON/DOP	under writing

**Vol. 4 Seawater Analysis II (Particulate Substances)**

Chap. 1	Particulate organic carbon (POC), particulate nitrogen (PN), and particulate phosphorus (PP)	G401EN:001-006	Takeshi YOSHIMURA
Chap. 2	Biogenic Silica	G402EN:001-003	Fuminori HASHIHAMA
Chap. 3	Carbon and Nitrogen Stable Isotopes in Particulate Organic Matter	G403EN:001-008	Yu UMEZAWA
Chap. 4	Phytoplankton Pigments	under writing	
Chap. 5	Prokaryote and Heterotrophic Nanoflagellates		
Chapter 5-1	Direct Counting Methods of Prokaryote and Heterotrophic Nanoflagellates by Epifluorescence Microscopy	G4051EN:001-006	Taichi YOKOKAWA
Chapter 5-2	Enumeration of prokaryotes by flow cytometry	G4052EN:001-004	Mitsuhide SATO
Chap. 6	Detremination of Micro Zooplankton	under writing	
Chap. 7	Primary Production	under writing	

**Vol. 5 Sediment Analysis**

Chap. 1	Collection of Marine Seidment	under writing
Chap. 2	Water Content and Porosity	under writing
Chap. 3	Ignition Loss	under writing
Chap. 4	Particle Size Distribution	under writing
Chap. 5	Major Components	under writing
Chap. 6	Pore Water	under writing

**Vol. 6 Plankton and Benthos**

Chap. 1	Plankton Net	G601EN:001-009	Hiroaki SAITO
Chap. 2	Benthos	G602EN:001-006	Shigeaki KOJIMA

**Vol. 7 Underway**

Chap. 1	pCO <sub>2</sub>	G701EN:001-007	Daisuke SASANO, Shinichiro NAKAOKA
Chap. 2	Acoustic Doppler Current Profilers	G702EN:001-005	Shinya KOUKETSU
Chap. 3	Bathymetry	under writing	
Chap. 4	Weather Observations	G704EN:001-085	Toshiya NAKANO

Chap. 5	Sea Ice	G705EN:001-040	Takenobu TOYOTA
Chap. 6	Incident radiation and aerosol optical thickness	under writing	
<b>Vol. 8 Measurements by sensors</b>			
Chap. 1	TSG	under writing	
Chap. 2	XBT/XCTD	under writing	
Chap. 3	Conductivity-Temperature-Depth profiler (CTD) (Blue-water measurements)	G803EN:001	Hiroshi UCHIDA
Chap. 4	Conductivity-Temperature-Depth profiler (CTD) (coastal-water measurements)	under writing	
Chap. 5	CTD Oxygen Sensor Calibration Procedures	G805EN:001	Hiroshi Uchida
Chap. 6	Fluorometer	under writing	
Chap. 7	Turbidity Sensor and Transmissometer	under writing	
Chap. 8	Light fields and optical properties	under writing	
Chap. 9	Lowered Acoustic Doppler Current Profiler (LADCP)	G809EN:001-007	Shinya KOUKETSU
<b>Vol. 9 Natural and artificial Radioactivity</b>			
Chap. 1	Radiometric Determination of Anthropogenic Radionuclides in Seawater samples	G901EN:001-011	Michio AOYAMA
Chap. 2	Marine Sediment	under writing	
Chap. 3	Marine Macro Organisms	under writing	
Chap. 4	Plankton and Benthos	under writing	
<b>Vol. 10 Marine Pollutant Observation</b>			
Chap. 1	Heavy Metals	under writing	
Chap. 2	Oil·Hydrocarbon	under writing	
Chap. 3	Microplastics (Surface Water Trawl Surveys for Small Debris Items)	GX03EN:001-009	Takashi MIYAO
Chap. 4	Floating Marine Pollutants (Shipboard Sighting Surveys for Macro-Debris Items)	GX04EN:001-009	Takashi MIYAO
Chap. 5	Persistent Organic Pollutants	under writing	
Chap. 6	New Persistent Organic Pollutants (2009~)	under writing	

List of editors of each volume

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## Ocean variables and the International system of units (SI)

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### 1. Introduction

The International System of Units (SI) consists of seven base units for length, mass, time, electric current, thermodynamic temperature, amount of a substance, and luminous intensity. The corresponding and clearly defined units (and their symbols) are the meter (m), kilogram (kg), second (s), ampere (a), kelvin (K), mole (mol), and candela (cd).

An instrument is calibrated with a working standard, and the working standard is calibrated with a reference standard that is more precise (smaller uncertainty) than the working standard. If there is an unbroken chain of calibration that reaches the International System of Units (SI), the result of a measurement with the instrument is said to be traceable to the SI.

Instruments used for all ocean observations should, in principle, be explicitly traceable to the SI. For each instrument there should be a system chart traceable to the SI. For example, Figure 1 shows a traceability system chart for mass at a research institution that would be relevant if a scientist were performing certification work with reference material for nutrient concentrations in seawater. As shown in Figure 1, all of the instruments used to measure weight, temperature, pressure, and the ambient humidity in the room containing the electronic balance must have a certificate that is traceable to the SI.

The word "uncertainty" has been used since the early 1990s to characterize the reliability of measurement data. Conventionally, concepts such as "error" and "accuracy" have been used to characterize the reliability of a measurement. The terminology, however, has varied among scientific fields and between countries. The Certificate in Investment Performance Measurement Program has tried to unify the methods used to assess and express the reliability of measurement data. The result has been a publication by the Joint Committee for Guides in Metrology (JCGM) that reflects the opinions of the seven major international organizations involved in scientific measurements: "Evaluation of measurement data—Guide to the expression of uncertainty in measurement" (JCGM 100, 2008).

This document is often referred to as the Guide to Uncertainty in Measurement or GUM. The GUM describes in detail the procedure for the quantitative assessment of the uncertainty of a measurement, i.e., the degree of ambiguity of the knowledge obtained from a measurement. The basic idea is to allocate the uncertainty to two components. Type A uncertainty is associated with conventional statistical analysis and is quantified by calculating the standard deviation of the data. Type B uncertainty is estimated from various sources of information other than the information used to calculate the type A uncertainty. The combination of the type A and type B uncertainty is the whole uncertainty. It is important to consider various metrics of the technical reliability of the measurement data. These metrics are then adapted for use in academic publications. An assessment of uncertainty is also required in the ISO 9000 document (Quality management systems—Fundamentals and vocabulary) of the International Organization for Standardization and the ISO 17025 document (General requirements for competence

of calibration and testing laboratories).

In section 2, ocean variables in this Guideline are described in the context of the seven SI metrics of length, mass, time, electric current, thermodynamic temperature, amount of substance, and luminous intensity. Section 2 discusses the relationship between the international system of measurement and certified reference materials. Section 3 provides an example of a comprehensive traceability system of mass at an institute.

## **2. Relationship between the observed ocean variables and the SI**

Table 1 shows the relationships between ocean variables and the SI described in Volumes 2, 3, and 9 of this guideline. When certified reference materials are obtained and associated methods of measurement for each ocean variable are used to calibrate measurement instruments, comparability of the results of measurements of ocean variables can be obtained. However, for a standard reference material such as the number of moles of a substance, it has been difficult in practice to ensure comparability of observed amounts. In addition, because receiving a calibration certificate is relatively expensive, full traceability cannot always be achieved.

However, an effort should be made to have measurements of ocean variables traceable to the SI. An exception is the liter, a non-SI metric of volume. In SI units, 1 liter should be 1 dm<sup>3</sup> or 10<sup>-3</sup> m<sup>3</sup>. Although a liter is not an SI unit, it is acceptable to continue to use liter as a metric of volume in an appropriate context (BIPM, 2006). However, the conversion from volume of water to mass of water requires knowledge of the density of the water. Therefore, a report of a quantity per liter should be accompanied by the temperature and salinity of the seawater at the time of the measurement.

Table1. Relationship between ocean variables and the SI

Observables	Reported volume unit	Alternative units	global standard	Standard instrument or standard substance	Basic quantity Symbol	Length L	Mass M	Time T	Electric current I	Temperature theta	Amount of substance N	Luminous intensity J
					Unit Symbol	meter m	Kilogram kg	Second s	ampere A	Kelvin K	mole mol	Candela cd
Time and Location			UTC WGS-84									
Physical observation												
Water temperature	Degree.C		ITS-90	SPRT							1	
Salinity			TEOS-10	IAPSO Standard seawater			1,-1					
Density	kg m-3		TEOS-10			-3		1				
Pressure	Pa							1	-2			
Transparency	m					1						
Water sampling analysis (Dissolved state)												
Dissolved oxygen	mol kg-1			NMIJ KIO3 SCOR, NMIJ and KANSO CRM				-1				1
Nutrient	mol kg-1							-1				1
Trace metal	nM											1
DIC	mol kg-1					-3						1
Talk	mol kg-1			SIO CRM								1
pH	µatm											1
pCO2	µatm											1
CFC, SF6	mol kg-1											1
C-13	‰			VPDB								1,-1
C-14	‰			"1890 wood"								1,-1
DOC/DON/DOP	mol kg-1											1
natural radioactivity and artificial radioactivity												
Sea water	Bq m-3											
Marine sediment	Bq kg-1	Bq kg-1										
Large organisms	Bq kg-1											
Plankton benthos	Bq kg-1											
UTC												
Universal Time, Coordinated												

### 3. An example of a complete traceability system

All observed ocean variables should, in general, be explicitly traceable to the SI. In the case of an instrument that measures mass, the traceability system chart should be available to facilitate traceability to the SI. As an example, Figure 1 shows the traceability system chart for mass measurements at a research institution that would be used to authenticate the certification of a reference material for nutrients in seawater. All weights and the balance to be used (Figure 1) as well as all the equipment for measuring room temperature, air pressure, and humidity must have a valid calibration certificate. This requirement implies that it is possible to calculate the whole uncertainty of the mass measurement. Table 2 shows the results of the calculation of the whole uncertainty associated with preparation of a certain solution. The results show that the uncertainties due to the weight and balance are small. However, the uncertainties due to the water temperature at the time of calibrating the volumetric flask and the room temperature at the time of making the buoyancy correction are relatively large. Thus, by estimating the magnitude of the uncertainty for each individual factor, it is possible to estimate the magnitude of the uncertainty of ocean variables, and it becomes possible to formulate a procedure to reduce the uncertainties of those variables.

Figure 1. An example of a complete traceability system chart for mass

International System of Units (SI)		National Institute of Standards and Technology (NIST)		Sato Co. Ltd		National Institute of Advanced Industrial Science and Technology (AIST)		
						IA Japan (ILAC)		
Weight	Ms-AMH-38-VAC	Digital thermometer	mirror cooling dew-point meter	Weight	1mg~200g	Model: TT12 precision temperature indicator	Weight	specified secondary standard instrument
	2188	7563-02	DewStar S-1S-8	Weight	100g~5kg	model number or capacity	Identification symbol C	
	1694248	52W-J0008	002E12	Weight	100g~5kg	Implement number	1mg~20kg	
				Weight	22229341	Certificate number		
		Digital multi-thermometer	Precision humidity generator		140081	Working standard platinum resistance thermometer		
		TR2114	SRH-IR135 (ADR)			WS-IPRT		
		73430473	0105-301/0901-404/0901-406			Implement number		
						Certificate number		
barometer	RPMA8A10 OKs	Data Logger		Electronic balance	CPA6202S	Thermometer	Weight	100g
	1453	SK-L200TH αD + SK-LTH α-2		Electronic balance	LP620S	PT sensor MODS002-01-PT-01 K-320	Weight	200g
	9154F	main body: 004970 sensor: 18294		Electronic balance	90608659	ST 2820057 MT12928010	Weight	11425455
		M18751			T-1402013	14-10522-000	Weight	141740
					T-1402009	Thermometer	Weight	141741
						PT sensor MODS002-01-PT-01 K-320	Weight	500g
						ST 2017002 MT12928014	Weight	1000g
						14-10523-000	Weight	11525550
							Weight	141743
							Weight	200mg ~
							Weight	30307406
							Weight	141739

Table 2. An example of an uncertainty budget for mass

		equipment	Type of uncertainty	Value
Weight g	Mass	100	Type A	100 g
	Mass	200	Type A	200 g
Non-linearity of the balance	Mass	BP210D_210g	Type B	200 g
Precision of the balance	Mass	200	Type A (10 times repeated)	199.99959 g
Buoyancy correction	room temperature		Type B (rectangle)	20 °C
	Atmospheric pressure		Type B (rectangle)	1013.25 hPa
	Humidity		Type B (rectangle)	50 %
Water temperature	Water temperature		Type B (rectangle)	20 °C

		Uncertainty	Divisor (probability distribution)		Sensitivity coefficient	standard uncertainty relative value(%)
Weight g	Mass	0.000045 g	2	2	1	0.0000225
	Mass	0.0001 g	2	2	1	0.000025
Non-linearity of the balance	Mass	0.0002	$\sqrt{3}$	1.732050808	1	0.0000577
Precision of the balance	Mass	5.67646E-05 g	1	1	1	0.0000284
Buoyancy correction	room temperature	0.5 °C	$\sqrt{3}$	1.732050808	3.87E-06 ml·ml <sup>-1</sup> ·K <sup>-1</sup>	0.00000559
	Atmospheric pressure	0.081 hPa	$\sqrt{3}$	1.732050808	1.04E-06 ml·ml <sup>-1</sup> ·hPa <sup>-1</sup>	4.82E-09
	Humidity	3 %	$\sqrt{3}$	1.732050808	9.26E-08 ml·ml <sup>-1</sup> ·% <sup>-1</sup>	0.000000321
Water temperature	Water temperature	0.04 °C	2	2	0.000118 ml·ml <sup>-1</sup> ·K <sup>-1</sup>	0.0000118

## References

BIPM (Bureau international des poids et mesures). 2006. The International system of units (SI), 8th edition.

JCGM (Joint Committee for Guides in Metrology) 100. 2008. Evaluation of measurement data – Guide to the expression of uncertainty in measurement, 1st edition.

## Acronym

UTC	Universal Time, Coordinated
WGS-84	World Geodetic System, WGS1984
ITS-90	The International Temperature Scale 1990
TEOS-10	Thermodynamic Equation Of Seawater - 2010 (TEOS-10)

## **Essential Ocean Variables (EOVs) of GOOS**

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The Global Ocean Observing System (GOOS), being sponsored by UNESCO/IOC, WMO, UNEP, and ICSU, is a programme to coordinate observations around the global ocean for climate, ocean health, and real-time services. In accordance with the "Framework for Ocean Observing", GOOS defines Essential Ocean Variables (EOVs) of physics, biogeochemistry, biology and ecosystems, the methods of many of these measurements being guided in this Guideline.

The latest information and the list of GOOS EOVs are available with their specification sheets at the following web site.

[http://goosocean.org/index.php?option=com\\_content&view=article&id=14&Itemid=114](http://goosocean.org/index.php?option=com_content&view=article&id=14&Itemid=114)

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## **Data Publication and International Exchange**

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### **1. Introduction**

Oceanographic data show observational values, which are attached to dates, times, and positions. Recent developments and improvements to observational techniques and instruments have been associated with data accuracy and quality control, so that the importance of the related information increases the exchange, sharing and integration of oceanographic data. This chapter therefore describes a mechanism of international exchange and sharing in the ocean sciences and other science fields for oceanographic data observed by research vessels and other ships. In this chapter, ‘data’ includes observed data and related information; the latter indicates ‘metadata’ – data of data – if needed. Specimens such as marine organisms or sea bottom materials are also included in data but only numerical data are treated in this chapter.

### **2. Importance of Data Publication**

There is no doubt about the importance of data for all scientific research. Working Group on Scientific Data Network Facilitation (2004) expressed that geophysics is a subject which studies the accumulation of unexpected and not replicable phenomena on the earth and vicinities since 4.6 billion years ago, so as many continuous observations as possible are required, all records should be archived and collected in principle and left as the common heritage of mankind. What is true for geophysics is true for the marine sciences as well. UNESCO’s IOC established the IODE in 1961 because common understanding in the world through oceanographic data exchange and sharing among the member states is important.

### **3. Outline of Data Publication and International Exchange**

The result of various observations by research vessels, including training, survey, chartered and voluntary ships, is published by a chief scientist or someone who belongs to an organization (hereafter, data originator) as cruise reports through preliminary and cruise summary reports. Real time data is reported before publication of their reports. In the past, observed data were recorded on paper as an expression in numerical form of visual observations, while more recently, massive amounts of digital data are being stored directly on mass storage devices, such as optical-magnetic disks or flash memory, for developing instruments and improving accuracy. Moreover, observed data is directly distributed by data originators with the spread of the network system, i.e., full-time connection and unlimited access to the Internet, and cruise reports are also outputted in digital document formats such as PDF.

The NODC, established under a program of the IODE of the IOC/UNESCO, has the role of

providing national oceanographic data and information in a usable format to a wider user community and for international exchange. The JODC was established in the Hydrographic Department of the Japan Maritime Safety Agency (presently, the Hydrographic and Oceanographic Department of the Japan Coast Guard) in 1965 in accordance with a resolution adopted by the IOC/UNESCO in 1961 as well as reports from the Council for Marine Scientific Technology in 1963 and 1964. The JODC provides various oceanographic data through its JODC Data On-line Service System (J-DOSS) and also submits its data to the NCEI (formerly, National Oceanographic Data Center of the United States) which houses the World Data Center for Oceanography under the ICSU WDS according to an IODE recommendation of the WOD project. Japanese data occupy 11% of total observations in the WOD, placing Japan second of 96 countries for contributions. Long time archiving is also one of the roles of the NODC in order to prevent data loss by principle investigator transfer or retirement, or the discontinuance of data distribution or archiving in his/her organization due to various reasons. Particularly in Japan, it is necessary to pay attention to the potential of lost data and information due to a natural disaster such as an earthquake or tsunami. It is therefore strongly recommended to submit the published data and metadata to the JODC.

On the other hand, several data management offices of international projects have continued to collect specific data and information after the end of the projects. The CDIAC Ocean Carbon Data Management Project was organized in 1993 and collects discrete and underway measurements of ocean carbon dioxide from various platforms, e.g., research vessels, commercial and volunteer ships, and buoys. The CCHDO delivers the highest quality global CTD and hydrographic data, including ocean carbon dioxide and its related parameters, which have been acquired during WOCE, CLIVAR and other international oceanographic research programs. The OBIS was created as the information component of the Census of Marine Life and serves to document the ocean's diversity, distribution and abundance of life, and is one of the projects of the IODE at the present. These data management offices target specific parameters and therefore should be called special data centers, against the NODC which collects all oceanographic data. The IODE cooperates with the special data centers for data collection and distribution, and recommends registering as an ADU, a new component of the IODE similar to the NODC. The Japan Ocean Biogeographic Information System Center of JAMSTEC is a Japan regional OBIS node that was registered as an ADU of the IODE in January 2015. It is also recommended that data originators submit their own data to the special data centers at the same time. As for the ICSU World Data System, the IODE is a network member, and some organizations in IOC member states are regular members: the World Data Service for Oceanography in the United States, the World Data Center for Oceanography in NODCs in China and the Russian Federation, the Flanders Marine Institute, the Data Centre in Belgium, and Ocean Networks Canada.

#### **4. National Oceanographic Programs and Cruise Summary Reports**

As complementary information until the Cruise Report is published, it is recommended that NOPs and CSRs be submitted.

A NOP provides information on when, where and what kind of oceanographic cruises are planned

at domestic research organizations, in order to enhance the effective use of oceanographic research opportunities and oceanographic data. NOPs are registered and searched on the Marine Information Clearing House Network operated by the Hydrographic Department of the Japan Coast Guard. Please see <http://www.mich.go.jp/> for more information.

A CSR is intended to fill the gap between the first announcement of an oceanographic program and the eventual catalogue of data available to users. As timely inventories of data to ensure prompt exchange, the CSR contains such information as the ocean area where the investigation was carried out, a track chart of the cruise, the name of the person who holds the data, and the name and volume of the data written in the IOC's CSR form. Please see <http://www.jodc.go.jp/info/csr.html> for more information.

## **5. Standardization for Oceanographic Data Exchange**

The JODC collects oceanographic data from domestic research institutes and submits them to the NCEI every year. Those data are stored in the WOD. In addition, non-Japanese data are extracted from the WOD and registered to J-DOSS all together. Recently, oceanographic data and information exchange is being carried out for not only oceanography but also marine meteorology. The IOC established JCOMM in cooperation with the WMO in 1999, and JCOMM coordinates, and develops and recommends standards and procedures for, a fully integrated marine observing, data management and services system. In order to standardize data processing, formats and codes are required for promoting data and information exchange between researchers in oceanography and other sciences. The Task Team for Ocean Data Standards established under the Joint JCOMM/IODE Expert Team on Data Management Practices reviews the proposals for standardization of oceanographic data exchange practices, and accepted proposals are recommended and published in the IOC Manuals and Guides No. 54 series. At present, three recommendations have been published for country codes, representation of date and time, and a quality flag scheme. It would be useful if data originators would consider the above recommendations when publishing data.

### **5-1. Country Codes**

Adoption of ISO 3166-1 and 3166-3 is recommended for identifying countries (IOC, 2010). Japan is defined as JP and JPN in ISO 3166-1 alpha-2 and alpha-3, respectively. The former is known as a top level domain at the highest level in the hierarchical Domain Name System of the Internet, and the latter is often seen in broadcasts of games or events such as the Olympics and international soccer matches.

### **5-2. Representation of Date and Time**

Adoption of ISO 8601:2004 is recommended for representation of date and time (IOC, 2011). The basic date notation is YYYYMMDD, where YYYY is the year, MM is the month of the year between 01 (January) and 12 (December), and DD is the day of the month between 01 and 31 (e.g., 20150624 or 2015-06-24), and the time notation is hhmmss, where hh is the number of complete hours between

00 and 24 (note that 24 is only allowed to indicate the end of the calendar day), mm is the number of complete minutes between 00 to 59, and ss is the number of complete seconds between 00 and 60 (note that 60 is used to indicate a positive leap second; e.g., 141531 or 14:15:31). The recommended representation is therefore familiar to Japanese. For more examples, see IOC (2011). The time zone must be clearly specified. In particular, biological observation during a long voyage or transoceanic cruise often uses the ship's time, not JST or GMT, in order to distinguish between day and night, so it is difficult to standardize the date and time for data exchange.

### **5-3. Quality Flag Scheme**

Although development and improvement of observational techniques and instruments earn wider observation coverage and accurate data and information, it is difficult to avoid the inclusion of error data derived from the malfunction of instruments, and missing or failed measurements. Quality control by data originators is highly reliable and their results are extremely helpful for data exchange. After the error data are eliminated or flagged as 'bad data' by data originators in advance, doubtful data are detected through quality control by data centers or users and are flagged as 'questionable data'. Some of the questionable data will be changed to 'unknown data' if it is difficult to make a decision because there is little historical data and a lack of metadata. It is not to be denied that for observational records of unexpected and not replicable phenomenon, all data should be archived and published with quality flags. It is noted that missing value which has no missing flag, and vice versa, may be misunderstood for data processing.

There are many observed data exchange formats with quality flags, e.g., the WHP-exchange format (Swift and Diggs, 2008) is the de facto standard format for CTDs and bottle sampling data with nine level quality flags added by data originators. On the other hand, data center formats, such as the WOD and FETI of the J-DOSS, determine their own quality flags to header information and observed data in each depth, respectively, because historical data usually have no information for data quality. The problem here is that there is a different definition of the flags in each format, e.g., flag '0' means good in one format but flag '2' means good in another format. For that reason, the recommended quality flag scheme (IOC, 2013) defines five flags: 1 as good, 2 as unknown, 3 as questionable, 4 as bad, and 9 as missing at the primary level, and other results for quality control procedures or data processing in detail are described at the second or lower level. The other purpose for the above simplified quality flag scheme is to promote data exchange between researchers in oceanography and other sciences. There is no format which adopts this quality flag scheme at present, but the Ocean Data View (ODV; Schlitzer, 2015) defines quality flags similar to the primary level flags of the above scheme, and the ODV can import 14 different existing formats with its own quality flags.

### **5-4. Ship Codes and Cruise Numbers**

There is no proposal for standardization of ship codes and cruise numbers at present, but a de facto standard that has been adopted by several data centers exists. It facilitates the management of observed data and metadata from each cruise, so ship codes and cruise numbers are required to identify the cruises. A cruise number assigned by a data originator is a primary, compatible identification of a

cruise by a ship with a timeline. For example, a cruise from September 23 to October 3 by the Ryofu Maru of the JMA is assigned as RF14-08, where RF is the ship code from the JMA, 14 means the year 2014, and 08 means the 8th cruise in 2014.

Former JODC ship codes were assigned as two alphanumeric characters, and the JODC now adopts the international radio call sign for ship identification because it is possible that the old ship codes will expire in the near future. On the other hand, the NCEI assigns three kinds of ship codes: the NODC code, which consists of two figures for the country code and two alphanumeric characters for ship identification, the WOD code with three or more figures, and a call sign (see <http://www.nodc.noaa.gov/General/NODC-Archive/platformlist.txt>). The NODC codes also synchronize with ICES ship codes, which are assigned by the ICES data center. For example, the Ryofu Maru of the JMA has been assigned 49UP, 49RY and 49UX from newest to oldest. Note that the country code in the NODC code is an original one, different from ISO 8601:2004 described in the previous section.

As for cruise identification, the CCHDO and CDIAC use an EXPOCODE that consists of a NODC ship code and cruise departure date (Swift and Diggs, 2008). For example, the EXPOCODE is automatically determined as 49UP20140923 for the above-mentioned cruise of the Ryofu Maru of the JMA. Nevertheless the data originator's cruise number should be recorded on database as metadata because of compatibility and continuity.

## 6. Data Policy

The intellectual property rights to the observed data basically belong to the organization that provides the facilities and equipment, and the data originator usually has a priority of data use rights in order to research, study and write documents. Unfortunately, its term is frequently unclear in regulation and several further parameters need a long time to fix values by calibration or quality control, so data publishing is often behind schedule. Working Group on Scientific Data Network Facilitation (2004) pointed out that some data originators are not so conscientious to publish and share observed data using the public facilities and equipment, so they often monopolize the data.

The NODC basically follows the IOC data exchange policy (IOC, 2003), while the JODC requests that users express that they have used its materials and submit one copy of the document and product to the JODC, and no reproduction of data can be provided to a third party without permission. As described in a previous section, the JODC submits their data to the NCEI in order to have it merged with that of the WOD, according to IODE recommendations of the WOD and GODAR, and the JODC shares WOD data with the J-DOSS.

## 7. Data Citation

Cruise reports are published with oceanographic data, and the scientific findings are also published in scientific journals through the peer review process. Both are used as references in other reports and articles; however, it is difficult to refer to oceanographic data because there is no citation information

in the data or databases such as the WOD and the J-DOSS, so the referenced data is mentioned in thanks at the end of an article or in the acknowledgements, which means the referenced data is not countable in the number of citations. The IODE therefore discussed this problem in cooperation with the SCOR, the BODC, and the MBLWHOI (Leadbetter et al., 2013).

On the other hand, the ocean carbon synthesis community has established projects, such as SOCAT for ocean surface and GLODAPv2 for ocean interior, and results of data synthesis from both projects were submitted to the ESSD, a data publication journal. Furthermore, the CDIAC archives other products from the projects, including technical documents and all cruise data, attached citations and DOIs. An increase in the number of references for oceanographic data will be rated highly by data originators and managers. The ESSD and DOIs are also important for digitizing historical data, and identification and citation of massive databases. The data citation issue is also being discussed in one of the working groups of the RDA in cooperation with researchers of other scientific fields.

## 8. Concluding Remarks

In this paper, we have explained that observed data and metadata are submitted to the NODC of the IODE so that the data is available for international exchange and sharing among researchers in the ocean sciences and other science fields. In addition, it is also important to recover or digitize historical data that were recorded to analog media such as paper using recent technologies, but it is not so easy to guarantee resources for such a project. The IODE created the GODAR project in order to search for and rescue historical data in the IOC member states, and the JODC conducted GODAR-WESTPAC, one of the regional GODAR projects. The global and regional GODAR projects have been terminated but GODAR has been recommended by the IODE again and their results have been summarized in the WOD.

## Acknowledgements

We would like to thank staff of the JODC for useful comments and suggestions.

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### Abbreviations and Acronyms

ADU	Associate Data Unit
BODC	British Oceanographic Data Centre
CCHDO	CLIVAR and Carbon Hydrographic Data Office
CDIAC	Carbon Dioxide Information Analysis Center
CLIVAR	Climate and Ocean: Variability, Predictability, and Change
CSR	Cruise Summary Report
CSV	Comma-Separated Values
CTD	Conductivity, Temperature and Depth profiler
DOI	Digital Object Identifier
ESSD	Earth System Science Data
FETI	Format of Exchange and Translation for Integration
GLODAPv2	Global Ocean Data Analysis Project Version 2
GMT	Greenwich Mean Time
GODAR	Global Oceanographic Data Archeology and Rescue
ICES	International Council for Exploration of the Sea
ICSU	International Council for Science
IOC	Intergovernmental Oceanographic Commission of UNESCO
IODE	International Oceanographic Data and Information Exchange of IOC of UNESCO
JAMSTEC	Japan Agency for Marine-Earth Science and Technology
JCOMM	Joint WMO-IOC Technical Commission for Oceanography and Marine Meteorology
JMA	Japan Meteorological Agency
JODC	Japan Oceanographic Data Center
JST	Japan Standard Time
MBLWHOI	Marine Biological Laboratory, Woods Hole Oceanographic Institution

NCEI	National Center for Environmental Information (USA)
NODC	National Oceanographic Data Center
NOP	National Oceanographic Program
OBIS	Ocean Biogeographic Information System
PDF	Portable Document Format
RDA	Research Data Alliance
SCOR	Scientific Committee on Oceanic Research
SOCAT	Surface Ocean CO2 Atlas
UNESCO	United Nations Educational, Scientific and Cultural Organization
WDS	World Data System
WHP	WOCE Hydrographic Programme
WMO	World Meteorological Organization
WOCE	World Ocean Circulation Experiment
WOD	World Ocean Database

## **Calculation of the Thermophysical Properties of Seawater (2010)**

A manual of “Calculation of the Thermophysical Properties of Seawater (2010)”, Manuals and Guides 56, IOC, can be obtained from a GO-SHIP web site as below.

[http://www.go-ship.org/Manual/TEOS-10\\_Manual\\_06Jul10.pdf](http://www.go-ship.org/Manual/TEOS-10_Manual_06Jul10.pdf)

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## Water temperature

○Toshiya NAKANO (Japan Meteorological Agency)

Water temperature (temperature) is the most fundamental physical properties of sea water with pressure and salinity in the International Thermodynamic Equation Of Seawater–2010 (TEOS–10). The vertical and horizontal distributions of temperature are associated with the thermal and dynamic structure in the ocean. Temperature is a key variable that supports oceanic condition, ocean and weather prediction, and air-sea interaction leading to understanding and forecasting short- and long-term climate variability.

### 1. Definition and Unit

In 1990 the International Practical Temperature Scale 1968 (IPTS–68) was replaced by the International Temperature Scale of 1990 (ITS–90) defined by 17 fixed temperature points. The triple point of water (273.16K or 0.01°C) remains unchanged on ITS-90, however, the definition of boiling point of water at the atmospheric pressure falls to 99.974°C (Rusby, 1991). Since many algorithms for calculating physical properties of seawater had been based largely on IPTS-68, it was necessary to discriminate between IPTS-68 and ITS-90. In the field of oceanography it is common to distinguish using subscripts as  $t_{90}$  with the Celsius scale (°C). The relation between ITS-90 ( $t_{90}$ ) and IPTS-68( $t_{68}$ ) can be adequately represented by the expression

$$t_{90} = 0.99976 \cdot t_{68} \quad (1)$$

This linear transformation is accurate within  $0.5 \times 10^{-3}$  K throughout the oceanographic temperature range  $-2^{\circ}\text{C}$  to  $40^{\circ}\text{C}$  (Saunders, 1990). Temperature reported in the literature is used ITS–90 and be labelled  $t_{90}$ . In order to use the International Equation of State of Seawater 1980 (EOS–80) with  $t_{90}$  data,  $t_{68}$  may be calculated using

$$t_{68} = 1.00024 \cdot t_{90} \quad (2)$$

### 2. Calibration of thermometers

To ensure traceability to the national standards, all thermometers need to be calibrated by a standard device such as a Standard Platinum Resistance Thermometer (SPRT). The standard device also needs to be calibrated by a higher standard device or by ITS-90 fixed point cells. These higher standard device or fixed point cells need to be traceable to the national measurement standards maintained by the National Metrology Institute (e.g. National Metrology Institute of Japan [NMIJ] and National Institute of Standards and Technology [NIST]). The oceanographic temperature range is realized by use of a SPRT calibrated at the ITS-90 defining points of the water triple point, 0.01°C and the gallium melting point, 29.7646°C. Calibrations of thermometers are made regularly with the standard thermometer fully submerged in a water bath. The temperature correction that must be applied to the thermometer is

estimated for agreement with the reading of the standard thermometer.

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## **Salinity**

○Takeshi Kawano (Japan Agency for Marine-earth Science and Technology)

A manual of “Method for Salinity (Conductivity Ratio) Measurement” can be obtained from a GO-SHIP web site as below.

[http://www.go-ship.org/Manual/Kawano\\_Salinity.pdf](http://www.go-ship.org/Manual/Kawano_Salinity.pdf)

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## Density of Seawater

○Hiroshi UCHIDA (Research and Development Center for Global Change / Japan Agency for Marine-Earth Science and Technology)

The density of seawater is defined as its weight per unit volume; in accord with the International System of Units (SI), it is expressed in units of kilograms per cubic meter ( $\text{kg/m}^3$ ). The density of seawater may be estimated as a function of pressure, temperature, and Absolute Salinity by using the International Thermodynamic Equation of Seawater – 2010 (TEOS-10) or by direct measurement using a densitometer. Seawater density data used in the TEOS-10 standards were obtained from density measurements (Millero, 2010).

The density of seawater can be measured with a hydrostatic weighing apparatus or a vibrating-tube densitometer. The former measures the buoyancy of a sinker suspended in a seawater sample. The volume and mass of the sinker must be calibrated in advance. The latter measures the natural period of a vibrating glass tube filled with a seawater sample. The natural period is proportional to the density of the sample. A hydrostatic weighing apparatus can directly measure seawater density traceable to the SI without a reference solution. However, the measuring time for a sample is quite long (longer than several hours), and a hydrostatic weighing apparatus cannot be used on a moving ship. Use of a single-crystal silicon sinker has made it possible to reduce the measurement uncertainty to about  $0.001 \text{ kg/m}^3$  with traceability to national density standards based on silicon spheres. However, the tendency of a silicon sphere to dissolve in seawater is a problem that needs to be solved. In contrast, for a vibrating tube densitometer, which can be used on a moving ship, the measuring time for a sample is short (several minutes). Although a reference solution is required for calibration, the resolution of the densitometer is high ( $0.001 \text{ kg/m}^3$ ). Because the uncertainties of the commercially available, SI-traceable density reference solutions are large ( $0.01 \text{ kg/m}^3$  for pure water), a substitution method is normally used for seawater density measurements. In the substitution method, the density difference between a seawater sample and pure water is measured, and the density of pure water, calculated from the TEOS-10 standards, is added to the difference.

An international guideline for seawater density measurements based on vibrating tube densitometers is now in preparation [Wolf, H., S. Weinreben, H. Uchida and R. Pawlowicz: Best practice guide for the measurement of seawater density. The Joint Committee on the Properties of Seawater (IAPSO/SCOR/IAPWS)]. However, seawater densities measured by the substitution method relative to pure water tend to be lower (by as much as  $0.01 \text{ kg/m}^3$ ) than densities calculated from TEOS-10 standards (e.g., Uchida et al., 2011). Although the systematic differences are hypothesized to be the result of systematic errors in the TEOS-10 standards or nonlinearities of vibrating-tube densitometers, a consensus has not yet been reached on the cause of the systematic differences. To solve this problem, a precise determination of the density determined by hydrostatic weighing is planned for IAPSO Standard Seawater, the composition of which is considered to be close to the Reference Composition defined in the TEOS-10 standards.

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## **DETERMINATION OF DISSOLVED NUTRIENTS (N, P, SI) IN SEAWATER WITH HIGH PRECISION AND INTER-COMPARABILITY USING GAS-SEGMENTED CONTINUOUS FLOW ANALYSERS**

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The Global Ocean Ship-based Hydrographic Investigations Program (GO-SHIP) brings together scientists with interests in physical oceanography, the carbon cycle, marine biogeochemistry and ecosystems, and other users and collectors of ocean interior data to develop a sustained global network of hydrographic sections as part of the Global Ocean Climate Observing System. A series of manuals and guidelines are being produced by GO-SHIP which update those developed by the World Ocean Circulation Experiment (WOCE) in the early 1990s. One of a GO-SHIP manual, “DETERMINATION OF DISSOLVED NUTRIENTS (N, P, SI) IN SEAWATER WITH HIGH PRECISION AND INTER-COMPARABILITY USING GAS-SEGMENTED CONTINUOUS FLOW ANALYSERS” was published by Hydes et al. in 2010 (Hydes et al., 2010) with a set of nutrient standard operating procedures (NSOPs) that provide detailed information on key procedures that are necessary if best quality data are to be achieved consistently.

In this guideline, revised version of NSOP9 Rev1.0 as of 23 October 2016 is presented together with an original manual at [http://www.go-ship.org/Manual/Hydes\\_et\\_al\\_Nutrients.pdf](http://www.go-ship.org/Manual/Hydes_et_al_Nutrients.pdf) by Hydes et al. 2010.

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## **NSOP 9 Rev1.0 as of 23 October 2016 by Michio AOYAMA**

### **EXAMPLE SOP FOR SHIPBOARD OPERATION OF A CFA SYSTEM**

#### **1. SCOPE AND FIELD OF APPLICATION**

This SOP describes the standardized set up of one laboratory's system for the shipboard determination of nutrients (nitrate + nitrite, nitrite, phosphate and silicate).

An SOP of this type should be part of the meta-data reported at the end of each cruise. Each laboratory's SOP should be updated as necessary before a cruise and the procedures outlined in the SOP followed during the cruise.

#### **2. PRINCIPLE**

The purpose of this SOP is to provide a record of how a CFA system was operated during a cruise. It should ensure that work on the cruise is carried out in a consistent and reproducible manner. It should also ensure that key procedures that aid in maintaining the relative accuracy of data such as the calibration of volumetric ware and pipettes are carried and documented in traceable way prior to and post cruise.

#### **3. EXAMPLE STANDARD OPERATING PROCEDURES**

##### *3.1 Methods*

The analytical methods of the nutrients during this cruise are similar with previous cruises (Aoyama et al., 2005).

#### **4. INSTRUMENTS AND METHODS**

##### *4.1 Analytical detail using QuAAtro 2-HR systems (BL-Tech)*

Nitrate + nitrite and nitrite are analyzed according to the modification method of Grasshoff (1970). The sample nitrate is reduced to nitrite in a cadmium tube inside of which is coated with metallic copper. The sample stream with its equivalent nitrite is treated with an acidic, sulfanilamide reagent and the nitrite forms nitrous acid which reacts with the sulfanilamide to produce a diazonium ion. N-1-Naphthylethylene-diamine added to the sample stream then couples with the diazonium ion to produce a red, azo dye. With reduction of the nitrate to nitrite, both nitrate and nitrite react and are measured; without reduction, only nitrite reacts. Thus, for the nitrite analysis, no reduction is performed and the alkaline buffer is not necessary. Nitrate is computed by difference.

The silicate method is analogous to that described for phosphate. The method used is essentially that of Grasshoff et al. (1983), wherein silicomolybdic acid is first formed from the silicate in the sample and added molybdic acid; then the silicomolybdic acid is reduced to silicomolybdous acid, or "molybdenum blue," using ascorbic acid as the reductant. The analytical methods of the nutrients, nitrate, nitrite, silicate and phosphate, during this cruise are same as the methods used in (Kawano et al. 2009).

The phosphate analysis is a modification of the procedure of Murphy and Riley (1962). Molybdic acid is added to the seawater sample to form phosphomolybdic acid which is in turn reduced to phosphomolybdous acid using L-ascorbic acid as the reductant.

The ammonia in seawater is mixed with an alkaline containing EDTA, ammonia as gas state is formed from seawater. The ammonia (gas) is absorbed in sulfuric acid by way of 0.5  $\mu\text{m}$  pore size membrane filter (ADVANTEC PTFE) at the dialyzer attached to analytical system. The ammonia absorbed in sulfuric acid is determined by coupling with phenol and hypochlorite to form indophenols blue. Wavelength using ammonia analysis is 630 nm, which is absorbance of indophenols blue.

The flow diagrams and reagents for each parameter are shown in Figures 1 to 5.

#### **4.2 Nitrate + Nitrite Reagents**

Imidazole (buffer), 0.06 M (0.4 % w/v)

Dissolve 4 g imidazole,  $\text{C}_3\text{H}_4\text{N}_2$ , in ca. 1000 ml DIW; add 2 ml concentrated HCl. After mixing, 1 ml TritonTMX-100 (50 % solution in ethanol) is added.

Sulfanilamide, 0.06 M (1 % w/v) in 1.2M HCl

Dissolve 10 g sulfanilamide,  $4\text{-NH}_2\text{C}_6\text{H}_4\text{SO}_3\text{H}$ , in 900 ml of DIW, add 100 ml concentrated HCl. After mixing, 2 ml TritonTMX-100 (50 % solution in ethanol) is added.

N-1-Naphthylethylene-diamine dihydrochloride, 0.004 M (0.1 % w/v)

Dissolve 1 g NED,  $\text{C}_{10}\text{H}_7\text{NHCH}_2\text{CH}_2\text{NH}_2 \cdot 2\text{HCl}$ , in 1000 ml of DIW and add 10 ml concentrated HCl. After mixing, 1 ml TritonTMX-100 (50 % solution in ethanol) is added. This reagent is stored in a dark bottle.

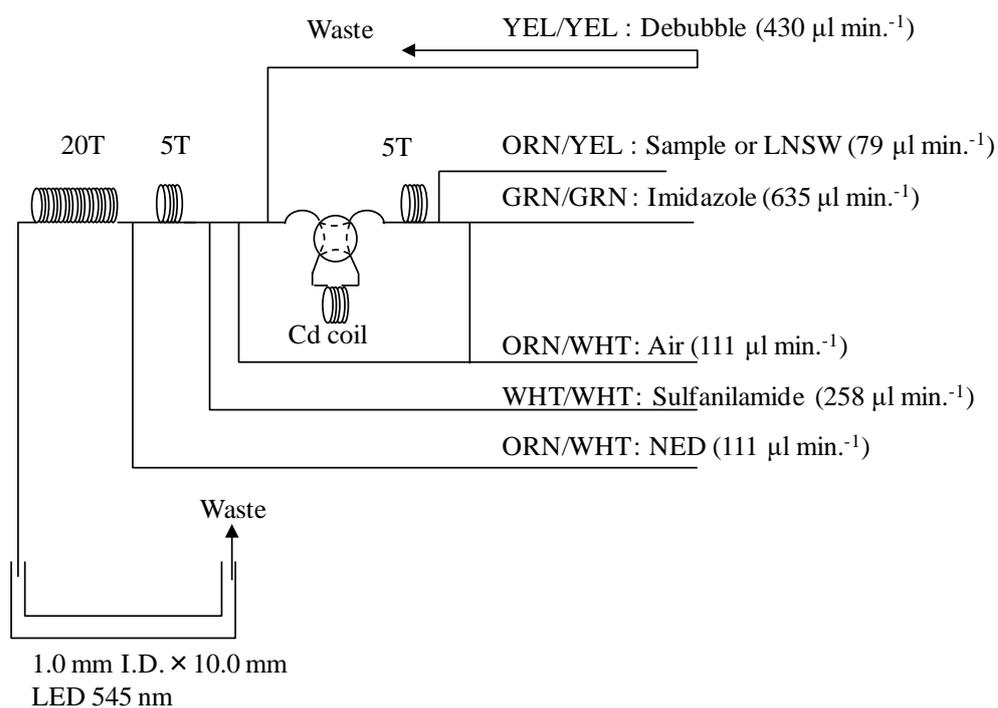


Figure 1  $\text{NO}_3+\text{NO}_2$  (1ch.) Flow diagram.

#### 4.3 Nitrite Reagents

Sulfanilamide, 0.06 M (1 % w/v) in 1.2 M HCl

Dissolve 10 g sulfanilamide, 4- $\text{NH}_2\text{C}_6\text{H}_4\text{SO}_3\text{H}$ , in 900 ml of DIW, add 100 ml concentrated HCl. After mixing, 2 ml Triton<sup>TM</sup>X-100 (50 % solution in ethanol) is added.

N-1-Napthylethylene-diamine dihydrochloride, 0.004 M (0.1 % w/v)

Dissolve 1 g NED,  $\text{C}_{10}\text{H}_7\text{NHCH}_2\text{CH}_2\text{NH}_2 \cdot 2\text{HCl}$ , in 1000 ml of DIW and add 10 ml concentrated HCl. After mixing, 1 ml Triton<sup>TM</sup>X-100 (50 % solution in ethanol) is added. This reagent is stored in a dark bottle.

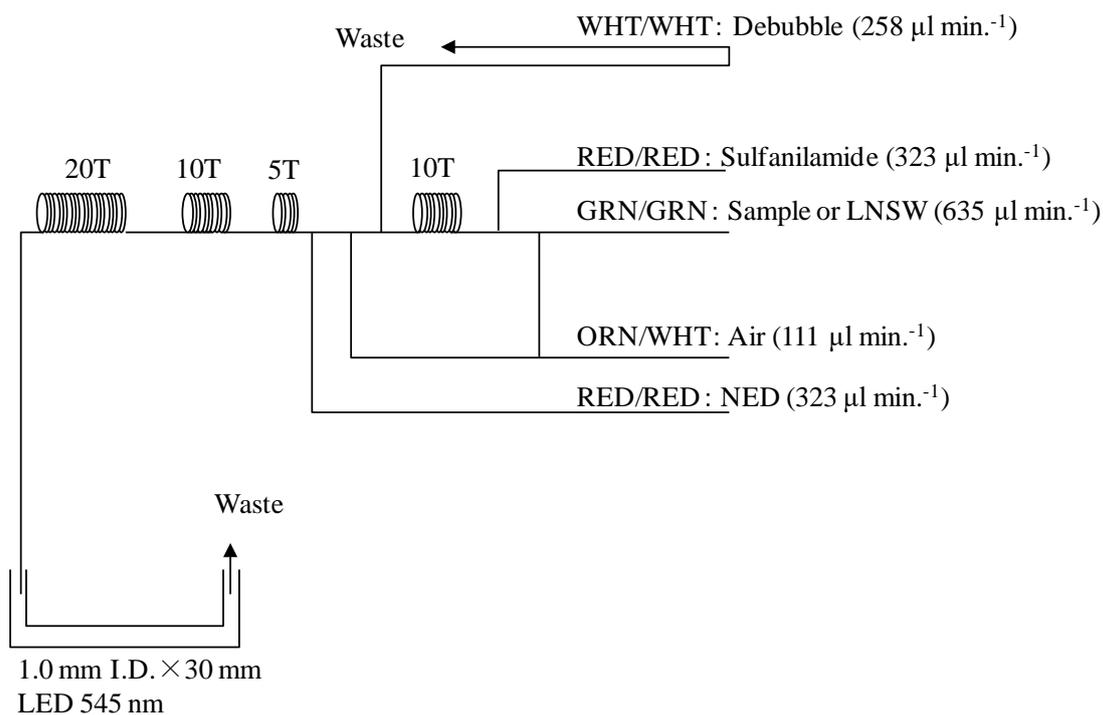


Figure 2  $\text{NO}_2$  (2ch.) Flow diagram.

#### 4.4 Silicate Reagents

Molybdic acid, 0.06 M (2 % w/v)

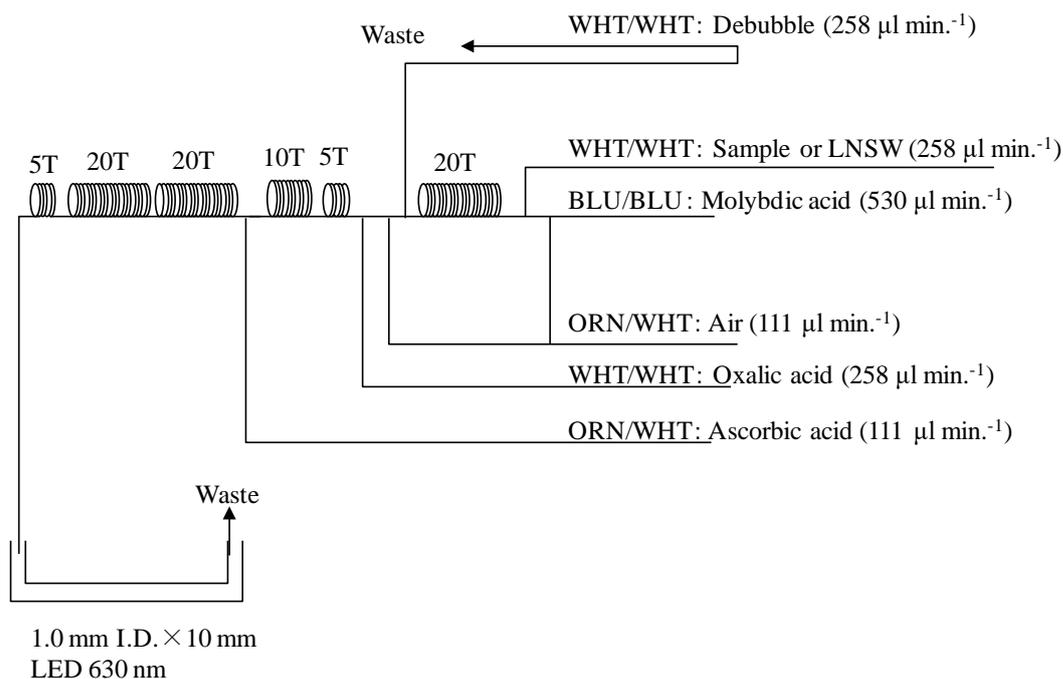
Dissolve 15 g disodium molybdate(VI) dihydrate,  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , in 980 ml DIW, add 8 ml concentrated  $\text{H}_2\text{SO}_4$ . After mixing, 20 ml sodium dodecyl sulphate (15 % solution in water) is added.

Oxalic acid, 0.6 M (5 % w/v)

Dissolve 50 g oxalic acid anhydrous,  $\text{HOOC:COOH}$ , in 950 ml of DIW.

Ascorbic acid, 0.01M (3 % w/v)

Dissolve 2.5 g L (+)-ascorbic acid,  $\text{C}_6\text{H}_8\text{O}_6$ , in 100 ml of DIW. This reagent was freshly prepared at every day.

Figure 3 SiO<sub>2</sub> (3ch.) Flow diagram.

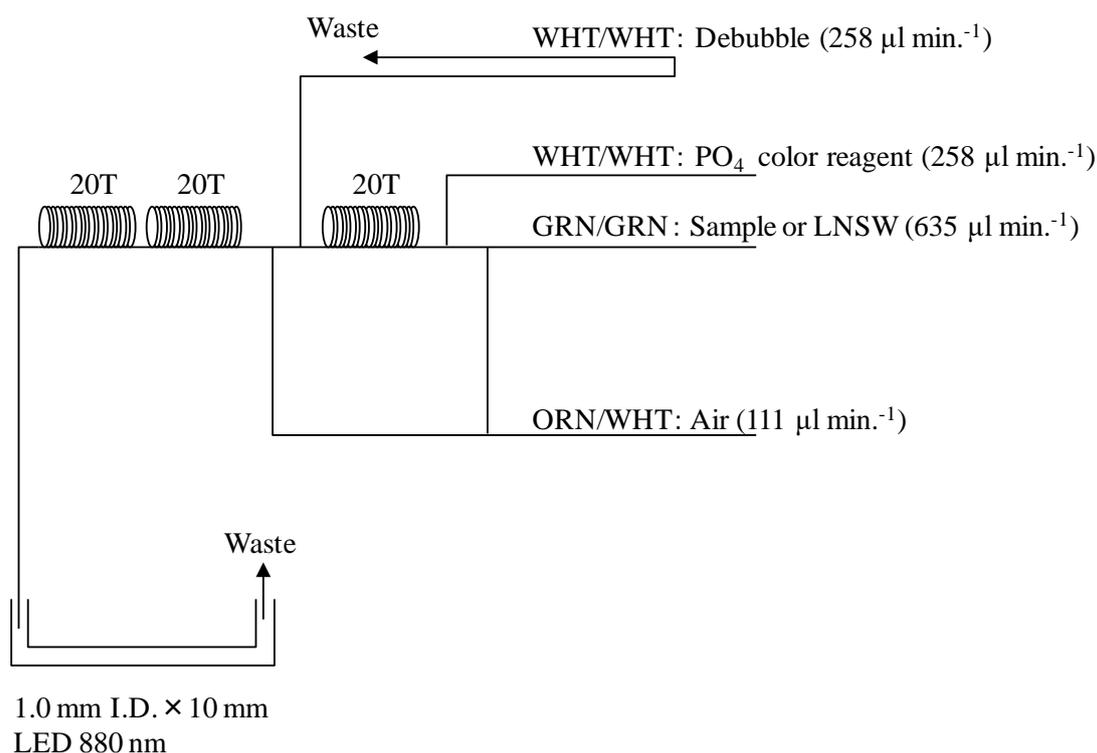
#### 4.5 Phosphate Reagents

Stock molybdate solution, 0.03M (0.8 % w/v)

Dissolve 8 g disodium molybdate(VI) dihydrate,  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , and 0.17 g antimony potassium tartrate,  $\text{C}_8\text{H}_4\text{K}_2\text{O}_{12}\text{Sb}_2 \cdot 3\text{H}_2\text{O}$ , in 950 ml of DIW and add 50 ml concentrated  $\text{H}_2\text{SO}_4$ .

##### Mixed Reagent

Dissolve 1.2 g L (+)-ascorbic acid,  $\text{C}_6\text{H}_8\text{O}_6$ , in 150 ml of stock molybdate solution. After mixing, 3 ml sodium dodecyl sulphate (15 % solution in water) is added. This reagent was freshly prepared before every measurement.

Figure 4  $\text{PO}_4$  (4ch.) Flow diagram.

#### 4.6 Ammonia Reagents

##### EDTA

Dissolve 41 g EDTA (ethylenediaminetetraacetic acid tetrasodium salt),  $\text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_8\text{Na}_4 \cdot 4\text{H}_2\text{O}$ , and 2 g boric acid,  $\text{H}_3\text{BO}_3$ , in 200 ml of DIW. After mixing, 1 ml Triton<sup>TM</sup>X-100 (30 % solution in DIW) is added. This reagent is prepared at a week about.

##### NaOH

Dissolve 5 g sodium hydroxide, NaOH, and 16 g EDTA in 100 ml of DIW. This reagent is prepared at a week about.

##### Stock Nitroprusside

Dissolved 0.25 g sodium pentacyanonitrosylferrate(II),  $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]$ , in 100 ml of DIW and add 0.2 ml 1N  $\text{H}_2\text{SO}_4$ . Stored in a dark bottle and prepared at a month about.

##### Nitroprusside solution

Mixed 4 ml stock nitroprusside and 5 ml 1N  $\text{H}_2\text{SO}_4$  in 500 ml of DIW. After mixing, 2 ml Triton<sup>TM</sup>X-100 (30 % solution in DIW) is added. This reagent is stored in a dark bottle and prepared at every 2 or 3 days.

### Alkaline phenol

Dissolved 10 g phenol,  $C_6H_5OH$ , 5 g sodium hydroxide and 2 g citric acid,  $C_6H_8O_7$ , in 200 ml DIW. Stored in a dark bottle and prepared at a week about.

### NaClO solution

Mixed 3 ml sodium hypochlorite solution, NaClO, in 47 ml DIW. Stored in a dark bottle and freshly prepared before every measurement. This reagent is prepared 0.3% available chlorine.

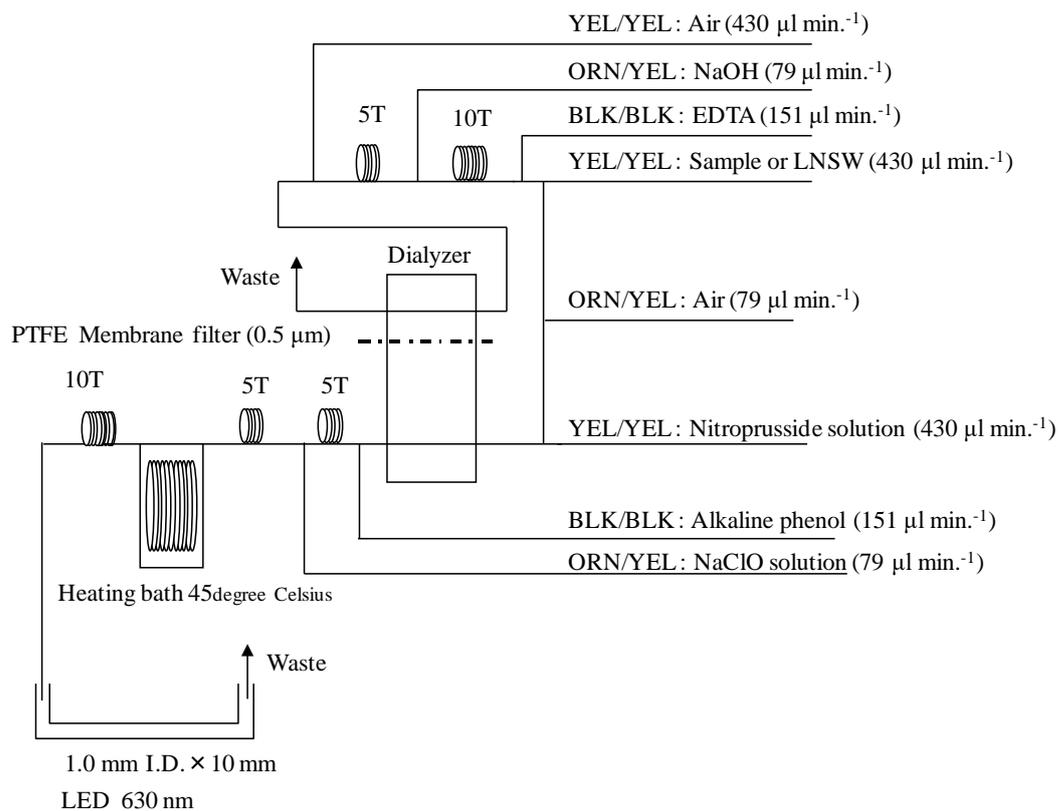


Figure 5  $NH_4$  (5ch.) Flow diagram.



## DETERMINATION OF DISSOLVED NUTRIENTS (N, P, SI) IN SEAWATER WITH HIGH PRECISION AND INTER-COMPARABILITY USING GAS-SEGMENTED CONTINUOUS FLOW ANALYSERS

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### ABSTRACT

The Global Ocean Ship-based Hydrographic Investigations Program (GO-SHIP) brings together scientists with interests in physical oceanography, the carbon cycle, marine biogeochemistry and ecosystems, and other users and collectors of ocean interior data to develop a sustained global network of hydrographic sections as part of the Global Ocean Climate Observing System. A series of manuals and guidelines are being produced by GO-SHIP which update those developed by the World Ocean Circulation Experiment (WOCE) in the early 1990s. Analysis of the data collected in WOCE suggests that improvements are needed in the collection of nutrient data if they are to be used for determining change within the ocean interior. Production of this manual is timely as it coincides with the development of reference materials for nutrients in seawater (RMNS). These RMNS solutions will be produced in sufficient quantities and be of sufficient quality that they will provide a basis for improving the consistency of nutrient measurements both within and between cruises.

This manual is a guide to suggested best practice in performing nutrient measurements at sea. It provides a detailed set of advice on laboratory practice for all the procedures surrounding the use of

gas-segmented continuous flow analysers (CFA) for the determination of dissolved nutrients (usually ammonium, nitrate, nitrite, phosphate and silicate) at sea. It does not proscribe the use of a particular instrument or related chemical method as these are well described in other publications.

The manual provides a brief introduction to the CFA method, the collection and storage of samples, considerations in the preparation of reagents and the calibrations of the system. It discusses how RMNS solutions can be used to “track” the performance of a system during a cruise and between cruises. It provides a format for the meta-data that need to be reported along side the sample data at the end of a cruise so that the quality of the reported data can be evaluated and set in context relative to other data sets.

Most importantly the central manual is accompanied by a set of nutrient standard operating procedures (NSOPs) that provide detailed information on key procedures that are necessary if best quality data are to be achieved consistently. These cover sample collection and storage, an example NSOP for the use of a CFA system at sea, high precision preparation of calibration solutions, assessment of the true calibration blank, checking the linearity of a calibration and the use of internal and externally prepared reference solutions for controlling the precision of data during a cruise and between cruises. An example meta-data report and advice on the assembly of the quality control and statistical data that should form part of the meta-data report are also given.

## CONTENTS

1.	INTRODUCTION.....	4
1.1	Guide to this Document.....	4
1.2	The RMNS approach.....	4
1.3	Definitions of Quality Control and Quality Assurance .....	5
2.	NUTRIENT ANALYSIS AND THE USE OF GAS – SEGMENTED CONTINUOUS FLOW ANALYSERS (CFA).....	5
2.1	Historical note.....	5
2.2	Basis-Colorimetry .....	6
2.3	Required procedures .....	6
2.4	Sample Collection and Storage .....	6
	2.4.1. Water Samplers.....	7
	2.4.2 Sampling Procedure and Precautions.....	7
	2.4.3 Sample Bottles .....	7
	2.4.4 Sample Storage .....	8
2.5	Analyser set up: key components, their function, and points to remember .....	8
	2.5.1 CFA hardware.....	8
	2.5.2 Assembly and maintenance .....	10
2.6	Preparation of reagents .....	10
	2.6.1 Specification of reagents.....	11
	2.6.2 Reagent containers .....	11
	2.6.3 Pure Water.....	11
	2.6.4 Wash and blank solutions .....	12
	2.6.5 Choice of blank and wash solutions .....	12
2.7	Preparation of calibration standard solutions .....	12

2.7.1.	Procedure for preparation of standard solutions.....	12
2.7.2	Volumetric Laboratory Ware.....	13
2.7.3	Pipettes .....	14
2.8	Check list of sources of error .....	14
3.	QUALITY ASSESSMENT .....	16
3.1	Precision and accuracy.....	16
3.2	Quality assessment techniques.....	16
3.3	Internal techniques .....	16
3.3.1	Duplicate measurements.....	16
3.3.2	Internal QC test solution .....	17
3.3.3	Tracking solutions.....	18
3.4	External techniques .....	19
3.4.1	Collaborative test exercises.....	19
4.	CALIBRATION PROCEDURES .....	20
4.1	Preparation of calibration solutions .....	20
4.2	Calibration of the nutrient analyzer .....	20
4.2.1	Overview .....	20
4.2.2	Working standards .....	21
4.2.3	Linearity of calibrations.....	21
4.3	Linearity problems.....	21
4.3.1	Illustration of non-linearity can occur based on data submitted to the INSS inter-comparison in 2008.....	21
5.	EXPERIENCE WITH USE OF RMNS SOLUTIONS .....	23
5.1	Example of improvement of comparability based on the use of RMNS solutions.....	23
6.	NUTRIENT ANALYSIS DATA AND META-DATA REPORTING.....	25
6.1	Check list for reporting nutrient data .....	25
7.	REFERENCES .....	25
8.	ACKNOWLEDGEMENTS.....	28

## APPENDIX: NUTRIENT STANDARD OPERATING PROCEDURES 29

NSOP 1	Applying air buoyancy corrections.....	29
NSOP 2	Gravimetric calibration of volumetric flasks and pipettes.....	33
NSOP 3	Preparation of calibration solutions.....	37
NSOP 4	Establishing the linearity of calibrations .....	40
NSOP 5	Determination of true blank value.....	47
NSOP 6	Improving the inter-run precision of nutrient measurement by use of a tracking standard .....	58
NSOP 7	Water sampling and sample storage for nutrients .....	61
NSOP 8	Low level nutrients - water sampling and sample storage .....	64
NSOP 9	Example NSOP for CFA operations at sea.....	67
NSOP 10	Preparation of control charts.....	75
NSOP 11	Statistical techniques used in quality assessment .....	81
NSOP 12	Requirements for reporting of nutrient meta-data .....	88

## 1. INTRODUCTION

### 1.1 Guide to this document

This document seeks to promote best practice in the use of any CFA system, to achieve optimum measurements of nutrients in seawater. It describes a systematic approach to achieving improved determination of seawater nutrients including appropriate analytical quality assurance procedures. This document does not provide a detailed guide to specific methodologies. We suggest that this document be used in conjunction with other more detailed description of how to successfully determine nutrients in seawater using a CFA system such as Aminot and Kerouel (2007) and Aminot et al. (2009).

Following the approach of Dickson et al. (2007) for the analysis of carbonate system parameters in seawater, specific recommended nutrient standard operating procedures (NSOPs) are appended. Some of these are closely based on the Dickson et al., (2007) procedures.

We provide recommendations for meta-data reporting of the quality control information gathered on a cruise. These should be followed by all laboratories reporting data for nutrients to national and international data centres.

We recommend that where possible common reference materials for nutrients in seawater (RMNS) are used by all laboratories (see below). This approach is required to improve the comparability of the global ocean nutrients data set.

### 1.2 The RMNS approach

To date no internationally agreed reference materials have been available for nutrient determinations in seawater, consequently significant discrepancies have been identified between data sets (e.g. Gouretski and Janke, 2001; Tanhua et al. 2009; Olafsson and Olsen, 2010). The quality and intercomparability of data would be much improved if reliable RMNS solutions were used.

In 2006 Michio Aoyama, of the Meteorological Research Institute, Japan working with Hidekazu Ota, of the General Environmental Technos Co., Ltd. (aka “KANSO Technos”) organised an inter-comparison study which included 55 different laboratories worldwide (Aoyama, 2007). The solutions used were prepared by KANSO Technos. They were natural seawaters containing a range of concentrations of nutrients, which were autoclaved and then bottled under the highest standards of cleanliness. Aoyama (2007) showed the solutions were sufficiently stable and consistent in their concentrations that they could be used as RMNS. Aoyama and Ota’s success was based on lessons learnt during the series of inter-comparison studies organised through ICES by Alain Aminot and Don Kirkwood (e.g. Aminot and Kirkwood 1995).

Extensive use of RMNS solutions will greatly improve the inter comparability measurements within and between laboratories. These materials along with the use of best practice in using analysis equipment and improved internal standardisation should make it commonly possible to achieve comparability of nutrient analyses to a level better than 1%. For example the use of a “tracking” reference material (see section 3.3.3) through a measurement campaign can improve the internal accuracy of measurements and the approach can be extended to link work on successive campaigns. To-date this approach has only been practiced by a few laboratories. Work by van Ooijen and Bakker in the Netherlands at the RNIOZ provides a clear demonstration of the effectiveness of this

approach.

### 1.3 Definitions Quality Control and Quality Assurance

A quality assurance programme consists of two separate related activities, quality control and quality assessment (Taylor, 1987).

**Quality control** — is the system of activities whose purpose is to control the quality of a measurement so that it meets the needs of users. The aim is to ensure that data generated are of known accuracy to a stated, quantitative degree of probability. The outcome is the provision of data that is dependable.

**Quality assessment/assurance** — is the system of activities that provide assurance that quality control is being done effectively. It provides a continuing evaluation of the quality of the analyses and of the performance of the analytical system.

The aim of quality control is to provide a stable measurement system whose outputs can be treated statistically, *i.e.*, the measurement is “in control” after “traceable” procedures have been followed. Any part of the procedure that can influence the measurement process has to be considered and should then be optimised (e.g. weighing and dispenser calibrations) and stabilized (e.g. laboratory temperatures) to the extent that is necessary (and practical) to obtain data of known quality. Measurement quality can be influenced by a variety of factors that are classified into three main categories (Taylor and Oppermann, 1986): management practices, personnel training and technical operations.

The first requirement of quality control is for the use of suitable and properly maintained equipment and facilities. Procedures should be standardised and documented so that all technical operations are carried out in a reliable and consistent manner. (Good laboratory management, and appropriate training of individual analysts, is essential to the production of data of high quality (see Taylor and Oppermann, 1986; Taylor, 1987; Vijverberg and Cofino, 1987; Dux, 1990), these aspects are not discussed further here.)

Such procedures should be complemented by the use of Good Laboratory Practices (GLPs), Good Measurement Practices (GMPs) and Standard Operating Procedures (SOPs). Both GLPs and GMPs should be developed and documented in each laboratory. They should identify critical operations that can cause variance or bias and seek to minimise their effects. SOPs describe the way specific operations or analytical methods should be carried out. They can form the basis for effective reporting of how particular work was carried out.

## 2. NUTRIENT ANALYSIS AND THE USE OF GAS-SEGMENTED CONTINUOUS FLOW ANALYSERS (CFA)

### 2.1 Historical note on CFA

In the late 1960s to meet the demands for the analysis of 10s to 100s of samples per day marine scientists followed the lead set in medical labs and began to automate chemical measurements. Early progress was made using the CFA system invented in 1957 by Skeggs (Skeggs, 2000; Atlas et al.,

1971). These systems have evolved to become the method of choice for the determination of nutrients in seawater (Mee, 1986, Aminot and Kerouel 2007). However there is evidence that data quality fell after GEOSECS due to the increased use of automated analytical equipment (Gouretski and Jancke, 2001). Serious systematic errors can occur when a system is used by insufficiently trained people treating it as a “black box”. Therefore to achieve high quality data, it is essential that an informed and skilled approach is taken to using the equipment and recording of appropriate meta-data.

## 2.2 Basis - Colorimetry

During the first half of the 20<sup>th</sup> century a number of methods were developed for the determination of the then recognised nutrient elements in seawater (nitrogen, phosphorous and silicon). These were based on the formation of coloured dye, the intensity of the colour of which was proportional to the concentration of the particular nutrient compound in the seawater being analysed. These methods progressed from colour assessment by eye to measurements using spectrophotometers (Strickland and Parsons, 1972). The generally simple nature of the methods meant that they could be easily adapted for use with the new “Auto-Analyzer” systems (Atlas et al., 1971). The key assumption in colorimetric analysis is that the amount of colour formed by the chemical reaction carried out is proportional to the amount of the analyte present in the solution. Ideally a linear relationship can be arrived at between the two. A “physical law” the Beer-Lambert law describes the relationship. The absorbance of the solution is directly proportional to the concentration of the colour formed and the path length in the measurement cell. (In turn this assumes that the method used produces a colour intensity, which is proportional to the concentration of the analyte in the seawater.) The absorbance is the negative logarithm of the ratio of the amount of light leaving the solution divided by the amount of light entering the solution. This can be measured in a spectrophotometer but in CFA system only the light leaving the solution is measured. In the first systems this was approximated to an absorbance by reading the values recorded on log scaled chart paper. From AA-II type systems onwards, logarithmic amplifier hardware has been used to linearise the output of the detector photocell.

There are therefore three factors in the use of a CFA method that determine how well the out put can be calibrated – (1) the reaction conditions must be such that the colour formed is proportional to the concentration of the analyte, (2) the amount of colour formed must be below the level beyond which the Beer-Lambert law no longer holds, (3) the electronics of the detector must produce an undistorted linearization of the output signal.

## 2.3 Required procedures

Each stage in the generation of data for the concentration of nutrients in seawater requires attention. In this document we provide an overview of these stages. In addition we have prepared a set of NSOPs for key stages.

## 2.4 Sample Collection (see NSOP 7)

Nutrients are present in the oceans in a wide range of concentrations. Care must be taken across this concentration range to ensure that the concentrations measured represent the in situ concentrations actually present at the time of sampling. Particular care is required in the case of the extremely low concentrations present in oligotrophic surface waters. Such samples can be contaminated during

sampling and sample storage. Microbial films form on sampler and sample bottle walls in short times, hours to a few days. Such films can take up or release nutrients. Nutrients vary widely in biochemical and *in vitro* reactivity. Gordon et al. (1993) considered that nitrite and phosphate are the most labile while silicate appears to be the least reactive. Nitrite concentrations in seawater samples and standard solutions can change markedly in a few hours under common storage conditions. However, silicate samples and standards can often be stored at room temperature (in the dark) for days with little detectable change.

#### 2.4.1 Water Samplers (NSOP 7)

At the beginning of a cruise leg and at weekly intervals during a cruise, the water samplers should be inspected for evidence of contamination and damaged components. Any rust should be removed and damaged components replaced. Microbial films should be removed using a soft sponge and a strong surface active phosphate free cleaning agent, such as Decon 90. (Brushes, and scouring agents and pads must not be used as they will damage the surface of the sampler and increase the likelihood of future contamination).

#### 2.4.2 Sampling Procedure and Precautions (NSOP 7)

The sampling procedure is important. Sample containers should be rinsed three times with the seawater being sampled, filling the bottle approximately 1/3 full each time, shaking with the cap loosely in place after each partial filling and then emptying the rinse water. Finally, fill the sample container 3/4 full (to allow for expansion if samples have to be frozen) and screw or press the cap on firmly.

During sampling, care must be taken not to contaminate the nutrient samples with fingerprints. Fingerprints contain measurable amounts of PO<sub>4</sub>. In particular, hands washed with soap are a common source of phosphate contamination. You should not handle the end of the sample draw tube, nor touch the inside surfaces of the sample container. Cigarette smoke is also known to contaminate samples. Avoid contamination with seawater, rainwater or other spurious materials dripping off the rosette or water samplers.

If gloves are worn during sampling these must be tested for their potential to introduce contamination. This testing needs to cover the contamination potential for all the different determinands being collected during a cruise.

#### 2.4.3 Sample Bottles (NSOP 7)

The largest errors in nutrient analysis tend to be due to a poor choice of sample containers, compounded by inappropriate storage.

Seawater as it comes from the sampling apparatus on the ship is a relatively sterile solution, particularly when sampled below the thermocline. It is therefore a gross error to put samples into non-sterile containers. That is any container other than an autoclaved one that has been used previously. It is appropriate to use disposable containers and to use them once and once only. If appropriate sterile containers are used samples collected directly into them can be stable for several days or more if stored in the dark in a refrigerator. All containers used must be checked for potential contamination prior to use.

#### 2.4.4 Sample Storage (NSOP 7)

Ideally nutrient samples should be analysed immediately after sampling to avoid any possibility of biological growth or decay in the samples. It is important that the time at which a sample was measured is recorded in the meta-data. This will allow discrepant data resulting from in appropriately long storage to be identified.

In practice samples may be stored (in the dark in cool/refrigerated conditions) for several hours to days except when sampling waters in peak bloom conditions. Under these conditions immediate filtering is advised. This advice does not apply to measurements of ammonium and for work at low concentrations when rapid analysis is advised.) Remember! “Cleanliness is next to Godliness”.

If storage is necessary for more than two to three days, samples should be frozen as soon after collection and as rapidly as possible. Before freezing ensure that sample bottles are no more than 3/4 full and firmly capped. A deep freezer (at least -20 °C) should be used. Good air circulation around the bottles in the freezer is important. Sample bottles should be retained in labelled gridded racks, so that they can be easily found and sorted for analysis when the time has come to measure them.

Samples should be thawed in air. Water baths should not be used because of the danger of contamination from tap water. As the sample melts and comes to room temperature its volume goes through a minimum and the resulting low pressure in the containers can suck in contaminating water from a water bath.

Samples for the determination of Si should be allowed to stand for at least 24 hours at room temperature for de-polymerisation to occur (Macdonald et al., 1986; Zhang and Ortner, 1998). For work at higher concentrations ( $>40 \mu\text{M kg}^{-1}$ ) you should check that your freezing and thawing procedures are appropriate.

### 2.5 Analyser set up: key components, their function, and points to remember

#### 2.5.1 CFA hardware

For a fuller introduction to CFA systems and practical guidance on their use in the analysis of seawater the reader should consult Aminot and Kerouel (2007) and Aminot et al (2009).

The general components of a CFA are illustrated schematically in Figure 1.

In a CFA system a multi-channel peristaltic pump moves samples and chemical reagents in a continuously flowing stream. The sample stream is segmented with air (or nitrogen) bubbles. This reduces mixing between adjacent segments (Zhang, 1997) and enhances mixing of the reagents within the sample stream. The segmented stream passes through a system manifold -a series glass coils appropriate to the individual method, in which mixing and time delays are accomplished. The sample-reagent mixture reacts chemically to produce a coloured compound whose light absorption is proportional to the concentration of nutrient in the sample. Finally the amount of light transmitted through the coloured solution is measured by a flow-through colorimeter located at the end of the flow path. Some methods use fluorometric rather than colorimetric detection, in these cases the output from the fluorimeter should be directly proportional to the concentration of the determinand. A fundamental difference between manual and CFA procedures is that complete colour development

is not required with CFA. Since all standards and samples are pumped through the system at the same rate and in constant proportion to the colour developing reagents, all samples and standards should achieve identical degrees of colour development. However, this aspect can introduce errors from any factor introducing fluctuation in the rate of colour development, e.g. laboratory temperature, sample salinity, variable flow in the pump and variable segment lengths which effect the efficiency of mixing and reagent ratios. For these reasons, analyses requiring high precision are adjusted give as near complete reaction as possible.

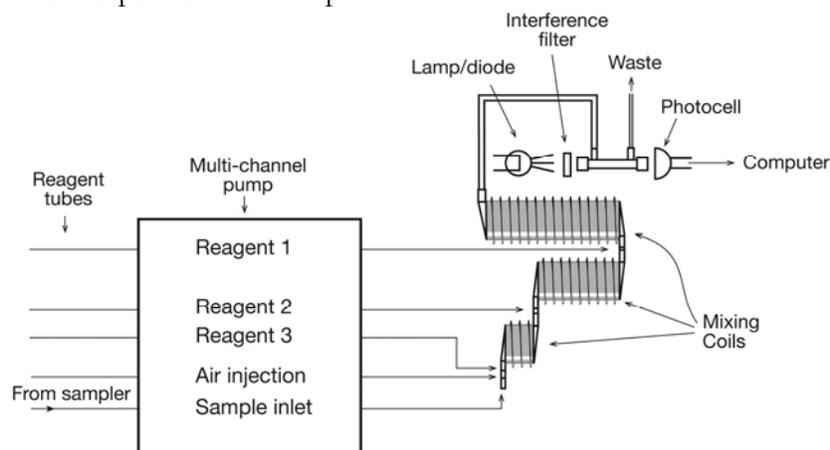


Figure 1. Schematic of a CFA system

*Sampler.* The system starts with an automated sampler. A sipping-needle moves at preset time intervals between sample containers and a “wash solution”. This sampler must be robust enough that motion on board a ship will not stop it operating reliably. The “wash solution” provides the baseline measurement at the start and end of the run and a marker between samples so that each can be identified discretely. It is not necessary to use the wash solution to provide a baseline measurement between samples.

*Peristaltic pump:* A peristaltic pump is the heart of the CFA system, it simultaneously pumps samples, reagents and air (or nitrogen) bubbles through the system. The higher the quality of the pump, the better the precision of the analyses is. The tubes used on the pump have different internal diameters (to give them different flow rates) but the same wall thickness (so that tubes with different flow rates can be used side by side on the one pump). Manufactured internal diameters of tubes do vary sufficiently from batch to batch from their nominal ones, so that the apparent sensitivity of method can change by a few percent when pump tubes are replaced. Aging and stretching of the tubes over time and warming of tubes during a run can also cause apparent shifts in sensitivity for the same reason. This is because changes in volume being pumped in individual tubes changes the relative proportion of the sample to reagents. All replacements of tubes on the pump need to be recorded so that the cause of such shifts in sensitivity can be later identified (see NSOP 12 on meta-data reporting).

*Reaction Manifolds:* For each analytical method a manifold (or cartridge) is built up of appropriately selected injection fittings, helical glass mixing coils and heating baths. A key component is the air injection system (which vary with manufacturer). These must be carefully maintained so that bubbles of the appropriate size are injected regularly at the appropriate rate, which is ideally 3 air bubbles in each loop of the helical glass coils.

*Colorimeter:* From the manifold the reacted-coloured solution passes through the colorimeter. In a standard AA-II (Technicon) type system the light source is a filament bulb and the selection of analytical wavelength is achieved by passing the light through a suitable interference filter. In an AA-II type system a debubbler is used to remove the air bubbles before they can enter the detector cell. The individual segments begin to mix at this point. The detector cell is essentially a glass tube inline with the light source and the detector, which is a photo-diode (or photo-tube in newer systems). The solution is brought in and out of the light path by bends in the glass tube. This means that a detector cell is not optically perfect and some light path refractive distortion can occur (Froelich and Pilson, 1978; Dias et al., 2006). If the wash and sample are of different densities spurious signals can be generated at the beginning and end of each peak as light is refracted by the gradient formed as they mix. Newer systems tend to have better optics and in some cases the bubbles are allowed to pass through the flowcell. Noise from the bubble is either removed by gating the light source in time with the bubbles or by electronically processing the signal to detect and remove the bubble signals.

### 2.5.2 Assembly and maintenance

For satisfactory results the components must be arranged with several ideas in mind.

- (1) The path lengths between sampler and pump, pump and manifold etc. must be minimised. This is especially true of sections of the flow streams that are not segmented by air bubbles, e.g. the lines between the sampling needle and the pump. A long un-segmented stream can lead to excessive mixing between samples and wash water.
- (2) Transmission tubing connected to reagent pump tubes should have a diameter similar to the pump tube or up to 30% smaller. Transmission tubing carrying the bubble-segmented stream should have the same diameter as the glass manifold fittings or up to 30% less. This also applies to the waste line carrying bubbles from the colorimeter; a regular bubble pattern should be maintained throughout its length. As the diameter relative to the volume increases, resistance to pumping increases and surging can develop in the flow. This induces noise.
- (3) All components should be arranged to avoid hydraulic pressure heads along the flow stream. Hydraulic heads tend to generate surging in the flow. It is not good practice to locate reagent reservoirs on shelves over the CFA, or have drain tubes of small diameter, which go directly into receptacles on the floor.
- (4) Avoid "dead volumes" in the flow channels. Dead volumes are usually introduced by de-bubblers and gaps in the butt joints between glass fittings.
- (5) A regular bubble pattern is essential for a noise-free output signal. Achieving good bubble patterns depends upon the system cleanliness and on ensuring that all plastic tubing through which bubbles pass, including the waste line from the detector, is wetted. Good bubbles appear round at the front and back, whereas in non-wetting conditions the bubbles appear straight at the back. At the end of each day's operation the reagent and manifold line should be flushed with pure water followed by a phosphate free cleansing agent such as Decon 90, then by pure water again.

### 2.6 Preparation of reagents

### 2.6.1 Specifications of reagents

Problems with reagents purity should be minimised by using “Analytical Grade” reagents. Due to the way a CFA system works small amounts of contamination can be tolerated, as they will produce a constant offset in the reagent baseline, which equally affects samples and standards equally. Reagent contamination is a problem when it produces sufficient absorbance to push the total absorbance into a nonlinear range. The reagent absorbance relative to water should be measured regularly. In general, the higher the reagent absorbance, the higher the detection limit of the method.

When weighing and packaging "pre-weighed" solid reagents for work at sea, the label of each package should identify the batch of chemical from which the weighing was done. A corresponding record should be kept of the name of the manufacturer and lot number from the label of the original container. Good practice, when making up the reagent solutions, is to record when and from what source each batch of reagent was prepared and the time and date when its use was begun. Such information can be invaluable for tracing sources of problems arising from "bad batches" of reagents or improperly formulated or weighed reagents.

### 2.6.2 Reagent containers and their maintenance

Containers should be convenient to use and easy to clean. The use of glass should be kept to minimum to avoid silica contamination by glass dissolution (Zhang et al., 1999). Tap water must never be used because of the high levels of Si and  $\text{NO}_3$  it usually contains. Use generous quantities of pure water for cleaning and Decon 90, if necessary. Once a container is clean – it should be kept clean by sealing it – simply put the lid back on – it does not need to be dry. In some laboratories atmospheric ammonium can cause contamination problems. Regular cleaning of storage containers reduces variance in the analytical results, as reagents degrade more slowly in well-maintained bottles than in dirty ones. When solutions are transferred all spillages on the outside of bottles should be cleaned off. The biggest danger resulting from poor cleanliness is that molybdenum blue stains on the necks of bottles are allowed to form. If this contamination gets into the reagent solution it will go blue through an auto-catalytic reaction. The acidified molybdate reagent used in the determination of Si throws a white precipitate as it ages. This is easily controlled at sea where the solution is replaced regularly by simply rinsing the molybdate solution bottles with pure water. If a precipitate does form in the bottle it can be dissolved with a solution of 10 % Decon 90 in pure water.

### 2.6.3 Pure Water

Dependably pure water is a necessity for nutrient work. The use of distilled water should be avoided because it can be contaminated by Si (from glass stills) and N-compounds (ammonium and nitrogen oxides) absorbed from the atmosphere during its production. Water prepared by reverse osmosis followed by deionisation should be used where possible. Such systems are now commonly available on research ships. Ideally the water should be of 18 megohm.cm specific resistance. If possible pure water should not be stored because, as noted for distillation, ammonium and nitrogen oxides can be absorbed from the ships atmosphere. Similarly glass containers should be avoided due to Si contamination (Zhang et al, 1999). Note: Sonicating pure water to degas it can sonochemically produce measurable concentrations of nitrite from dissolved nitrogen gas.

### 2.6.4 Wash and blank solutions

All CFA systems tend to suffer from spurious signals when solutions of different density are present in the detector cell. Therefore a wash solution must have a matrix with similar optical density to that of the seawater samples being measured.

The wash solution is also commonly used for the preparation of the calibration standards. In a CFA system a chemical reaction may not be complete when the coloured solution passes through the detector cell. The sample matrix can affect the rate of colour formation. The apparent sensitivity of the method could be different between standards and samples if the compositions of the wash solution and samples are significantly different. You **MUST** check if the methods you are using give different apparent sensitivities when standards made up in pure water, seawater or sodium chloride solution.

### 2.6.5 Choice of blank and wash solutions

The ideal wash solution and matrix for preparation of calibration standards is natural seawater of similar salinity to the samples being measured and which contains undetectable or low concentrations of the analytes. Some laboratories are in the fortunate position of being able to collect, store and validate a large volume of natural seawater with low concentrations of nutrients. This water is then used at sea as both the wash solution and for the preparation of working standards. Such water should be collected and filtered through a filter having a pore size of 1 microns or smaller and then be stored in the dark for several months to stabilise. Before it is used the nutrient concentration in the aged water should be checked, ideally by a more sensitive method than the one that will be used for during the cruise.

Sodium chloride solution containing 40 g l<sup>-1</sup> has been used successfully as artificial seawater (ASW) wash and for the preparation of standards, as it has the same refractive index as seawater at salinity 35 and for most analyses the rates of the reaction are not significantly different from those in seawater. Whether LNS or ASW are used as the wash they are effectively taken to be the “zero” standard, therefore meticulous attention must be paid to monitoring the quality of these waters with respect to their nutrient content. Details of how this should be done are provided in NSOP 10. When ASW is prepared from sodium chloride each batch of sodium chloride needs to be checked. Although contamination with respect to PO<sub>4</sub> and NO<sub>3</sub> is rare it does occur, but more common is contamination by Si. This can be as large as a few µM kg<sup>-1</sup> and requires the rejection of batches of sodium chloride.

With the advent of newer instrumentation with better flowcell optics, a number of laboratories are using pure water as the baseline wash water (Aminot et al. 2009). It may be used for the sampler wash when the values recorded from it are not used in the calculation of the sample concentrations, because a separate “zero” standard of LNS or ASW is used.

## 2.7 Preparation of calibration standard solutions

### 2.7.1 Procedure for preparation of standard solutions

CFA systems determine a concentration in terms of mass of determinand per volume of solution relative to a series of standard solutions. The concentration determined is therefore at the

temperature at which the standard solutions were prepared. It is this temperature that should to calculate the density of the sample, when converting from  $\mu\text{M l}^{-1}$  to  $\mu\text{M kg}^{-1}$ .

Primary (concentrated) standards are prepared using analytical-grade salts and ultra-pure water. Working standards are prepared in either nutrient-depleted natural seawater or artificial seawater. The accuracy of the preparation of the standard solution is critical. To achieve high quality measurements the salts must be dried and ground carefully before weighing. Salts should be dried in an oven at 105 °C for 2 hours then cooled in a desiccator. Higher temperatures should not be used for drying to avoid decomposing the salts. If salts are not dried prior to weighing, errors of 2-3 % can arise. Weighing should take into account air buoyancy (NSOP 1). The primary and secondary standards should be made up and diluted into volumetric flasks whose volumes have been checked. Dilution of primary standards must be done using calibrated pipettes of known reliability (NSOP 2). Please note well: The use of un-calibrated plastic volumetric ware and lack of attention to solution temperature at the time of making up standards can lead to aggregate errors on the order of three percent or even greater.

### 2.7.2 Volumetric Laboratory Ware (NSOP 1 & 2)

To ensure the accuracy of calibrations all volumetric glass and plastic-ware need to be gravimetrically calibrated. You can do this better than the manufacturer will do.

Temperature effects upon volumes contained by borosilicate glass volumetric ware are well documented and volumes at ship and shore laboratory temperatures can be computed (NSOP 2, Lembeck, 1974).

You should make yourself aware of the likely errors that can result from changing laboratory temperatures. The weights obtained from the calibration weighing must be corrected for the density of water and air buoyancy. The gravimetrically calibrated volumes must be used in computing concentrations of standard solutions.

Plastic (polypropylene) volumetric flasks must be gravimetrically calibrated within 2-3 °C of the temperature at which they will be used. Gordon et al (1993) reported that the volumes of plastic volumetric flasks calibrated in the OSU laboratory can be stable over several years' time. However, the volumes of all plastic volumetric flasks must be checked before each cruise. If they have been dried in an oven the volume can be permanently shifted by as much as 1 %.

Because of the better stability of Pyrex compared to plastics with respect to thermal expansion and because of the slow attack by DIW, Pyrex is recommended for preparation of the concentrated "primary" calibration standard solution. Exposure time to the Pyrex should be kept to minimum. Gordon et al. (1993) reported that Pyrex volumetric flasks gave initial dissolution rates of 0.03 to 0.045  $\mu\text{M kg}^{-1}$  silicate per minute into LNSW and no detectable dissolution into DIW." Similarly, Zhang et al (1999) demonstrated that dissolution from glassware can introduce micromolar silicate within a few hours. The extent of dissolution depends upon contact time, salinity and pH of solution, and the size and shape of the containers." Therefore, glass for the initial dissolution of primary standards and then transfer solution immediately into plastic (polycarbonate) containers that have a low transpiration rate for water.

### 2.7.3 Pipettes (NSOP 2)

Fixed volume pipettes should be used. Pipettes with adjustable volume are not recommended for use at sea as the precision of these pipettes would need to be checked each time their volume was changed and this cannot be done at sea.

All pipettes should have nominal calibration tolerances of 0.1% or better. Each pipette must be gravimetrically calibrated in order to verify and improve upon this nominal tolerance. This should be done before and after each cruise.

All persons preparing standards on the cruise should be trained in the use of pipettes. Their ability to obtain good precision with the pipettes should be checked by an exercise in which they do multiple pipetting and weighing of each aliquot pipetted.

## 2.8 Check list of sources of error

1. Impurity of salts used to prepare standards can be a major source of error. For example it was traditionally assumed sodium hexafluorosilicate was only 96 % pure (Strickland and Parsons 1972). Where possible new standards should be compared with old and with materials prepared by other labs. A number of errors can occur with the preparation and dilution of primary and secondary standards. These errors may in some cases be relatively small in themselves but can accumulate.
2. Weighing – the air buoyancy correction is 0.1 %.
3. Volumetrics - grade A glassware tolerances range from 0.16 % at 25 ml to 0.04 % at 1000ml. User calibration can reduce this error to 0.01 %.
4. Volumetrics - plastic can permanently shrink if heated (in for example a drying oven). The volume change can exceed 1%.
5. Change in volume of glassware with temperature – the volume of Pyrex volumetric flask calibrated at 20 °C will reduce by 0.015 % if cooled to 5 °C
6. Change in volume of an aqueous solution with temperature - the volume of a solution will increase by 0.2 % if warmed from 5 °C to 20 °C.
7. “Eppendorf” type air displacement pipettes are commonly used. These have precision of 0.1% if used carefully. The accuracy expected to be about 0.1 % of the stated value when the pipette is new.
8. Pipetting cold solution in an air displacement pipette can cause an increase in the volume by 5 % if a pipette at 20 °C is used to take solution from a bottle stored in a refrigerator.
9. Errors can arise in the output from CFA systems from the potential errors in calibration listed above and also from mechanical performance of the system. These errors (considered below) are difficult to quantify but can be minimized by using appropriate procedures and careful attention to details. Some modern systems have software, which helps by checking the optical, thermal and hydraulic characteristics of the instrument before a run can be started.
10. It is important that the analyser should be run in a thermally stable environment and the analyzer should be fully “warmed up” before an analytical run is started.
11. A record should be kept of the baseline height and the absorbance produced by the top standard – as an indicator of possible changes in or contamination of reagent or wash water solutions.
12. The stability (noise) of the reagent baseline directly determines the detection limit. It should be measured and recorded regularly, so that shifts in performance can be identified.
13. “Carryover” of one sample to the next can occur depending on the manifold and the

sampling rate. It can be measured and corrected for when modern software packages are used. However for best performance particularly when samples with highly varying concentration are being run (say across the thermocline), the system should be adjusted to reduce carryover to a minimum, ideally <1% of the preceding peak height.

14. When a CFA system is working well, the variation between duplicate measurements of peak heights should be <0.2 % of the full-scale range of the analysis.
15. If a linear calibration curve is used to calculate non-linear absorbance signals, significant errors in nutrient data can occur. This is particularly true in samples whose concentrations are outside the range of calibrant concentrations. It can also be significant in the mid-range (causing errors of ~ 3 %) see section 4.3.1.
16. Major problems can occur even with the new software systems supplied with most new CFA systems, if that software is used thoughtlessly. Visual checks of peak shape, the position of peak picking and the plausibility of results should always be carried out.

**Table 1. Summary of errors listed above that are possible at different stages of a CFA based analysis**

Source of Error	%
Weighing	
Impure standard salt	4
Wet standard salt	3
Buoyancy	0.1
Volumetrics	
Heat distorted plastic	1
Not checked grade A	0.16
User calibrated	0.01
Temperature change glass (15 °C)	0.015
Temperature change water (15 °C)	0.200
Pipette - "Eppendorf" type	
Precision	0.1
Accuracy	0.1
Temperature effect 15 °C on air volume	5
CFA	
Inherent precision	0.1
Carryover	<0.5
Forcing a linear fit to non linear calibration data.	3
Reporting $\mu\text{M l}^{-1}$ as $\mu\text{Mkg}^{-1}$ or visa-versa	3

The errors listed above are summarised in Table 1. From this table it is clear that using consistent batches of pure salts for the preparation of standards is important for achieving consistent results. These salts must be prepared in a consistent manner including their drying and grinding before they are weighed (see NSOP 3). The potential total errors possible from preparing working standards from primary and secondary standards stored in a refrigerator, which are cold when pipetted should be noted and avoided. The next largest potential error is when a linear fit is forced on non linear calibration data (see section 4.3.1). It is also imperative that data are clearly reported as  $\mu\text{M l}^{-1}$  (this is the unit they are measured in at the temperature at which the calibration standards were prepared) or fully worked up as  $\mu\text{M kg}^{-1}$  taking into account the salinity of the sample (and the calibration temperature). Finally all volumetric ware must be checked and calibrated particularly plastic volumetric flasks and air displacement pipettes.

### 3. QUALITY ASSESSMENT

#### 3.1 Precision and accuracy

Precision is a measure of how *reproducible* a particular experimental procedure is. It can refer either to a particular stage of the procedure, *e.g.*, the final analysis, or to the entire procedure including sampling and sample handling. It is quantified by performing replicate measurements and estimating a mean and standard deviation from the results. Accuracy is a measure of the degree of agreement of a measured value with the “true” value. An accurate method provides unbiased results. Quantification of accuracy is only possible when the “true” value is known. In practice this is possible when certified reference solutions can be measured as part of the everyday analytical procedure.

#### 3.2 Quality assessment techniques

A key part of any quality assurance program is the statistical evaluation of the quality of the data output. There are both internal and external techniques for quality assessment (see Taylor, 1987). Key internal techniques are duplicated measurements, internal test samples, control charts and audits. While external techniques include, collaborative tests, exchange of samples, external reference materials and audits.

#### 3.3 Internal techniques

##### 3.3.1 Duplicate measurements

Duplicate measurements of an appropriate number of samples provide an evaluation of the precision that is being achieved. At least 10% of the samples should be measured in duplicate on each sample run. Differences between duplicates should be reported both as the true difference between the duplicates first minus the second value and the absolute difference. Ideally, one would analyse duplicate samples from all of the samples. Duplicates should be measured early and late in the run so that the difference measured gives an indication of drift in sensitivity occurring in the run and repeated as part of the next run to check for calibration differences between runs. A picture of variance during the cruise and for the whole cruise can then be built up, and recorded in the control charts for the cruise (NSOP 10).

As an example nitrate concentration differences between duplicate measurements for 4600 pairs during the cruise R/V Mirai MR0706 and MR0704 cruises are shown below. In this case, about half of the duplicate measurements were within 0.2 % for the samples with concentrations between 35 – 40  $\mu\text{M kg}^{-1}$  of nitrate.

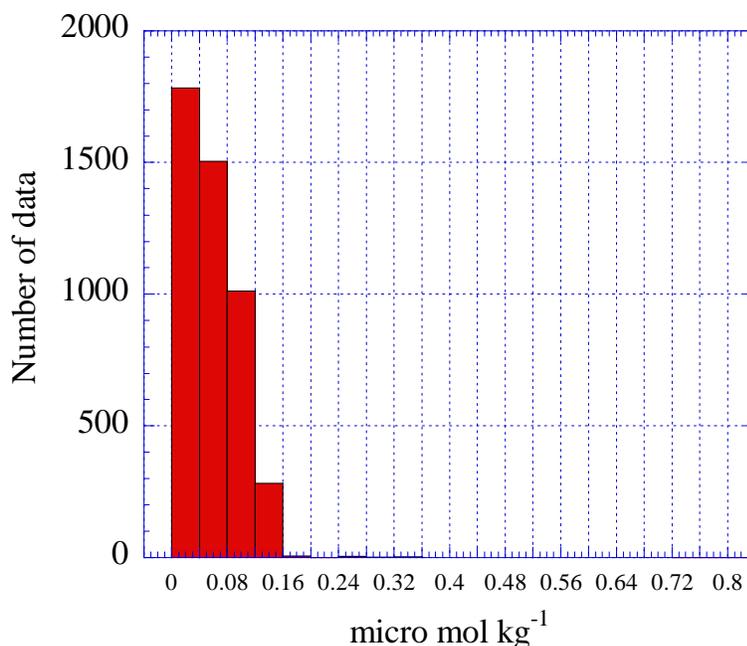


Figure 2. Nitrate concentration differences (absolute) between duplicate measurements of 4600 pairs during the cruise R/V Mirai MR0706 and MR0704 cruises. Nitrate concentrations were in the range between 35  $\mu\text{M}$   $\text{kg}^{-1}$  and 40  $\mu\text{M}$   $\text{kg}^{-1}$ . The width of the bar corresponds to 0.1% of the concentration of the samples.

### 3.3.2 Internal QC test solution

An internal test solution prepared in a laboratory can be used to monitor precision and bias (drift between runs over the length of a cruise), if the test solution value can be prepared with sufficient precision. Similarly if the material (standard solution) used is sufficiently stable for a sufficiently long period of time it can also be used to assess bias between cruises. An example of the use of such an internal standard is shown below. This is control chart (See NSOP 10) for repeated measurements made on standard prepared in the laboratory before the cruise. At the end of cruise the information in these charts allows the work on the cruise to be evaluated. This ensures that the work is being carried out appropriately and that the necessary documentation is being maintained.

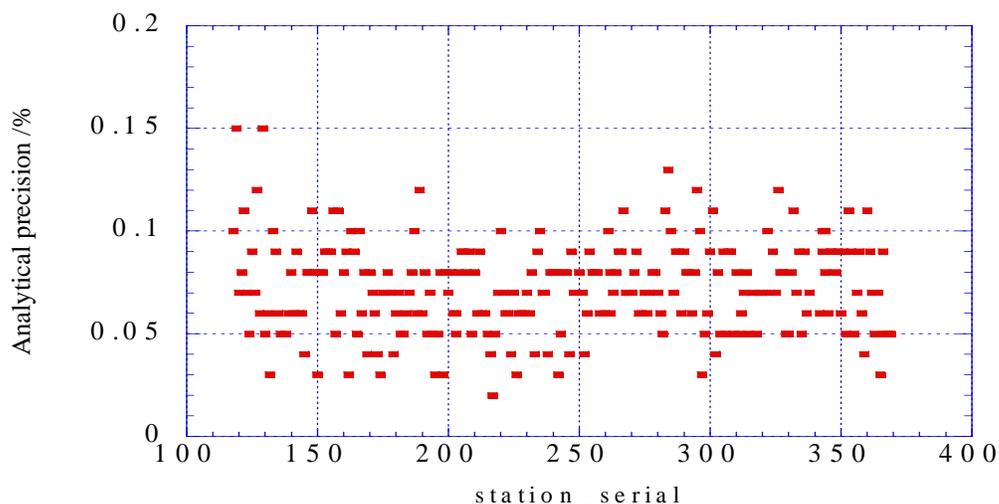


Figure 3. Control chart showing the variation of the precision (standard deviation) of determinations of nitrate at 250 stations during the R/V Mirai MR0706 cruise. A test solution prepared in the laboratory before the cruise was used. It was measured 11 times at equally spaced intervals during each run (Aoyama et al., 2008).

### 3.3.3 “Tracking” QC solutions (NSOP 6)

The use of an internal standard has been further developed by Van Ooijen and Bakker at NIOZ. Their procedure is to prepare a sufficient quantity of concentrated standard solution which is preserved by the addition of mercuric chloride. It is prepared independently of the standards used to calibrate individual analysis runs. An appropriate dilution is then made up for use on each run of the auto-analyser. The volume prepared is such that the results from a series of cruises can be compared. In practice duplicate samples are measured within an analysis run and the measurement of the same samples is repeated on the following run. The standard deviation in the difference between duplicates tends to be higher between runs rather than within runs. This deviation between runs can be reduced if the data is adjusted on the basis of the measurements of a “tracking” or reference solution.

The “tracking” solution is prepared by a one step dilution; this means that the reproducibility will be about 0.1 % due to the inherent errors of pipetting. (Note: The use of the tracking standard is only allowed if its value is in the same range as the samples in the field, and in a range of about 60-80 % of full scale values.) The tracking standard is prepared and measured as part of each analysis run. At the end of the cruise a mean value for the tracking is calculated and the data for each run is adjusted by the ratio of value for the tracking standard on that run to the mean value of the tracking standard for the whole cruise.

The value of the approach is shown in the data from a southern ocean cruise in Table 2. The procedure was - for each CTD cast a sample from the bottom depth was measured in duplicate and then re-measured in the next station run. On each run the tracking sample was measured. At the end of the cruise a statistical check was made by calculating the RMS of the duplicate difference before and after correction. Table 2 shows that the RMS difference was smaller following adjustment by the tracking standard ratio. This suggests that the adjustment improved the inter-run precision achieved

over the cruise.

Table 2. Comparison of RMS values of the difference between duplicate samples measured on sequential runs before and after adjustment relative to the value of a tracking standard measured throughout the cruise. Data from the ANTXV/4 cruise on RV "Polarstern" in the Weddell Sea. Absolute RMS value and a % of range of concentrations measured.		
	raw data RMS	corrected data RMS
Si	0.80 $\mu\text{M kg}^{-1}$	0.57 $\mu\text{M kg}^{-1}$
	0.70 %	0.47 %
NO <sub>x</sub>	0.20 $\mu\text{M kg}^{-1}$	0.16 $\mu\text{M kg}^{-1}$
	0.60 %	0.48 %
PO <sub>4</sub>	0.013 $\mu\text{M kg}^{-1}$	0.010 $\mu\text{M kg}^{-1}$
	0.60 %	0.44 %

### 3.4 External techniques

#### 3.4.1 Collaborative test exercises

External evidence for the quality of the measurement process is important for several reasons. First, it provides the most straightforward approach for assuring the compatibility of the measurements with other laboratories. Second, errors can arise over time that internal evaluations cannot detect. External quality assessment techniques, however, should supplement, but not replace, a laboratory's ongoing internal quality assessment program. Collaborative test exercises have over the years helped greatly to improve comparability between laboratories (see Aminot and Kirkwood 1995; Aminot and Kerouel 1995; Aoyama et al., 2008).

Reference materials are stable substances for which one or more properties are established sufficiently well to calibrate a chemical analyzer, or to validate a measurement process (Taylor, 1987). Ideally, such materials are based on a matrix similar to that of the samples of interest, in this case, seawater. Reference materials test the full measurement process (though not the sampling). The most useful reference materials are those for which one or more properties have been *certified* as accurate, preferably by the use of a definitive method in the hands of two or more analysts.

A Reference Material for Nutrients in Seawater (RMNS) is now produced in Japan by the General Environmental Technos Co., Ltd. These are available in large batch sizes with a long shelf life (>3 years), which allows comparison between cruises that may be a few years apart. They are based on "real seawater" and have been shown to have a homogeneity of better than 0.2% (Aoyama et al., 2010). They can also be made with appropriate concentrations and nutrient ratios to cover work in shelf seas and different oceans by collecting water from these regions and sending it to the General Environmental Technos Co., Ltd. for processing.

The solutions can be used by individual laboratories as internal tracking standards to improve the run-to-run comparability during measurements campaigns.

Recommendations for the use of RMNS solutions are made in Section 5.

## 4. CALIBRATION PROCEDURES

### 4.1 Preparation of calibration solutions (NSOP 3)

Working standard solutions for calibration of the analyser are prepared by serial dilution of primary standard solution. The primary standard solution is prepared at sea by dissolution of pure, crystalline standard materials, pre-weighed ashore. Preparation of the solutions is done using calibrated volumetric ware and pipettes (NSOP 2). Standard concentrations must be calculated for the exact masses taken, not the nominal weights. This includes correcting for air buoyancy (NSOP 1). The timing and frequency of preparation of standards should be consistent and carefully recorded. A complete and detailed record should be kept of all the identities of the pipettes, and volumetric flask used for preparation of each standard along with the label information for each pre-weighed standard used and the date and time of preparation of primary and secondary standards. It is expected that primary standard solutions of nitrate, phosphate and silicate should be stable for the duration of a normal hydrographic cruise lasting about a month. However to provide a check on the possible deterioration of the primary standards, new ones should be prepared every two weeks. The results from the "new" and "old" standards should be compared and used along with information from "tracking" standards to identify if deterioration of the primary standards has occurred.

Serial dilution of the primary standards may require the preparation of an intermediate secondary standard. This will be prepared in pure water. It may be expected to be stable for several days if stored in a refrigerator, but it is best prepared daily.

### 4.2 Calibration of the nutrient analyzer

#### 4.2.1 Overview

Calibration of the analyser should be performed on each analytical run. This is necessary to take into accounts shifts in the sensitivity of the system due to changing conditions such as laboratory temperature, aging of pump tubes and degradation of the reagents. Calibration is normally carried out by:- (1) measuring a set of standards at the start of the run, (2) at regular intervals measuring the position of the baseline (3) repeatedly measuring a chosen solution- a "drift" standard (normally at 75 % of full scale) at regular intervals during the run to check for changes (drift) in sensitivity.

The relative response of the system to nitrate relative to nitrite can change due to change in the efficiency of the cadmium column used to reduce nitrate to nitrite. A pair of standards one containing a high concentration of nitrate and the other an equivalent concentration of nitrite should be run and the results compared to assess the reduction efficiency of the cadmium column. If the efficiency is too low (<90%) or erratic the column should be replaced.

To determine the amount of carryover from one sample to the next a high standard followed by two low ones should be run. The difference between the heights of the two low standards divided by the height of the high standard gives the carryover factor (Zhang, 1997).

The concentration in each sample can then be calculated once the analytical run has been done and the data recorded.

Modern CFA systems are now usually supplied with software that, based on a protocol, allows the

peaks to be detected and their height measured ignoring spikes in the data. The software links peaks to the types of samples and standards being measured at a given position in the run. It then calculates the concentration of nutrients in the samples taking into account the concentrations of standards, drifts, column efficiency and peak carryover.

#### 4.2.2 Working standards

The concentration of the working standards should cover the range expected in the sampled waters. Prior to cruise this can be found in historical data sets such as ocean atlases. The range to be used must be decided before a cruise and not changed between legs. A minimum of four working standards should be made up for each run. The range of concentrations should be evenly divided across the range of expected concentrations.

#### 4.2.3 Linearity of calibrations (NSOP 4)

In CFA work, systems are usually adjusted so that a near linear calibration can be used to compute sample concentrations. However, the linearity of method needs to be checked, particularly when working at high concentrations. With old instruments, small changes in flow volumes when changing tubes or changes in light source output can push a method response into the nonlinear range. Even with newer instruments we need to know the range of linearity for each method. The set up of the analyser should be adjusted by using an appropriate ratio of sample to reagents so that over the concentration ranges to be measured the analyser gives as close to linear response as possible. This should be checked to ensure the mid-scale offset from a straight line is <0.2%, use of a quadratic fit to the calibration data may be required to achieve this.

### 4.3 Linearity problems

#### 4.3.1 Illustration of non-linearity based on data submitted to the INSS inter-comparison in 2008

For calibrating the data from a CFA system, if a laboratory bases its calibration on using only two known concentrations and a base line value then it can only derive a linear function, “ $y = ax + b$ ” from the calibration data. If three or more levels of calibration solution are run then either a linear function or quadratic function ( $y = ax^2 + bx + c$ ) can be fitted to the calibration data. The choice should be based on experience of the output of the system. If a quadratic fit does give a better fit it should be checked to see if this is a true result or one generated by an error such the use of an inaccurate pipette. To check this a larger number (~10) of standards should run as samples and the raw peak heights examined (See NSOP 4).

The 2008 Inter-laboratory comparison study provided an opportunity to assess the non-linear problem based on the results returned for the common RMNS solutions analysed. A number of the laboratories provided a description of their calibration procedures including the number of standards run and the type of fit (linear or quadratic) applied to the data.

In Figure 4 the results reported by the different laboratories are compared as the difference between each laboratories results and the result determined by a laboratory that measured five calibration standards and then applied a quadratic fit to derive the calibration equation. The comparison is made for two different groups of laboratories. The first group (Group 1) of three laboratories measured five calibration standards and derived a calibration equation by a linear fit to the data. The second

group (Group 2) of four laboratories used only two standards and a linear fit.

The data in Figure 4 indicate that the maximum deviation between the laboratories was about  $0.6 \mu\text{M kg}^{-1}$  in the mid range of the samples at  $20 \mu\text{M kg}^{-1}$ . This was for two of the Group 2 laboratories, the deviation for the other two Group 2 laboratories was about  $0.3 \mu\text{M kg}^{-1}$ . For the Group 1 laboratories the difference was smaller around  $0.1 \mu\text{M kg}^{-1}$  (0.5 %).

These results suggest that the Group 2 laboratories have not paid enough attention to linearising the output of the set up of their CFA systems, while the Group 1 laboratories had better set up systems. Assuming there is not a problem with the linearity of the reference laboratory set up, it also suggests that there is true residual non-linearity in the calibration of the Group 1 systems and that more consistent data would be achieved if a quadratic fit was applied by the Group 1 laboratories.

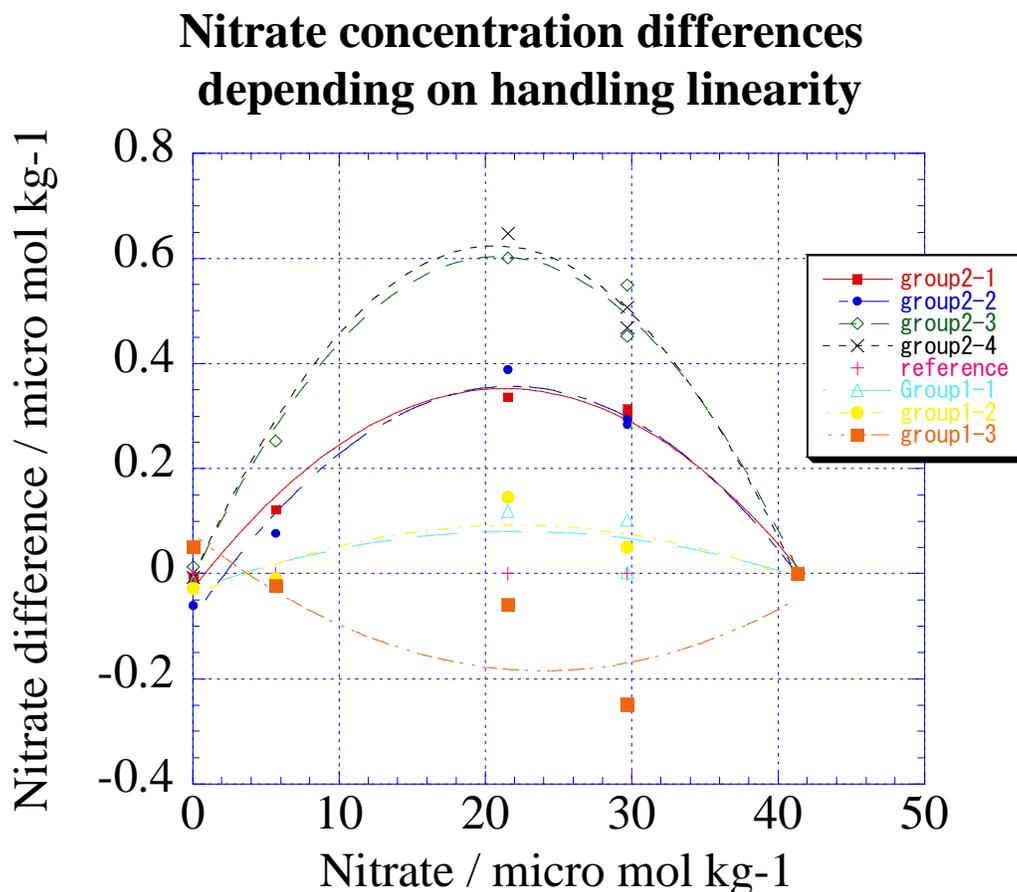


Figure 4. Plot of difference between nitrate concentration values reported by individual laboratories and the reference laboratory. The reference laboratory measured five standards and applied a quadratic fit. Group 1, laboratories measured 5 standards and applied a linear fit, Group 2 laboratories measured two standards and applied linear fit.

## 5. EXPERIENCE WITH USE OF RMNS SOLUTIONS

The purpose of RMNS solutions is to improve the consistency of measurements within a cruise and between cruises. They are limited resource so need to be used in conjunction with internal standard solutions produced by each laboratory. The relative accuracy of in house standard solutions can then be validated when the RMNS values from a cruise are compared the values reported by other laboratories who have used the same batch of RMNS solutions.

RMNS solutions are potentially more homogeneous than “tracking solutions” prepared from dry salts (see section 3.3.3 and NSOP 6). They should be used either in place of or alongside a laboratory’s internally prepared tracking solution. RMNS samples would be measured on each analytical run and the data would be used at the end of the cruise to adjust the data for the cruise in the same manner as is done when a tracking standard is used (NSOP 6). In the cruise meta-data all the RMNS values should be reported along with the mean, median and standard deviation.

Inter-comparison exercises have shown evidence that discrepancies arise between different laboratories if inappropriate assumptions are being made about the linearity of calibration data. (See section 4.2.3 above). So that such non-linearity can be detected, a minimum of three RMNS solution at low, mid and top of the range should analysed at regular intervals during a cruise. Reporting these data in the meta-data at the end of cruise will allow non linearity to be identified when comparisons are made to the data reported by other laboratories who have measured the same RMNS solutions.

### 5.1 Example of improvement of comparability based on the use of RMNS solutions

Figure 5 shows concentrations of nitrate in the North Pacific Ocean at the crossing point of four WOCE cruises for the WOCE lines P3 line and P14 (within 250 km of 24 °N - 180 °E). These were in 1985 (P3), 1993 (P14), 2005 (P3) and 2007 (P14). During the P3 cruise in 1985 and P14 cruise in 1993, nutrients measurements were done using an in-house calibration standard. During the P3 and P14 reoccupation cruises in 2005 and 2007, a set of RMNS were used as calibration standard throughout the cruises. Figure 5.1 shows a much closer agreement between reoccupation cruise than between the earlier P3 and P14 cruises.

Figure 6 shows that the use of the RMNS solutions produces data with tighter N:P ratio but also significant shift in the value of the ratio, from 15 to less than 14.5 at depth of 5000 metres.

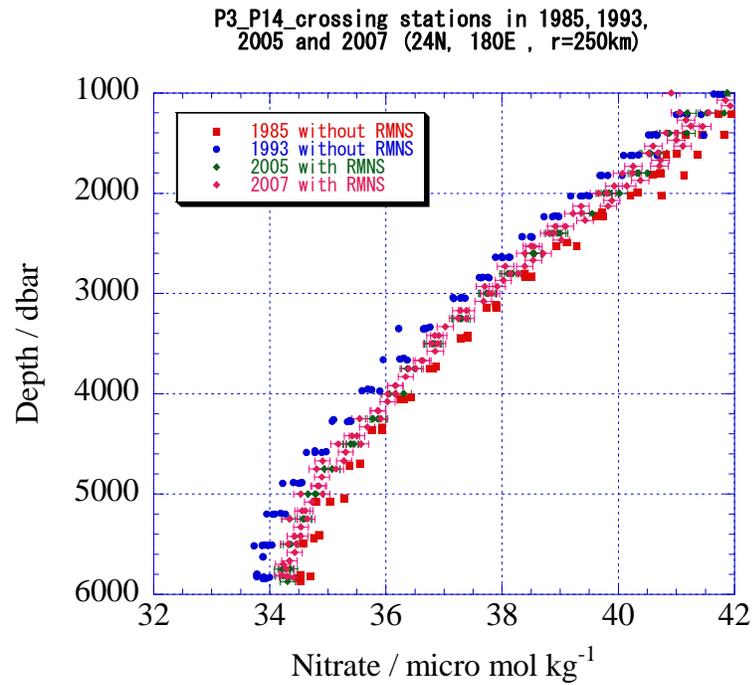


Figure 5. Profiles of nitrate concentration in the North Pacific Ocean at the crossing of P3 line and P14 line carried out in 1985 (P3), 1993 (P14), 2005 (P3) and P14(2007).

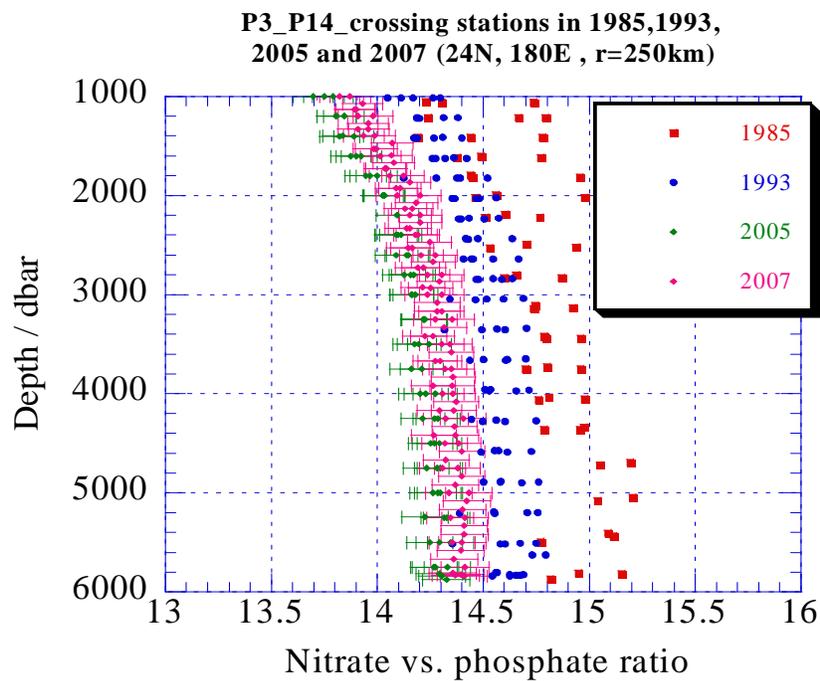


Figure 6. Profiles of N:P ratio at the crossing of 1985 (P3), 1993 (P14), 2005 (P3) and P14(2007).

## 6. NUTRIENT ANALYSIS DATA AND META-DATA REPORTING

(See NSOP 12 - Meta-data reporting)

### 6.1 Check list for reporting nutrient data

Adequate and accurate records must be kept of all the procedures used and the results of the quality assessments of each reported data set: All archived data should be reported with this “meta-data” attached in electronic format. Without the meta-data to document methods and QA/QC protocols, archived data are of limited use.

The material to be archived should include

Samples results:

- Header file showing what was measured (variables/parameters, units)
- Time and location of sample taken (time; latitude; longitude; station identifier)
- Time the sample was measured
- Raw nutrient data
- Nutrient data adjusted for tracking and RMNS results
- Clear statement that the data are reported as  $\mu\text{M l}^{-1}$  or  $\mu\text{M kg}^{-1}$

Quality control results:

- Control charts
- Precision from duplicates in and between runs
- Tracking solution data
- RMNS data
- Record of calculations and adjustments

Meta-Data on how the measurements were carried out:

- How the measurements were made (equipment, calibration, methodology *etc.*, with references to literature, if available);
- Who measured it (name and institution of the analyst(s) and Principal Investigator responsible for the data);
- Quality assurance report
- Data records

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## 8. ACKNOWLEDGEMENTS

In November 2007 Michio Aoyama of the Japanese Meteorological Research Institute organised a meeting in Tsukuba, Japan to discuss the establishment of an “International Scale for the Measurements of Nutrients in Seawater” (INSS) as part of a process that would improve the international comparability of nutrient measurements in seawater. This would be based on the development of seawater based reference solutions which could then be used by all laboratories making measurements of nutrients in seawater. The meeting also recognised that progress towards a higher level of intercomparability would be hindered if best practice were not followed by the laboratories using these new reference seawater materials. The meeting therefore agreed to work on an update of the Gordon et al (1993) protocols developed to support the WOCE hydrographic programme.

The aim was to explain and illustrate best practice, taking as their starting point as the excellent work of Lou Gordon and the team he led to prepare “A Suggested Protocol for Continuous Flow Automated Analysis of Seawater Nutrients (Phosphate, Nitrate, Nitrite and Silicic Acid) in the WOCE Hydrographic Program and the Joint Global Ocean Fluxes Study by Louis I. Gordon, Joe C. Jennings, Jr. Andrew A. Ross, James M. Krest (1993) WOCE Hydrographic Program Office, Methods Manual WHPO 91-1”.

The format of the new document follows that of Dickson et al. (2007) [Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.) 2007. *Guide to best practices for ocean CO<sub>2</sub> measurements*. PICES Special Publication 3, 191 pp.] in having a central text accompanied by a set of individual standard operating procedures (SOPs). The content of several of our nutrient standard operating procedures (NSOPs) follows very closely the content of ones of Dickson et al. Andrew Dickson very kindly supplied the original “Word” files for the carbonate SOPs for use in preparing the NSOPs.

## APPENDIX: NUTRIENT STANDARD OPERATING PROCEDURES (NSOPS)

**NSOP 1. APPLYING AIR BUOYANCY CORRECTIONS**

## 1. SCOPE AND FIELD OF APPLICATION

If uncorrected, the effect of air buoyancy is frequently the largest source of error in mass measurements. This procedure provides equations to be used to correct for the buoyant effect of air. An air buoyancy correction should be made in all high accuracy mass determinations.

## 2. PRINCIPLE

The up-thrust due to air buoyancy acts both on the sample being weighed and on the counterbalancing weights. If the sample and weights are of different densities and hence of different volumes, it will be necessary to allow for the resulting difference in air buoyancy to obtain an accurate determination of mass.

## 3. REQUIREMENTS

## 3.1 Knowledge of the air density at the time of weighing

For the most accurate measurements, the air density is computed from a knowledge of air pressure, temperature, and relative humidity. Tolerances for the various measurements are given in Table 1.

Table 1. Tolerances for various physical parameters.

Variable	Uncertainty in computed air density	
	$\pm 0.1\%$	$\pm 1.0\%$
Relative humidity (%)	$\pm 11.3\%$	–
Air temperature (°C)	$\pm 0.29$ K	$\pm 2.9$ K
Air pressure (kPa)	$\pm 0.10$ kPa	$\pm 1.0$ kPa

Barometer accurate to  $\pm 0.05$  kPa,  
 Thermometer accurate to  $\pm 0.1$  °C,  
 Hygrometer accurate to 10 %.

An error of 1 % in air density results in an error of approximately 1 part in  $10^5$  in the mass corrected for air buoyancy. Although meteorological variability can result in variations of up to 3 % in air density, the change of pressure (and hence of air density) with altitude can be much more significant. For measurements of moderate accuracy, made at sea level and at normal laboratory temperatures, an assumed air density of  $0.0012 \text{ g cm}^{-3}$  is often adequate.

## 3.2 Knowledge of the apparent mass scale used to calibrate the balance

There are two apparent mass scales in common use. The older one is based on the use of brass weights adjusted to a density of  $8.4 \text{ g cm}^{-3}$ , the more recent one on the use of stainless steel weights adjusted to a density<sup>1</sup> of  $8.0 \text{ g cm}^{-3}$ .

### 3.3 Knowledge of the density of the sample

The density of the sample being weighed is needed for this calculation. The procedure for computation of air density is as follows:

The density of air in  $\text{g cm}^{-3}$  can be computed from measurements of pressure, temperature, and relative humidity (Jones, 1978):

$$\rho(\text{air}) = \frac{3.4848 (p - 0.0037960U \cdot e_s)}{273.15 + t} \times 10^{-3} \quad (1)$$

where

$p$  = air pressure (kPa),  
 $U$  = relative humidity (%),  
 $t$  = temperature ( $^{\circ}\text{C}$ ),

$e_s$  = saturation vapor pressure (kPa),

$$e_s = 1.7526 \times 10^8 \exp[-5315.56/(t + 273.15)]. \quad (2)$$

Computation of mass from weight:

The mass,  $m$ , of a sample of weight,  $w$ , and density,  $\rho(\text{sample})$ , is computed from the expression

$$m = w \left( \frac{1 - \rho(\text{air})/\rho(\text{weights})}{1 - \rho(\text{air})/\rho(\text{sample})} \right) \quad (3)$$

(see Annex of NSOP1 for the derivation).

Example calculation:

The following data were used for this calculation<sup>2</sup>:

weight of sample,  $w = 100.00000 \text{ g}$ ,

density of sample,  $\rho(\text{sample}) = 1.0000 \text{ g cm}^{-3}$ .

Weighing conditions:

$$\begin{aligned} p &= 101.325 \text{ kPa (1 atm)}, \\ U &= 30.0 \%, \\ t &= 20.00 \text{ }^{\circ}\text{C}, \\ \rho(\text{weights}) &= 8.0000 \text{ g cm}^{-3}. \end{aligned}$$

<sup>1</sup> Strictly, these densities apply only at  $20 \text{ }^{\circ}\text{C}$ . The conversion factor from the “apparent mass” obtained by using these values to “true” mass is defined by the expression

$$Q = \frac{\rho(\text{weights})(D_{20} - 0.0012)}{D_{20}[\rho(\text{weights}) - 0.0012]}$$

where  $D_{20}$  is the apparent mass scale to which the weights are adjusted. This factor may be considered as unity for most purposes.

<sup>2</sup> The seemingly excessive number of decimal places is provided here so that users of this procedure can check their computation scheme.

Computation of air density

$$e_s = 2.338 \text{ kPa,}$$

$$\rho(\text{air}) = 0.0012013 \text{ g cm}^{-3}.$$

Computation of mass

$$m = 100.10524 \text{ g.}$$

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### ANNEX NSOP 1: DERIVATION OF THE EXPRESSION FOR BUOYANCY CORRECTION

An expression for the buoyancy correction can be derived from a consideration of the forces shown in Figure 1. Although the majority of balances nowadays are single-pan, the principles remain the same, the difference being that the forces are compared sequentially using a force sensor rather than simultaneously using a lever. At balance, the opposing forces are equal:

$$m_1g - V_1\rho(\text{air})g = m_2g - V_2\rho(\text{air})g \quad (4)$$

where  $g$  is the acceleration due to gravity and  $\rho(\text{air})$  is the density of the air at the temperature, pressure, and humidity of the weighing operation. Note that  $m_2$  is the “weight” of a sample whose true mass is  $m_1$ .

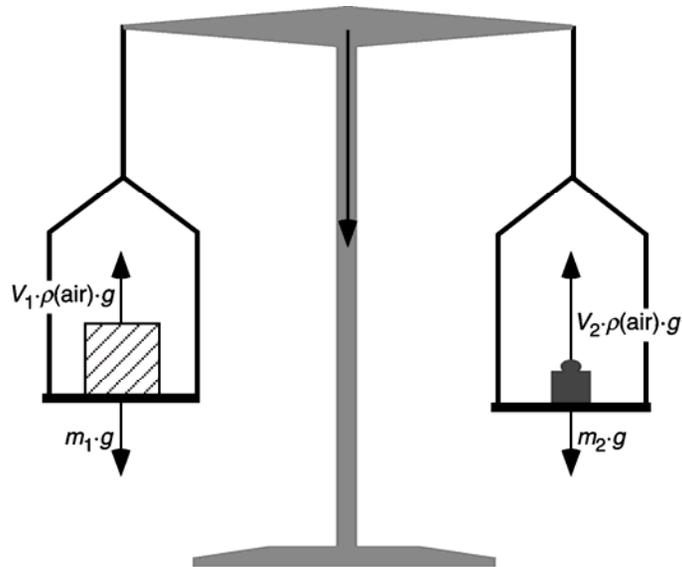


Figure 1. Forces on sample (1) and weights (2) when weighing in air.

As

$$V = m / \rho, \quad (5)$$

we can rewrite equation (4) as

$$m_1 - m_1 \rho(\text{air}) / \rho_1 = m_2 - m_2 \rho(\text{air}) / \rho_2. \quad (6)$$

This equation can be rearranged to obtain the expression

$$m_1 = m_2 \frac{1 - \rho(\text{air}) / \rho_2}{1 - \rho(\text{air}) / \rho_1}. \quad (7)$$

Equation (7) is the basis of the expression used for air buoyancy correction (Schoonover and Jones, 1981; Taylor and Oppermann, 1986):

$$m = w \frac{1 - \rho(\text{air}) / \rho(\text{weights})}{1 - \rho(\text{air}) / \rho(\text{sample})} \quad (8)$$

where  $w$  is the “weight” of the sample in air and  $m$  is the true mass.

Equation (6) can also be rearranged to give

$$m_1 = m_2 + m_2 \rho(\text{air}) \left( \frac{m_1}{m_2} \frac{1}{\rho_1} - \frac{1}{\rho_2} \right). \quad (9)$$

As  $m_1 \approx m_2$ , equation (9) is almost identical to the commonly quoted expression for buoyancy correction,

$$m = w + w \rho(\text{air}) \left[ \frac{1}{\rho(\text{sample})} - \frac{1}{\rho(\text{weights})} \right] \quad (10)$$

(Woodward and Redman, 1973; Dean, 1985). An approximate value of  $0.0012 \text{ g cm}^{-3}$  for  $\rho(\text{air})$  is often used with this expression; this is appropriate to measurements of moderate accuracy made at sea level pressures and at normal laboratory temperatures.

## NSOP 2. GRAVIMETRIC CALIBRATION OF VOLUME CONTAINED IN VOLUMETRIC FLASKS AND PIPETTES USING WATER

### 1. SCOPE AND FIELD OF APPLICATION

This procedure describes how to calibrate the volume of solution contained by volumetric flasks, pipettes or other containers capable of being filled to a reproducible mark. This is expressed as the volume contained at a standard temperature (usually 20.0°C). This procedure is capable of achieving a reproducibility of better than 0.01% (1 relative standard deviation).

“Eppendorf” type air displacement pipettes are commonly used along with volumetric flasks for the preparation of calibration solutions. These have precision of 0.1 % if used carefully. The accuracy expected to be about 0.1 % of the stated value when the pipette is new. Their precision and accuracy should be checked on a regular basis.

### 2. PRINCIPLE

The mass of water contained by the flask at a measured calibration temperature is used to compute the volume of water contained at that temperature. The volume that would be contained at the standard temperature (20°C) can be calculated by taking account of the volumetric expansion of the flask. The volume of liquid contained at any desired temperature can be calculated in a similar fashion.

Warning. This requires that the temperature of the calibration solution is known. Taking solutions directly from a refrigerator and preparing a standard solution should be avoided for this reason. Similarly pipetting cold solution in an air displacement pipette can cause an increase in the volume by 5 % if a pipette calibrated at 20°C is used to pipette a solution at 5°C. This because the cold solution once in the pipette can cause the air above it to contract.

### 3. APPARATUS

- Analytical balance capable of weighing the quantity of water contained with a sensitivity of 1 part in  $10^5$  while having the capacity to weigh the water together with the container being calibrated.
- Thermometer accurate to  $\pm 0.1$  °C.
- Container large enough to retain more than 10 aliquots dispensed by the pipette being calibrated.

### 4. REAGENTS

- Deionised water in equilibrium with the temperature of the laboratory.

### 5. PROCEDURE CALIBRATION OF VOLUMETRIC FLASKS

- Weigh the clean dry empty container together with the associated closure.
- Fill the container being calibrated to the mark with pure water, allowing the temperature of the container and contained water to reach an equilibrium value. Note this temperature.

- Close the container and reweigh it.

## 6. CALCULATION AND EXPRESSION OF RESULTS

### 6.1 Volume of the water contained at the calibration temperature

Compute the weight of the water contained from the difference between weights of the filled and empty container:

$$w(\text{H}_2\text{O}) = w(\text{filled container}) - w(\text{empty container}). \quad (11)$$

Compute the mass of water contained, correcting for air buoyancy (see NSOP 1):

$$m(\text{H}_2\text{O}) = w(\text{H}_2\text{O}) \left( \frac{1 - \rho(\text{air})/\rho(\text{weights})}{1 - \rho(\text{air})/\rho(\text{sample})} \right). \quad (12)$$

The volume contained at the noted temperature ( $t$ ) is

$$V(t) = m(\text{H}_2\text{O})/\rho(\text{H}_2\text{O}, t). \quad (13)$$

The density of air-saturated water in the temperature range 5 to 40 °C is given by the expression (Jones and Harris, 1992)

$$\begin{aligned} \rho_w / (\text{kg m}^{-3}) = & 999.84847 + 6.337563 \times 10^{-2} (t/^\circ\text{C}) \\ & - 8.523829 \times 10^{-3} (t/^\circ\text{C})^2 + 6.943248 \times 10^{-5} (t/^\circ\text{C})^3 \\ & - 3.821216 \times 10^{-7} (t/^\circ\text{C})^4 \end{aligned} \quad (14)$$

where  $t$  is the temperature on ITS 90<sup>3</sup>. To achieve an accuracy of 1 part in 10<sup>4</sup>,  $t$  must be known to within 0.5 °C.

### 6.2 Volume that would be contained at an alternate temperature

To convert the volume contained at one temperature ( $t_1$ ) to a standard or alternate temperature ( $t_2$ ), we need to take account of the thermal expansion of the container being used. For Pyrex-like glasses (Corning 7740, Kimble KG-33, Schott Duran, Wheaton 200, *etc.*) the coefficient of linear expansion  $a_l$  is  $32.5 \times 10^{-7} \text{ K}^{-1}$ ; for glasses such as Kimble KG-35,  $a_l$  is about  $55 \times 10^{-7} \text{ K}^{-1}$ .

The coefficient of volumetric expansion,

$$\alpha_v = (1 + \alpha_l)^3 - 1 \approx 3\alpha_l, \quad (15)$$

is used to calculate the corrected volume at the alternate temperature,

$$V(t_2) = V(t_1) [1 + \alpha_v (t_2 - t_1)]. \quad (16)$$

This correction is negligible for all except the most precise work; unless  $t_2 - t_1$  exceeds 10 °C or if plastic ware is used.

Example calculation:

The following data were used for this calculation:

<sup>3</sup> The International Practical Temperature Scale of 1968 (IPTS 68) has been superseded by the International Temperature Scale of 1990 (ITS 90). A simple equation can be used to relate the two over the oceanographic temperature range 0 to 40 °C (Jones and Harris, 1992):

$$t_{90} = 0.0002 + 0.99975 t_{68}.$$

The small difference in temperature scales is typically not important to the calibration of glassware for the procedures in this Guide.

$w(\text{H}_2\text{O}) = 996.55 \text{ g}$ ,  
 calibration temperature =  $23.0^\circ\text{C}$ ,  
 $\rho(\text{H}_2\text{O}, 23.0^\circ\text{C}) = 0.997535 \text{ g cm}^{-3}$ ,  
 $\alpha_l = 32.5 \times 10^{-7} \text{ K}^{-1}$ ,

weighing conditions:

$\rho(\text{air}) = 0.0012 \text{ g cm}^{-3}$ ,<sup>4</sup>  
 $\rho(\text{weights}) = 8.0 \text{ g cm}^{-3}$ .

Correct weight of water to mass:

$$\begin{aligned}
 m(\text{H}_2\text{O}) &= 996.55 \times \frac{1 - 0.0012/8.0}{1 - 0.0012/0.997535} \\
 &= 997.60 \text{ g}.
 \end{aligned}$$

Compute volume of water contained at the calibration temperature of  $23.0^\circ\text{C}$ :

$$\begin{aligned}
 V(23.0^\circ\text{C}) &= 997.60/0.997535 \\
 &= 1000.07 \text{ cm}^3.
 \end{aligned}$$

Compute volume that would be contained at the standard temperature of  $20.0^\circ\text{C}$ , i.e., the standard calibrated volume:

$$\begin{aligned}
 V(20.0^\circ\text{C}) &= 1000.07 \left[ 1 + 3(32.5 \times 10^{-7})(20.0 - 23.0) \right] \\
 &= 1000.04 \text{ cm}^3.
 \end{aligned}$$

Compute volume that would be contained at  $25^\circ\text{C}$ .

$$\begin{aligned}
 V(25.0^\circ\text{C}) &= 1000.04 \left[ 1 + 3(32.5 \times 10^{-7})(25.0 - 20.0) \right] \\
 &= 1000.09 \text{ cm}^3.
 \end{aligned}$$

### 6.3 Calibration of micro-litre pipettes

- Weigh the clean dry empty container.
- Dispense 10 aliquots of deionised water recording the weight of each aliquot
- Correct the weight of each aliquot for air buoyancy (see NSOP 1).
- Calculate the precision achieved and record the precision and accuracy of the pipette

### 6.4 Quality assurance

To ensure that the volume contained is in control, the amount contained should be measured regularly and a property control chart maintained of the volume corrected to  $20^\circ\text{C}$  (see N SOP 10).

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Batista, E., Pinto, L., Filipe, E. and van der Veen, A.M.H., 2006. Calibration of micropipettes: Test

<sup>4</sup> This value is appropriate to measurements of moderate accuracy made at sea level pressure (1 atm) and at normal laboratory temperatures ( $\sim 20^\circ\text{C}$ ). For a more accurate value see NSOP 12, Equation (1).

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doi:10.1016/j.measurement.2006.05.01.

### NSOP 3. PREPARATION OF CALIBRATION SOLUTIONS FOR USE WITH CFA SYSTEM

#### 1. SCOPE AND FIELD OF APPLICATION

This NSOP describes the preparation of the solutions needed to calibrate a segmented continuous flow analyser (CFA) used to determine dissolved nutrients - ammonium, nitrate, nitrite, phosphate and silicate.

#### 2. PRINCIPLE

Primary standard solutions are prepared from dry salts in the laboratory in sufficient quantity that replicate aliquots can be taken to sea and stored on land for a cross check subsequent to the cruise. The primary solution is diluted sequentially to achieve solutions at appropriate concentrations. The dry materials are weighed following NSOP 1 to achieve weight *in vacuo* and dissolved in and diluted in volumetric flasks which have been calibrated following NSOP 2.

#### 3. APPARATUS

- Pestle and Mortar
- Drying oven
- Desiccator
- Analytical balance capable of weighing the quantity of salt with a sensitivity of 1 part in  $10^5$  while having the capacity to weigh the water together with the container being calibrated,
- Thermometer accurate to  $\pm 0.1$  °C
- Calibrated volumetric flasks
- Calibrated pipettes

#### 4. REAGENTS

- Pure (18 megohm.cm RO-deionised) water.
- Appropriate nutrient salts
- Low nutrient seawater (LNS) or sodium chloride solution (40 g/l) if LNS of sufficient quality and quantity is not available.

#### 5. GENERAL CONSIDERATIONS

The primary standard materials must be chemically pure, reagent grade or primary standard grade chemicals, they should be dried at 105°C for 2 hours and cooled in a desiccator before weighing. Before drying the salts may need to be finely crushed using a carefully cleaned mortar and pestle; they must not be ground.<sup>5</sup>

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<sup>5</sup> Crushing is accomplished with use of minimum force, rocking the pestle back and forth over a small amount of the material to be crushed. Grinding is defined here as a vigorous circular movement of the pestle against the mortar, with maximum or strong force. Grinding can impart considerable energy to the material being ground, sufficient to

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Weights must be corrected to *in-vacuo* in order to achieve 0.1 % accuracy which is desirable given the reproducibility attainable with CFA. The weights given below are nominal. If, for efficiency, exact weights are not taken, careful track must be kept of the exact weights placed in each "pre-weigh" container, air buoyancy corrections made, and actual concentrations used in subsequent computations of concentrations. Adjust the concentrations of the primary standards suggested below to be appropriate for the range of concentration in the samples you will be working with.

The label of each "pre-weigh" container should identify the batch of chemical from which the weighing was done. A record should be kept of the manufacturer and lot number from the label of the original container. When making up the reagent solutions, when and from what source each batch of reagent was prepared and the time and date when its use is begun should be recorded.

## 6. PREPARATION OF PRIMARY STANDARDS

When a set of primary standards is prepared the working concentrations obtained from these standards should be compared with those of the previous set of primary standards. If the absorbances of new working standards do not agree within 0.3 % of the values from the previous standards the test should be repeated and if discrepancies are still present appropriate new primary standard should be prepared and possible reasons for the miss-match must be investigated and the finds recorded.

6.1 Nitrite: *Primary nitrite standard* (5,000  $\mu\text{M l}^{-1}$ ). Use analytical-grade sodium nitrite ( $\text{NaNO}_2$ ; 69.00  $\text{g mol}^{-1}$ ). (If the purity differs from 100 % but is certified, increase the mass to be weighed proportionally. Do not use an old product (Hansen and Koroleff, 1999). Weigh 0.345 g for 1,000 ml of solution. When the salt is completely dissolved, transfer it to a clean glass or plastic storage bottle. Store at ambient temperature, in the dark, and renew each month. Never add acid or mercury as a preservative, because they accelerate nitrite loss (Aminot and K erouel, 1996).

6.2 Nitrate: *Primary nitrite standard* (5,000  $\mu\text{M l}^{-1}$ ). Dry (105  $^\circ\text{C}$ , 1 hour) analytical-grade potassium nitrate ( $\text{KNO}_3$ ; 101.11  $\text{g mol}^{-1}$ ), then let it cool in a desiccator. Weigh 505.6 mg for 1,000 ml of solution. (note: if the  $\text{KNO}_3$  purity differs from 100% but is certified, increase the weighted mass proportionally). When the salt is completely dissolved, mix the solution and transfer it to a clean glass or plastic storage bottle: Store at ambient temperature and in the dark. The solution is stable for at least 1 year provided no evaporation occurs (Aminot and K erouel, 1996).

6.3 Ammonium: Use analytical-grade ammonium sulphate ( $(\text{NH}_4)_2\text{SO}_4$ ; 132.14  $\text{g mol}^{-1}$ ). (This is preferred to  $\text{NH}_4\text{Cl}$ , which is slightly hygroscopic; note that ammonium sulphate contains two ammonium groups per molecule.) If its purity differs from 100 % but is certified, increase the mass to be weighed proportionally.

6.4 Phosphate: Use analytical-grade potassium dihydrogenphosphate ( $\text{KH}_2\text{PO}_4$ ; 136.09  $\text{g mol}^{-1}$ ). If its purity differs from 100% but is certified, increase the mass to be weighed proportionally.

6.5 Silicate: Use analytical-grade sodium hexafluorosilicate ( $\text{Na}_2\text{SiF}_6$ ; 188.06  $\text{g mol}^{-1}$ ) in a fine powder of purity  $\geq 99$  % (e.g., Carlo Erba 480005 or Fluka 71596). If its purity differs from 100 %

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cause chemical change in some cases. The need for crushing is to fracture coarsely crystalline material into a fine, uniform powder so that water trapped in coarse crystals can evaporate during the drying process.

but is certified, increase the mass to be weighed proportionally. *Primary silicate standard* ( $5,000 \mu\text{M l}^{-1}$ ) Weigh 940.3 mg and transfer it using ultra-pure water into a 1,000 ml plastic volumetric flask. Add about 800 ml of ultra-pure water and leave under magnetic stirring to ensure complete dissolution (up to several hours at  $\sim 20^\circ\text{C}$ ). Remove with care the stirrer magnet while properly rinsing it, then adjust the volume, mix the solution, and transfer it to a plastic bottle. In a tightly closed bottle, this standard is stable for several years at ambient temperature (Aminot and K  rouel, 1996).

## 7. PREPARATION OF WORKING STANDARDS

The dilution of the primary standard will be determined by the range of concentrations required to cover the concentrations to be encountered on a particular cruise.

It is often convenient to prepare a secondary standard in pure water which is then diluted to provide the working standards. This secondary standard can be a mixed standard. This reduces the amount of pipetting required to prepare the working standards.

Secondary standards should be prepared daily.

Working standards should not be retained for more than 8 hours.

## 8. REFERENCES

- Aminot, A., and R. Kerouel. 1996. Stability and preservation of primary calibration solutions of nutrients. *Marine Chemistry* 52:173-181.
- Hansen, H. P., and F. Koroleff. 1999. Determination of nutrients. In *Methods of seawater analysis*, K. Grasshoff, K. Kremling and M. Ehrhardt, eds. Wiley-VCH, Weinheim, Germany.

## NSOP 4. ESTABLISHING THE LINEARITY OF STANDARD CALIBRATIONS

### 1. SCOPE AND FIELD OF APPLICATION

If insufficient attention is paid to the appropriateness of fitting a linear calibration equation to auto-analyser data, errors of several percent can be generated in the mid-range of the data. Examples of this are provided in section 4.3 of the main manual.

The tests suggested here should be carried out whenever a method is set up or modified in order to establish whether a linear or quadratic equation gives the better slope fit to the data. It is particularly important to carry out such tests when sample concentrations are analysed, which are higher than your normal concentration range. Some laboratories have run such tests on a regular basis during cruises to control the behaviour of their system, as particularly when working in high concentration ranges close to the end of the linear range of a method changes such as a contaminated reagent could shift the output into a non-linear range.

### 2. PRINCIPLE

Non-linearity in the output from an auto-analyser can come from two sources:

- (1) True non Beer-Lambert Law non-linearity, i.e., when the absorbance of a reacted solution exceeds that for which the particular method is linear. (In this case the method should become linear if the reaction mixture is diluted.)
- (2) A non-linear output related to the linearization performed by the electronics of the detector. (In this case the method will not become linear if the reaction mixture is diluted.)

The linearity of a method can be tested by running an appropriate number of standard solutions over the concentration range of interest and then examining the spread of residual differences between the data, and the best fit linear and quadratic calibration equations when fitted to that data.

The degree of likely error can then be estimated at the mid-point of the calibration range ideally this offset should be <0.5 %.

### 3. REQUIREMENTS

- An auto-analyser system
- System software set to provide raw data output for peak heights
- Ten standard solutions
- Spreadsheet or statistical software to calculate best fit and residuals

### 4. METHOD

1. Set up the auto-analyser to run the method of interest over the required concentration range.

2. Load the system table (and sample tray) with an appropriate number of standards at the start of the run for the particular peak height measurement software to work. Load the system with the ten standards. Set up the sample table and tray, or programme the X-Y sampler if being used, so that each sample is measured sufficient times to assess the noise of the run and to take into account variations resulting from peak height carryover. For ten samples numbered 0 to 9 the order might be 0123456789 9876543210 9876543210 0123456789.
3. Run the samples and download the peak heights for the ten standards at the end of the run
4. Load the results into Excel or similar software.
5. Plot sample concentration against peak height.
6. Calculate the best fit for both linear and quadratic equations.
7. Then calculate the residual difference between the observed and the best fit data points.
8. Plot the residual values against the concentration of the standards. (For a good fit the residuals should vary around zero with spread similar to the precision of the method.)

## 5. EXAMPLE RESULTS

Example results are presented below.

Table 1. Example data for linearity check			
Std conc.	Peak height	Linear fit	Quadratic fit
Analyser data		Calculated residuals	
0	0	0.0	0.0
1	100	2.2	-1.2
2	200	4.4	-1.4
3	300	6.6	-0.4
4	400	8.7	1.8
5	495	5.9	0.0
6	590	3.1	-0.6
7	685	0.3	-0.1
8	780	-2.5	1.5
9	870	-10.3	-0.8
0	0	0.0	0.0
1	102	4.2	0.8
2	202	6.4	0.6
3	303	9.6	2.6
4	404	12.7	5.8
5	500	10.9	5.0
6	596	9.1	5.4
7	691	6.3	5.9

8	785	2.5	6.5
9	876	-4.3	5.2
0	0	0.0	0.0
1	98	0.2	-3.2
2	198	2.4	-3.4
3	297	3.6	-3.4
4	396	4.7	-2.2
5	490	0.9	-5.0
6	584	-2.9	-6.6
7	679	-5.7	-6.1
8	775	-7.5	-3.5
9	864	-16.3	-6.8
0	0	0.0	0.0
1	101	3.2	-0.2
2	199	3.4	-2.4
3	302	8.6	1.6
4	397	5.7	-1.2
5	498	8.9	3.0
6	587	0.1	-3.6
7	685	0.3	-0.1
8	783	0.5	4.5
9	867	-13.3	-3.8
		sum of residual differences	
		72.7	-6.2

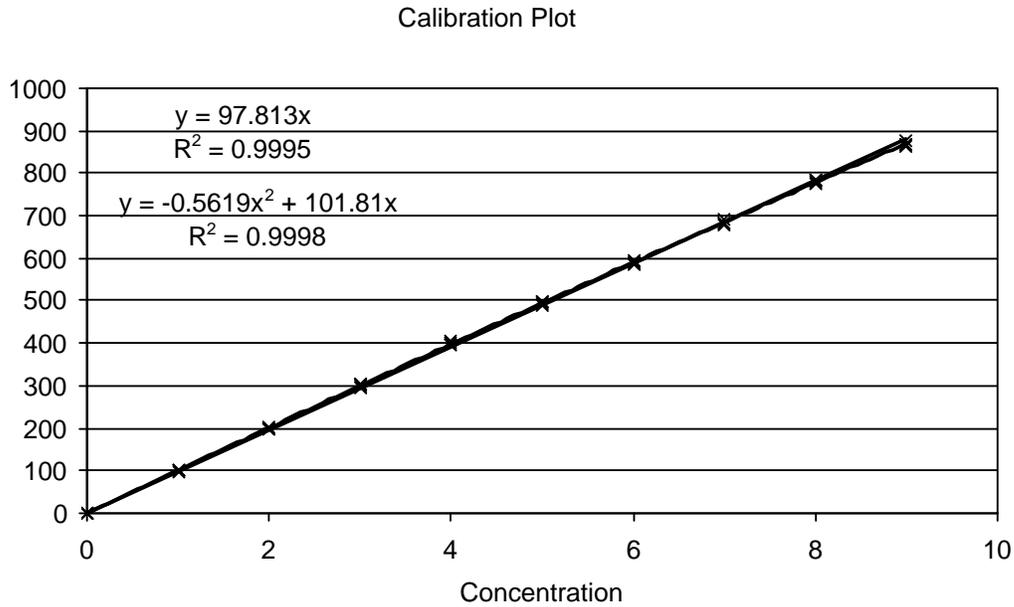


Figure 1. Plot of analyser data from Table 1, this shows the trend lines for both linear and quadratic equation fits. In this example the data appears to be linear and the  $R^2$  values are close to 1.0 in both cases

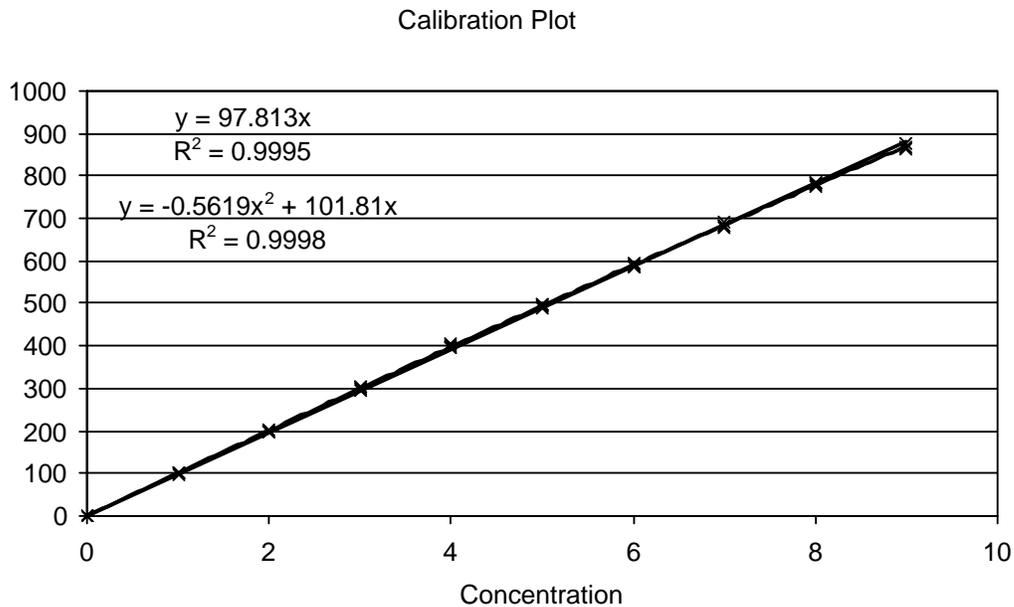
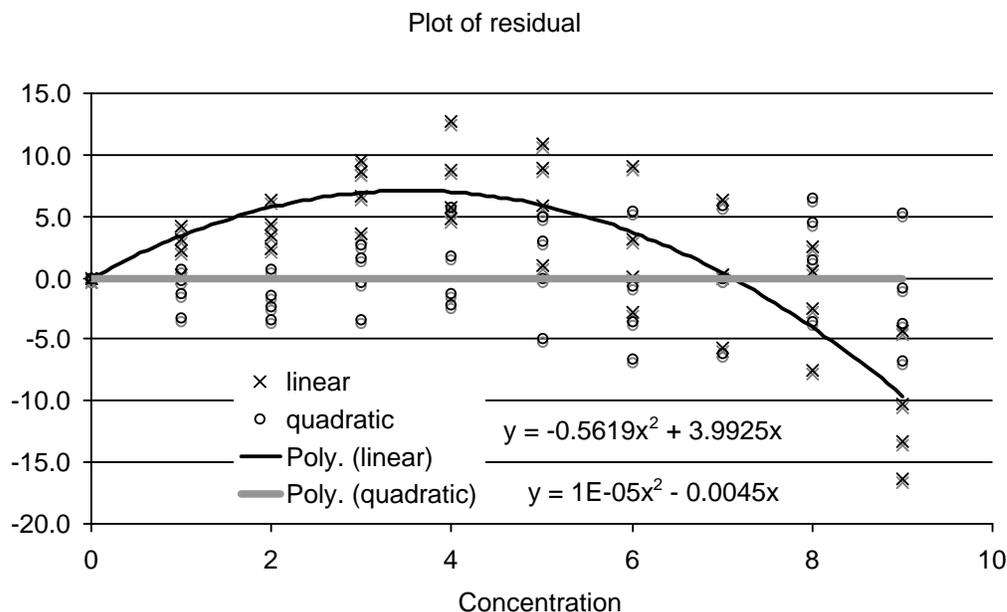


Figure 2. Plot of the residual difference between the measured values at each standard concentration and the best fit value calculated from equations for a linear and a quadratic fit to the data (top). A quadratic fit was then applied to both the linear or quadratic sets of residual data (bottom).



## 6. DISCUSSION

The data in Table 1 shows a method that gives a linear response up to the mid-point of the concentration range over which it is being applied. When both linear and quadratic equations are fitted to the data, relatively high  $R^2$  values of 0.9995 and 0.9998 respectively, are returned, and the method appears to be close to linear.

Plotting the residual values between the observed data and the best fit value of the peak height gives a magnified view of the differences (Figure 2). Clearly, when a linear fit forced through the origin is applied to the data then the values at intermediate concentrations are over estimated, but underestimated at high concentrations. The sum of the residuals is 73 in this case. Less bias is shown in the residuals estimated with the quadratic equation and the data is scattered around zero with the sum of the residuals also close to zero at -6.

Fitting a quadratic equation to the plotted residuals in Figure 2 suggests that the estimate using a linear fit would be 0.7 % high at mid concentrations and 1.0% low at high concentrations.

## 7. CONCLUSIONS

Before an analytical method is run on 'real' samples then the linearity of the output for all concentrations, should be checked. This is best done by looking at the residual differences between the observed concentration of standard solutions, and the value obtained when applying the calibration equation to the measured peak heights of the those standard solutions. The most appropriate equation (linear or quadratic) for calibrating the data can then be decided.

Note: If the quadratic equation gives a better fit the method can then be adjusted to run with a greater degree of dilution to see if the results become more linear. This will identify, if the non-linearity seen is due to an absorbance which is beyond the Beer-Lambert Law limit of the method,

or due to an inherent problem with the linearity of output from the detector of the auto-analyser.

## NSOP 5. DETERMINATION OF TRUE SAMPLE BLANK VALUE

### 1. INTRODUCTION

#### 1.1 Definition of the blank value

Following Taylor (1990) an analytical blank can be defined in the following way:

A blank is the measured value for the apparent concentration of a determinand obtained when the determinand is not present in the sample at the time of measurement, that is to say the measured value for the component is due to artefacts. The blank value (which may be positive or negative) should be deducted from a measured value to give a net value due to the actual quantity of the determinand contained in the sample. The blank measurement must be made in such a way that the applied correction is valid.

In order to obtain the true value for a determination it is essential to have access to the signal that would be obtained for a sample when the concentration of the determinand is zero. The sources of blank errors and problem specific to CFA analyses are considered below. It is essential that laboratories employ consistent procedures for assessing the blank values appropriate to the instrumentation that they are using and for the type of samples they are working with.

#### 1.2 Choice of approach for CFA to determining blank values

For work with samples with a small range of refractive index difference (generally open ocean samples) the approach can be simpler than when working with samples from river plumes and estuaries. Consequently two operating procedures are presented. Particularly when working at low concentrations it is critical important that the correct approach is used.

#### 1.3 Artifacts contributing to the blank

Four types of artifacts can contribute to the blank. The additive nature of these artifacts is illustrated in Figure 5.1. In order of priority for CFA analyses they are:

- *Contamination of the baseline water* - All baseline solutions (natural or artificial saline water or pure water) used have the potential for varying degree of contamination - even pure water can be contaminated during handling and storage.
- *Refractive index blank.* - A signal is generated by optically imperfect flowcells. This changes as the salinity of the samples varies (e.g. phosphate in an AAI type system an error of 0.04  $\mu\text{M}$  for a 10 units change in salinity can be typical). This signal is usually positive, but negative signals can occur. In addition transient signals can be generated at the start and end of peaks when light is reflected off the interface between waters of different density in the flow cell. This is the Schlieren effect. It can occur even in instruments in which the segmenting air bubble passes through the cell.
- *Sample turbidity* - A signal is generated by particles suspended in the sample. (Note: reaction with the reagents may modify the turbidity relative to that in the untreated sample.)

- *Reagent blank* - A signal is generated by contamination of the chemical reagents used in the analysis and their optical characteristics (absorbance or fluorescence). In CFA because the reagent blank affects samples and the baseline equally it can usually be discounted, except in the case of a contamination event that makes the method more non-linear than would normally be expected by significantly increasing the total absorbance of the reacted solution.

#### 1.4 General formulae

Let us define the optical components of the baseline and of sample peaks.

**RB** = reagent blank.

**RIb** = saline water baseline refractive index signal.

**RI<sub>s</sub>** = sample refractive index blank.

**C<sub>b</sub>** = height corresponding to the concentration of the determinand in the baseline.

**C<sub>s</sub>** = height corresponding to the net concentration of the determinand in the sample.

**T** = sample turbidity (for unfiltered samples).

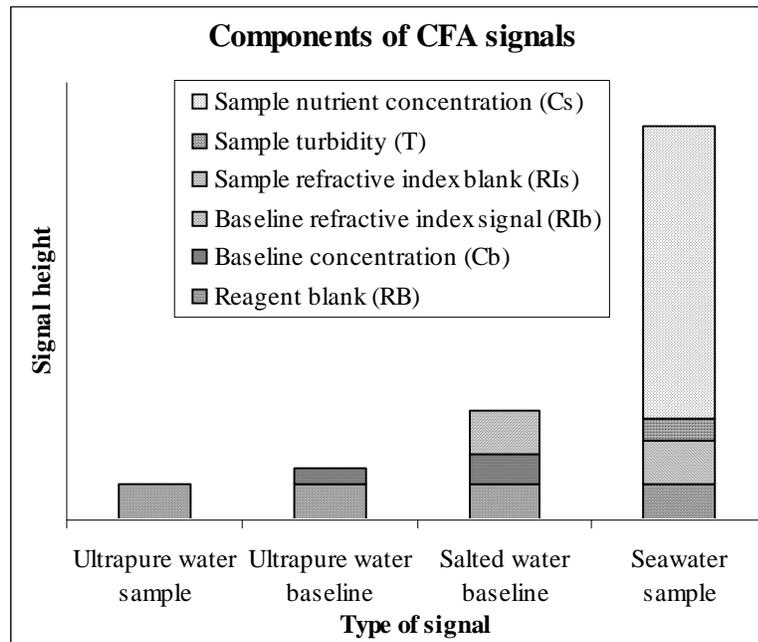


Figure 1. Components of baselines and peak signals in CFA.

Now, the signal height can be expressed as follows (for simplification the constant electronic signal expected for a reagent blank equal to zero has been omitted):

The height for the **baseline** is:

$$H_b = C_b + RB + RI_b \quad (1)$$

The height for a **sample peak** is:

$$H_s = C_s + RB + RI_s + T \quad (2)$$

The sample height relative to the baseline is:  $H_{s(\text{measured})} = (H_s - H_b)$ , i.e., when combining (1) and (2):

$$H_{s(\text{measured})} = C_s + RB + RI_s + T - (Cb + RB + RI_b)$$

$$H_{s(\text{measured})} = (C_s - Cb) + (RI_s - RI_b) + T$$

The height corresponding to the **net concentration in the sample** is:

$$C_s = H_{s(\text{measured})} + Cb - (RI_s - RI_b + T) \quad (3)$$

1.4.1 If the baseline is pure water:

In that case  $RI_b = 0$ , hence:

$$C_s = H_{s(\text{measured})} + Cb - [RI_s + T] \quad (4)$$

$Cb$  is determined by difference between the baseline signal and the signal of a freshly drawn ultra pure water ( $PW_0$ ). If using pure water as baseline  $Cb$  has to be determined by comparison with freshly drawn water on a regular basis. This can be done as an “extra” cup on the run. Turbidity  $T$ , which is generally negligible for oceanic waters (and = 0 for filtered samples), is measured together with the  $RI_s$  (see operating procedure NSOP 5.1).

The great advantage of using pure water to provide the baseline is that pure water is easily available and  $Cb$  should be close to zero. The disadvantages are:- (1) that the refractive index blank potentially has to be determined for each saline sample, if the optics of the analyser used generate a significant refractive index blank; (2) if a Schlieren effect occurs, this can distort the peak shape and consequently increase the time each sample must be sampled for in order to achieve a stable peak height.

1.4.2 If the baseline is saline water:

The idea of using a saline baseline is principally to minimise the Schlieren effect and also to avoid having to make a correction for the refractive index blank, when the samples have similar salinity.

$$C_s = H_{s(\text{measured})} + Cb - [(RI_s - RI_b) + T] \quad (3)$$

However if the salinity of samples varies significantly and/or turbidity is not negligible, all artefacts must be determined. How large a “significant” variation in salinity is, is highly dependent on the instrumentation, the particular method being used and the precision required from it.

In equation (3),  $(RI_s - RI_b)$  is the difference between the refractive index signal of the sample and the saline baseline. This relative refractive index blank is small and ideally  $(RI_s - RI_b) = 0$  when the baseline and the samples have similar salinities. This particular case is generally achieved when working with ocean waters where the range of salinity encountered on a particular cruise will usually be small and  $T$  will also be effectively zero. In that particular case, equation (3) becomes:

$$C_s = H_{s(\text{measured})} + Cb \quad (5)$$

In which case the critical aspect is the determination of  $Cb$ .

1.4.3 Determination of  $Cb$  and  $RI_b$

Both  $Cb$  and  $RI_b$  must be monitored. The refractive index blank  $RI_b$  should be relatively

invariant if the same instrument and method are being used. However  $C_b$  can potentially change each with each batch of solution used as the baseline.

This can be achieved by making a set of measurements of the baseline and samples with and without colour development, so that the contribution to the signal of the background signal due to contamination and refractive index variations can be distinguished. For this a set of so called “RIs reagents” are used (see section 1.5 of this NSOP). The heights  $C_b$  and  $RI_b$  are converted into concentrations by determining the sensitivity of the method  $S$  (in terms of peak measured per unit concentration of the determinand).

For work with a saline water baseline four measurements of the baseline are made after the sensitivity has been determined. The procedure is described in NSOP 5.2.

### 1.5 The RIs (colour free) reagents

The refractive index blanks ( $RI_s$  and  $RI_b$ ) are the absorbances resulting from the differences in optical properties between fresh water and seawater in the absence of any colour development (Loder and Glibert, 1977; Froelich and Pilson, 1978). The refractive index depends on salinity, but also on reaction conditions which may affect the refractive index or the turbidity, such as the total salt content and the pH of the reagents. To measure a  $RI_s$ , the signals from fresh and seawater must be compared in conditions as close as possible to those of the analysis but without any colour development. This requires that what we call ‘RIs reagents’, are prepared. They omit the one component, which is indispensable for colour development.

By using RIs reagents, the optical properties of the medium are kept as similar as possible to those prevailing during the colour forming reaction. It is sometimes recommended, that pure water can be used for the RIs reagents. However this fails to accurately determine refractive index differences. Similarly, it is important to keep the wetting agent in the solutions to maintain the hydraulic stability of the flow and as the wetting agent often contributes the refractive index. Note: that if a reagent contains only the chemical that should be removed, the corresponding RIs reagent is then pure water. For example, the chemicals removed from the RIs reagents are - in the molybdenum blue methods for phosphate and silicate it is the molybdate, and in the Benschneider and Robinson’s method for nitrite (and nitrate) it is the NED (Aminot et al., 2009).

### 1.6 Recommendation

To obtain accurate values of sample concentrations several corrections have to be applied to the raw values of sample peak heights. Minimising their number and reducing the analysis steps reduces cumulative errors and increases data quality. Working with a pure water (18 megohm.cm) baseline can help achieve this aim if the instrumentation and the method being used allow it. This requires that optical artefacts such as the Schleiern effect remain small enough to allow satisfactory measurements of the peak heights.

## NSOP 5.1 DETERMINATION OF THE BLANK VALUE WHEN WORKING WITH SALINE SAMPLES AND USING PURE WATER TO PROVIDE THE BASELINE MEASUREMENT.

### 1. SCOPE AND FIELD OF APPLICATION

This NSOP describes the sequence of measurements that need to be made when pure water is used to provide the baseline measurement in a CFA system. It is appropriate for any type of water, provided the Schlieren effect does not excessively distort the peak shape.

### 2. PRINCIPLE

Freshly drawn pure (18 megohm.cm) water is used and is assumed to contain negligible concentration of the determinand. This removes the uncertainty that is inherent in using a saline solution. In this procedure, raw sample concentrations are computed from raw heights and only the slope of the calibration curve, before the sample blanks are subtracted to provide the net concentrations.

Section 1.4 of NSOP 5 shows that for baseline concentration, the size of the sample refractive index (*RI<sub>s</sub>*) effect and the sample turbidity (*T*) must be determined.

$$Cs = Hs_{(measured)} + Cb - [RIs + T] \quad (4)$$

Where

<b><i>H<sub>s</sub></i></b>	= height of sample peak
<b><i>H<sub>b</sub></i></b>	= height of pure water baseline with complete reagents
<b><i>H<sub>b</sub>PW<sub>0</sub></i></b>	= height of freshly drawn pure water sample with complete reagents,
<b><i>C<sub>b</sub></i></b>	= height corresponding to the concentration of the determinand in the baseline. $Cb = Hb - HbPW_0$
<b><i>H<sub>b</sub>(RIs)</i></b>	= height of pure water baseline with RIs reagents
<b><i>H<sub>s</sub>(RIs)</i></b>	= height of sample peak with RIs reagents,

For each sample

$$(RIs + T) = (Hs(RIs) - Hb(RIs))$$

Figure 1 of this NSOP illustrates the determination of these artifacts.

### 3. EQUIPMENT

Standard Continuous Flow Analyzer (CFA) equipment

### 4. REAGENTS

- Stock standards of the specific nutrient
- Baseline solution - pure water

- Freshly drawn pure water of 18megohm.cm (See notes, section 6)
- Reagent solutions used in the specific analysis on the CFA instrument
- Reagent solutions (“RIs reagents”) the same as the above but with the essential colour forming chemical removed (See section 1.5 of NSOP 5.)

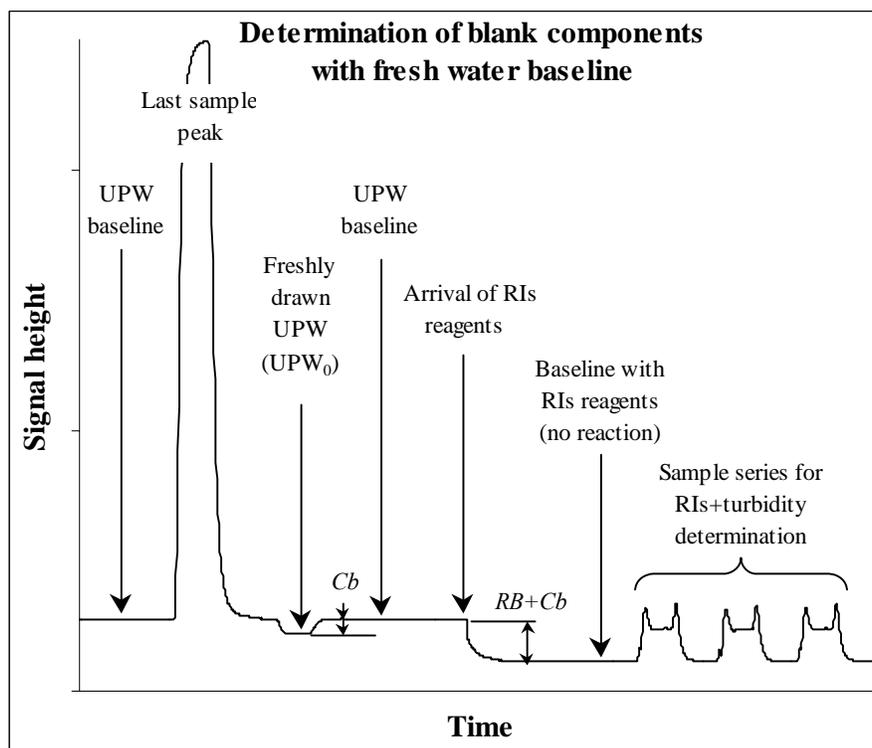


Figure 1. Analytical sequence for the determination of sample blanks using ultrapure water (UPW) for the baseline.  $C_b$  = baseline concentration;  $RB$  = reagent blank.

## 5. PROCEDURE

**Normal run including measurement of freshly drawn pure water.** During the run with normal reagents, freshly drawn pure water is analysed like a sample. Record the baseline ( $H_b$ ). The height of the pure water peak  $H_{bPW_0}$  is the signal at the true zero concentration level. The difference between this signal and the baseline level is height corresponding to the concentration of the determinand in the baseline, ( $C_b = H_b - H_{bPW_0}$ ). The terms  $C_b$  and  $H_{s_{(measured)}}$  in equation 4 are now known.

**Check on reagent blank.** After the last peak has passed with the normal (complete) reagents, replace the key colour-forming reagent(s) with the RIs reagent(s). Measure the new baseline  $H_b(RIs)$ . The difference between the baseline levels with normal reagents and with RIs reagents is equal to the sum of the baseline concentration and the reagent blank, i.e. ( $C_b + B_r$ ), using the previously defined terms.

**Determination of refractive index and turbidity signals of each sample.** Rerun the samples.

Record the peak heights  $H_s(RI_s)$ . For each sample  $H_s(RI_s)$  corresponds to the sum of refractive index and the turbidity,  $H_s(RI_s) = (RI_s + T)$  see equation 4.

**Calculation.** By combining the results of stages 1 and 3, equation 4 is now solved and  $C_s$  is now known for each sample and can then used in the normal way to calculate the results.

## 6. NOTES

Dissolved silicate can be present in ultra pure water even if the conductivity reading on the dispensing unit is 18 megohm.cm. Therefore the blank determination for silicate should be done with water obtained from equipment where the purification cartridges are known to have been recently replaced.

The omitted chemicals (“essential chemicals”) are:

$PO_4$	Ammonium Molybdate
Si	Ammonium Molybdate
$NO_x$	NED
$NO_2$	NED
$NH_4$	Sodium Hypochlorite

Usually,  $C_b = 0$ , but contamination may occur for ammonium (from handling) and silicate (from aged ion exchange resins).

In general, with oceanic waters of sample salinity and turbidity are similar so that differences of  $[RIB+T]$  among samples are insignificant and only a few representative samples need to be checked to obtain that value.

## NSOP 5.2 DETERMINATION OF THE BLANK VALUE WHEN WORKING WITH OCEAN WATER SAMPLES AND A SALINE BASELINE WATER OF SIMILAR SALINITY (LOW NUTRIENT SEAWATER LNS OR ARTIFICIAL SEAWATER ASW).

### 1. SCOPE AND FIELD OF APPLICATION

This NSOP describes the procedure to determine the nutrient concentration in a saline water used as the baseline solution in a CFA-system. It is appropriate for use with samples from oceanic waters where the experience has been that the instrumentation used is insensitive to the range of salinity and turbidity encountered.

### 2. PRINCIPLE

Signals contributing to the measured baseline are generated by the natural concentration of the determinand in and/or contamination of the LNS/ASW and by the optical properties of the solution in the flow cell. Freshly prepared pure water can provide a zero concentration reference but also might have an associated optically generated signal different to that of the saline baseline solution. The concentration of the determinand in the baseline solution must therefore be determined in two stages.

The need is to determine

***C<sub>b</sub>*** = height corresponding to the concentration of the determinand in the baseline.

***RI<sub>b</sub>*** = height of saline water baseline refractive index signal.

The sensitivity of the analysis (measured as peak height per unit of concentration) is determined in the normal way.

The nutrient concentration of LNS/ASW is then determined by measuring the signal of the baseline using the LNS/ASW and then freshly prepared pure water in conjunction with the standard combination of reagents. The difference gives a measure of  $(C_b + RI_b)$ . In the second stage the measurements of the baselines for LNS/ASW and pure water is repeated using a set of reagents with the essential colour-forming reagent removed (RIs reagents). The difference gives a measure of  $(RI_b)$ .

The following signals are measured:

***HS*** = net height of calibration standard

***S*** = sensitivity ( $HS / (\text{concentration of standard})$ )

***H<sub>b</sub>*** = height of saline water baseline with complete reagents,

***H<sub>b</sub>PW<sub>0</sub>*** = height of freshly drawn pure water baseline with complete reagents,

***H<sub>b</sub>(RIs)*** = height of saline water baseline with RIs reagents,

***H<sub>b</sub>PW<sub>0</sub>(RIs)*** = height of freshly drawn pure water with RIs reagents

### 3. EQUIPMENT

Standard Continuous Flow Analyzer (CFA) equipment

### 4. REAGENTS

- Stock standards of the specific nutrient
- Baseline solution - Low Nutrient Seawater or Artificial Seawater
- Freshly drawn pure water of 18 megohm.cm (See note, section 8.1)
- Reagent solutions used in the specific analysis on the CFA instrument
- Reagent solutions (“RIs reagents”) the same as the above but with the essential colour forming chemical removed (See note, section 8.2)

## 5. PROCEDURE

1. Prior to the blank determination prepare a standard in LNSW/ASW at an appropriate concentration for detecting small signals in *Cb* and *RIb* (see note, section 8.3).
2. Start up the CFA-system as normal, using the LNSW/ASW as a baseline and standard reagents.
3. Wait for stable baseline to be achieved.
4. Sample the standard for 120 seconds and adjust the gain appropriately (see note, section 8.3)
5. Wait for stable baseline to be achieved for at least 5 minutes record baseline (*Hb*).
6. Sample the standard for 120 seconds and record height (*HS*)
7. (When the peak of this standard shows on the screen) Exchange the LNS/ASW baseline solution for freshly drawn pure water. (During this exchange, introduce enough air into the system so you can see when this new reagent has gone through the flowcell by looking at a spike from these air bubbles. (see note, section 8.4)). Record a stable baseline for 5 minutes again (*HbPW<sub>0</sub>*)
8. Change the appropriate standard reagent bottle to the “RIs reagent” bottle, After the air bubble peak has been observed record a stable baseline for 5 minutes again (gives *HbPW<sub>0</sub>(RIs)*).
9. Change the baseline solution back to LNSW/ASW. After the air bubble has been observed record a stable baseline for 5 minutes again (gives *Hb(RIs)*).
10. Change the reagents back to the working reagents.

## 6. CALCULATION

1. Sensitivity  $S = HS / (\text{concentration of standard})$

2. Measure of “*Cb + RIb*”

$$(Hb - HbPW_0) = A$$

3. Measure of “*RIb*”

$$Hb(RIs) - HbPW_0(RIs) = B$$

Hence the correction that has to be added to the sample values i.e. the true blank is

4.  $Cb$  (height of true blank) =  $(A-B)$

5. True sample concentration =  $(Cm + Cb) / S$

Where  $Cm$  is the measured height of the sample peak above the baseline.

## 7. CAUTION

As the baseline water can be contaminated during handling and storage this means that depending on the working conditions, the background level of nutrients may not be the same in the LNS/ASW used to prepare the working standards as that in the baseline LNS/ASW, being pumped through the analyser - even though both came from the same bulk solution at some point in time. Effort should be made to minimise such discrepancies.

A way to do this is to determine the calibration (zero) blank as one of the set of measured calibration standards. It should be determined on LNS/ASW drawn from the bulk solution providing the baseline at the same time as the water used to prepare the spiked standards and should be handled and stored in the same way.

## 8. NOTES

1. Dissolved silicate can be present in ultra pure water even if the conductivity reading on the dispensing unit is 18 megohm.cm. Therefore the blank determination for silicate should be done with water obtained from equipment where the purification cartridges are known to have been recently replaced.
2. The omitted chemicals (“essential chemicals”) are:
 

PO <sub>4</sub>	Ammonium Molybdate
Si	Ammonium Molybdate
NO <sub>x</sub>	NED
NO <sub>2</sub>	NED
NH <sub>4</sub>	Sodium Hypochlorite
3. The calibration standard should be at an appropriate concentration for detecting small signals in *Cb* and *Rlb*. For measurement of at macro nutrient concentrations in ocean water for silicate and nitrate a standard concentration of 5 µM l<sup>-1</sup> would be appropriate and for phosphate 1 µM l<sup>-1</sup> with the peak height adjusted to give full scale “deflection”. Those determining micro-nutrient concentration would work with lower concentration standards.
4. Marking the change over by introducing extra air should be done with each change-over of baseline solution and reagents.

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## **NSOP 6. IMPROVING THE INTER-RUN PRECISION OF NUTRIENT MEASUREMENT BY USE OF A TRACKING STANDARD**

### 1. SCOPE AND FIELD OF APPLICATION

This NSOP describes the procedure developed at RNIOZ (Texel, Netherlands), which uses results from measurements of a stable artificial standard solution made through out a cruise to adjust the data and there by improve inter-run precision.

### 2. PRINCIPLE

The assumption are (1) that the preparation of so called “tracking” standard solution by single dilution of stable mixed standard solution can produce a solution containing concentrations of nutrients which are less variable than the calibration relationship measured on each run of a CFA system measuring nutrient during a cruise; (2) if this precisely prepared solution is measured on each run then the output from each run can be adjusted by the ratio between the concentration of the standard measured on that run and the average of all runs, in order to improve the overall precision of the measurements during the cruise.

### 3. EQUIPMENT

- Volumetric flask calibrated to 0.01 %
- Dispensing pipette calibrated and with a precision of 0.1 %
- Thermometer reading to 0.1°C

### 4. REAGENTS

- Mixed secondary standard containing know amounts of nitrate, phosphate and silicate preserved by the addition of Mercuric Chloride to a concentration of 0.02 g l<sup>-1</sup> (HgCl<sub>2</sub>)
- Low nutrient seawater or sodium chloride solution (40 g l<sup>-1</sup>)

### 5. PROCEDURE

1. Prior to each run of the CFA system prepare a fresh tracking standard recording the temperature. The concentration should be chosen to be about 80 % of that of the top standards.
2. During the run measure the standard a minimum of three times spaced through the run.
3. At the end of the run record the mean value for the standard.
4. Validate the use of the standard by measuring in duplicate the deepest sample taken from the rosette on the analytical run for the samples from that rosette cast.
5. For the validation repeat the measurement of the duplicated deep sample on the run for samples from the next cast.
6. During the cruise prepare a control chart for the values of the tracking standard.
7. At the end of the cruise calculate the mean value for the tracking standard.
8. For each analytical run calculate the ratio of the value of the tracking standard on that run to the mean value of the tracking standard for the cruise.
9. Adjust the values for each run by the ratio of the tracking standard values.

10. Compare the route mean square value of the difference between measurements of the deep sample duplicates measured in a run and the difference between run.
11. Use of the tracking standard is acceptable if the root mean square differences are smaller after adjustment to the tracker standard value.

## 6. NOTES

All details of this adjustment process and the control chart of the tracer values should be include as part of the cruise meta-data set.

Alternatively RMNS materials produced from the same batch can be used to the same effect as the tracking standards, however due to cost implications it is normally the case to use a tracking standard on this regular basis but then compare and check against a RMNS standard every few days during a cruise, hence this links the tracking standards and the RMNS solutions, and hence calibrates the tracking standard also. Details of these results should also be logged and compared as before.

## **NSOP 7. WATER SAMPLING AND STORAGE OF SAMPLES FOR THE DETERMINATION OF CONCENTRATIONS OF NUTRIENTS IN SEAWATER**

### 1. SCOPE AND FIELD OF APPLICATION

This NSOP describes how to collect discrete samples, from a Niskin or other water sampler, that are suitable for the analysis of dissolved nutrients - nitrate, nitrite, phosphate and silicate. It also describes how samples should be stored after collection if necessary.

### 2. PRINCIPLE

A sample of seawater is collected in a sterile plastic container that can be tightly sealed for short or long term storage.

### 3. APPARATUS

#### ***Water Samplers***

At the beginning of a cruise leg and at weekly intervals, the water samplers should be inspected for evidence of biological and damaged components. Any rust should be removed and damaged components replaced. Microbial films should be removed using a soft sponge and Decon 90. (Brushes, and scouring agents and pads must not be used as they will damage the surface of the bottle and increase the likelihood of future contamination).

#### ***Drawing tube***

Tygon tubing is normally used to transfer the sample from the Niskin to the sample container; however, if dissolved organic carbon samples are being collected from the same Niskins, then it may be necessary to use silicone tubing to prevent contamination from the Tygon. When oxygen samples are also being collected the drawing tube should be pre-treated by soaking in clean seawater for at least one day. This minimizes the amount of bubble formation in the tube when drawing a sample.

#### ***Sample container***

The largest errors in nutrient analysis tend to be due to poor choice of sample containers, compounded by inappropriate storage. Seawater as it comes from the sampling apparatus on the ship is a relatively sterile solution, particularly when sampled below the thermocline and will therefore be slow to change if placed in a sterile container. It is therefore a gross error to put samples into non-sterile containers. That is any container other than an autoclaved one that has been used previously. Disposable containers such as 30ml Coulter Counter vials provide a simple source of sterile containers when used once and then disposed of. It is essential that you check your chosen containers both for contamination and sterility.

### 4. PROCEDURE

#### 4.1 Introduction

The order in which different samples are taken from the Niskin bottle will be decided by the principal scientist on the cruise taking into consideration the stability of the components being sampled for. Nutrients are relatively stable and will normally be sampled towards the end of the process before salinity samples are drawn.

#### 4.2 Filling procedure

- Fill a sample vial rack - with an appropriate number of vials for the number of bottles on the rosette and the number of duplicates to be taken.
- Clearly label each sample vial with the bottle number and a unique identity for the cast.
- Check that the drawing tube is clean - replace if necessary
- Rinse the sample container — Rinse the container and its lid twice with half to a third the volume of the container of sample
- Fill the sample container three quarters full.
- Check the headspace — Check the vial has not been over filled, a head space is necessary if subsequently samples need to be frozen. Firmly tighten the container's lid

#### 4.3 Sample documentation

The following information must be recorded in the sampling logbook at the time of sampling:

- Time and date when taken.
- Full name of person who took sample.
- Location: an unambiguous designation of the station, cast, and the rosette position from which the sample was taken.
- Container designation: a number or alphanumeric symbol unique to the sample container; and the cruise.
- Comments: additional information such as conditions when sampling, problems with sample collection, etc.

### 5. SAMPLE STORAGE

During a cruise samples should be stored in a cool, dark, location (preferably refrigerated but not frozen) until use. Ideally nutrient samples should be analysed immediately after sampling to avoid any possibility of biological growth or decay in the samples.

#### 5.1 Sample storage with freezing

If storage is necessary for more than a two to three days samples should be frozen as soon after collection and as rapidly as possible. Before freezing ensure that sample bottles are no more than 3/4 full and firmly capped. A deep freezer (at least -20 °C) should be used. Good air circulation around the bottles in the freezer is important. Sample pots should be retained in labeled gridded racks, so that they can be easily found and sorted for analysis when the time has come to measure them. Samples should be thawed in air. Water baths should not be used because of the danger of contamination from tap water. As the sample melts and comes to room temperature its volume goes through a minimum the resulting low pressure in the containers can suck in contaminating water from a water bath.

Samples for the determination of Si should be allowed to stand for at least 24 hours at room temperature for de-polymerisation to occur. For work at higher (>40 mM/m<sup>3</sup>) concentrations you should check that your freezing and thawing procedures are appropriate.

## 6. QUALITY ASSURANCE

Duplicate sampling is recommended, both from the same sampler (e.g., Niskin bottle) and if possible, from two (Niskin) samplers tripped together at the same depth, to assess the quality of the sampling procedures.

It is important that the time at which a sample was measured is recorded in the meta-data. This will potentially allow discrepant data resulting from in appropriately long storage to be identified..

## **NSOP 8. WATER SAMPLING AND STORAGE OF SAMPLES FOR THE DETERMINATION OF LOW CONCENTRATIONS OF NUTRIENTS IN SEAWATER**

### 1. SCOPE AND FIELD OF APPLICATION

This SOP describes how to collect discrete samples, from a Niskin bottle or other water samplers that are suitable for the analysis of low concentrations dissolved nutrients - ammonium, nitrate, nitrite and phosphate. It also describes how samples should be stored after collection if this cannot be avoided. The recommendation is that samples are not stored.

### 2. PRINCIPLE

A sample of seawater is collected in a clean, 'aged', sterile plastic (HDPE) container that can be tightly sealed for short or long term storage.

### 3. APPARATUS

#### ***Water Samplers***

At the beginning of a cruise leg and at weekly intervals, the water samplers should be inspected for evidence of biological and damaged components. Only water samplers with external springs should be used. Any rust should be removed and damaged components replaced. Microbial films should be removed using a soft sponge and Decon 90. (Brushes, and scouring agents and pads must not be used as they will damage the surface of the bottle and increase the likelihood of future contamination).

#### ***Drawing tube***

For low concentration samples and dissolved organic carbon samples to transfer the sample from the Niskin to the sample container; "aged" silicon tubing should be used (Tygon tubing can generate contamination). When oxygen samples are also being collected the drawing tube should be pre-treated by soaking in clean seawater for at least one day. This minimizes the amount of bubble formation in the tube when drawing a sample.

#### ***Sample container***

The largest errors in nutrient analysis tend to be due to poor choice of sample containers, compounded by inappropriate storage. Seawater as it comes from the sampling apparatus on the ship is a relatively sterile solution, particularly when sampled below the thermocline. It is therefore a gross error to put samples into non-sterile containers. You must check and document how well the containers you use do their job with respect to both contamination and loss of nutrients.

### 4. PROCEDURE

#### 4.1 Introduction

The order in which different samples are taken from the Niskin bottle will be decided by the Principal Scientist on the cruise taking into consideration the stability of the components being sampled for. Nutrients are relatively stable and will normally be sampled towards the end of the process before salinity samples are drawn. However, for ammonium analysis and for nanomolar nitrate, nitrite and phosphate then the sampling should be as soon as possible to allow on-board analysis to take place

immediately. In this case the nutrients would be sampled after any gas sampling procedures. It is imperative that the gloves worn for nutrient sampling are clean and non-contaminating. They should be tested by leaving in Milli-Q water and then analysing to see if the gloves proposed actually leach any nutrients out. Any persons sampling before the nutrients should also wear the appropriate gloves.

#### 4.2 Filling procedure

- Fill a sample vial rack - with an appropriate number of vials for the number of bottles on the rosette and the number of duplicates to be taken.
- Clearly label each samples vial with a unique identity for the cruise, and the sampling event.
- Check that the drawing tube is clean – clean thoroughly or replace if necessary
- Rinse the sample bottle — Rinse the vial and its lid thrice (with a third to half the container volume sample).
- Fill the sample bottle — Fill the vial three quarters full.
- Check the headspace — Check the vial has not been over filled, a head space is necessary if subsequently samples need to be frozen. Firmly tighten the vial's lid.

#### 4.3. Sample documentation

The following information must be recorded in the sampling logbook at the time of sampling:

- Time and date when taken.
- Full name of person who took sample.
- Location: an unambiguous designation of the station, cast, and bottle number from which the sample was taken.
- Container designation: a number or alphanumeric symbol unique to the sample container; and the cruise.
- Comments: additional information such as conditions when sampling, problems with sample collection, etc.

### 5. SAMPLE STORAGE

Ideally nutrient samples should be analysed immediately after sampling to avoid any possibility of biological growth or decay in the samples. If necessary, samples should be stored in a cool, dark, location (preferably refrigerated but not frozen) until use, but ideally no longer than 1-2 hours.

#### 5.1 Sample storage with freezing

If storage is necessary samples should be frozen as soon after collection and as rapidly as possible. Before freezing ensure that sample bottles are no more than 3/4 full and firmly capped. A deep freezer (at least -20 °C) should be used. Good air circulation around the bottles in the freezer is important. Sample pots should be retained in labelled gridded racks, so that they can be easily found and sorted for analysis when they the time has come to measure them.

Samples should be thawed in air. Water baths should not be used because of the danger of contamination from tap water. As the sample melts and comes to room temperature its volume goes

through a minimum the resulting low pressure in the containers can suck in contaminating water from a water bath.

Samples for the determination ammonium, nitrate and phosphate are best measured as soon as the possible after thawing. It is recommended that a series of internal standards are added to the samples before freezing to act as a freezing ‘tracking standard’, this can show how well the samples have survived the freezing process and what artifacts have occurred as a result.

## 6. QUALITY ASSURANCE

Duplicate sampling is recommended, both from the same sampler (e.g., Niskin bottle) and, if possible, from two Niskin bottle samplers tripped together at the same depth, to assess the quality of the sampling procedures.

It is important that the time at which a sample was measured is recorded in the meta-data. This will potentially allow discrepant data resulting from inappropriately long storage to be identified..

## NSOP 9. EXAMPLE SOP FOR SHIPBOARD OPERATION OF A CFA SYSTEM

### 1. SCOPE AND FIELD OF APPLICATION

This SOP describes the standardised set up of one laboratory's system for the shipboard determination of nutrients (nitrate + nitrite, nitrite, phosphate and silicate). An SOP of this type should be part of the meta-data reported at the end of each cruise. Each laboratory's SOP should be updated as necessary before a cruise and the procedures outlined in the SOP followed during the cruise.

### 2. PRINCIPLE

The purpose of this SOP is to provide a record of how a CFA system was operated during a cruise. It should ensure that work on the cruise is carried out in a consistent and reproducible manner. It should also ensure that key procedures that aid in maintaining the relative accuracy of data such as the calibration of volumetric ware and pipettes are carried and documented in traceable way prior to and post cruise.

### 3. EXAMPLE STANDARD OPERATING PROCEDURES FOR SEA GOING AUTO-ANALYSER USE PREPARED BY MARINE WORKS, JAPAN LTD., FOR THE USE OF A "BRAN AND LUEBBE TRAACS 800" SYSTEM AT SEA

#### 3.1 Methods

The analytical methods of the nutrients during this cruise are similar with previous cruises (Aoyama et al., 2005).

**Nitrate + nitrite:** Nitrate + nitrite and nitrite are analyzed following a modification of the method of Grasshoff (1970). The sample nitrate is reduced to nitrite in a cadmium tube the inside of which is coated with metallic copper. The sample stream after reduction is treated with an acidic, sulfanilamide reagent to produce a diazonium ion. N-1-Naphthylethylene-diamine added to the sample stream to produce a red azo dye. With reduction of the nitrate to nitrite, both nitrate and nitrite react and are measured; without reduction, only nitrite reacts. Thus, for the nitrite analysis, no reduction is performed and the alkaline buffer is not necessary. Nitrate is computed by difference.

**Silicate:** The silicate method is analogous to that described for phosphate. The method used is essentially that of Grasshoff et al. (1983). Silicomolybdic acid is first formed from the silicate in the sample and molybdic acid. The silicomolybdic acid is reduced to silicomolybdous acid, or "molybdenum blue," using ascorbic acid.

**Phosphate:** The phosphate analysis is a modification of the procedure of Murphy and Riley (1962). Molybdic acid is added to the seawater sample to form phosphomolybdic acid which is in turn reduced to phosphomolybdous acid using L-ascorbic acid as the reductant.

The flow diagrams and reagents for each parameter are shown in Figures 1-4 below.

## Nitrate Reagents

Imidazole (buffer), 0.06 M (0.4 % w/v) Dissolve 4 g imidazole,  $C_3H_4N_2$ , in ca. 1000 ml DIW; add 2 ml concentrated HCl. After mixing, 1 ml Triton(R)X-100 (50 % solution in ethanol) is added.

Sulfanilamide, 0.06 M (1 % w/v) in 1.2M HCl. Dissolve 10 g sulfanilamide,  $4-NH_2C_6H_4SO_3H$ , in 900 ml of DIW, add 100 ml concentrated HCl. After mixing, 2 ml Triton(R)X-100 (50 % solution in ethanol) is added.

N-1-Naphthylethylene-diamine dihydrochloride, 0.004 M (0.1 % w/v). Dissolve 1 g NEDA,  $C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl$ , in 1000 ml of DIW and add 10 ml concentrated HCl. Stored in a dark bottle.

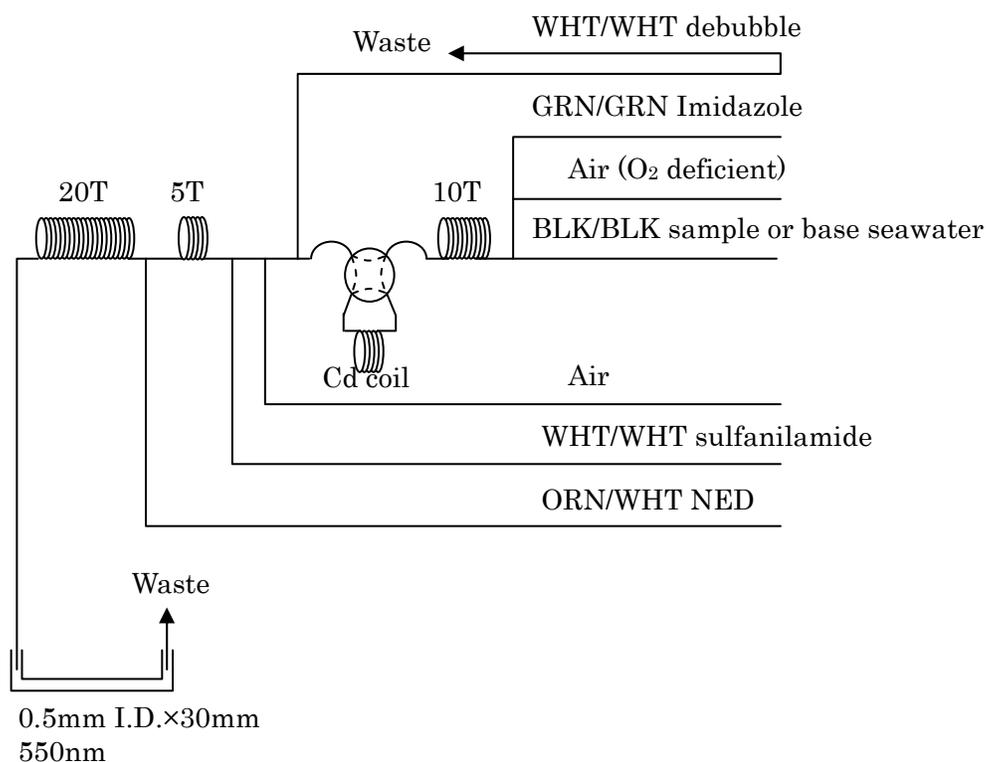


Figure 1. Flow diagram Nitrate + Nitrite.

## Nitrite Reagents

Sulfanilamide, 0.06 M (1 % w/v) in 1.2 M HCl. Dissolve 10g sulfanilamide, 4-NH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>3</sub>H, in 900 ml of DIW, add 100 ml concentrated HCl. After mixing, 2 ml Triton(R)X-100 (50 % solution in ethanol) is added.

N-1-Naphthylethylene-diamine dihydrochloride, 0.004 M (0.1 % w/v). Dissolve 1 g NEDA, C<sub>10</sub>H<sub>7</sub>NHCH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub> • 2HCl, in 1000 ml of DIW and add 10 ml concentrated HCl. Stored in a dark bottle.

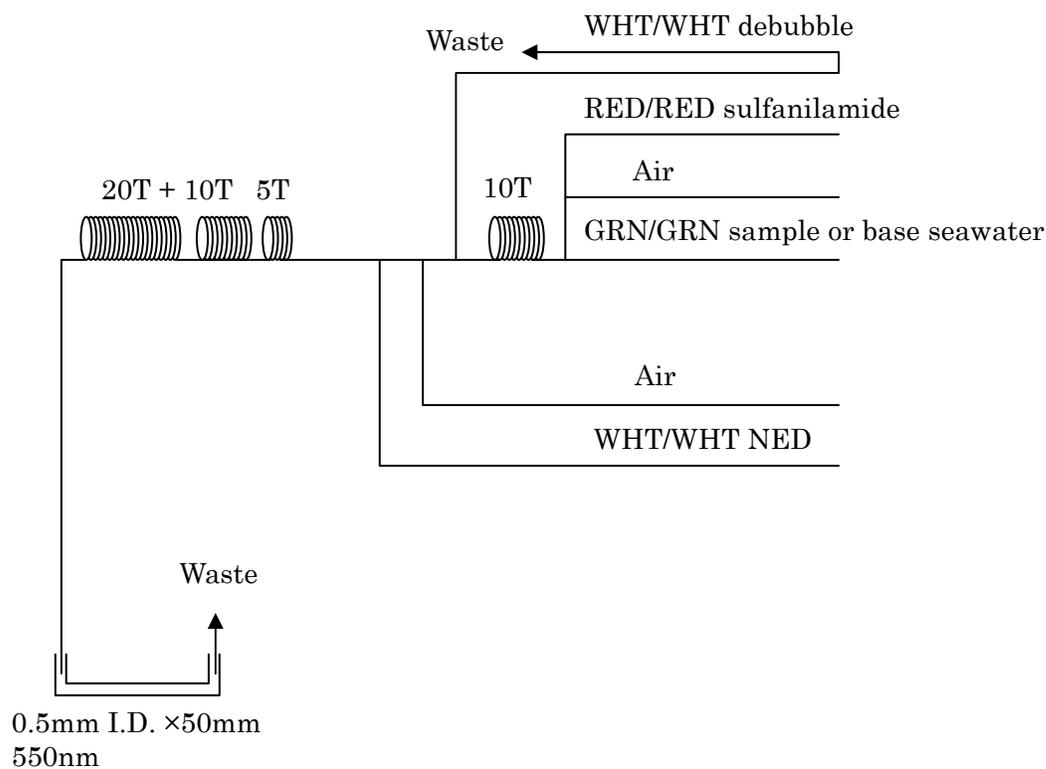


Figure 2. Flow diagram Nitrite.

## Silicate Reagents

Molybdic acid, 0.06 M (2 % w/v) Dissolve 15 g disodium molybdate(VI) trihydrate,  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , in 980 ml DIW, add 8 ml concentrated  $\text{H}_2\text{SO}_4$ . After mixing, 20 ml sodium dodecyl sulphate (15 % solution in water) is added.

Oxalic acid, 0.6 M (5 % w/v) Dissolve 50g oxalic acid anhydrous,  $\text{C}_2\text{H}_2\text{O}_4$ , in 950 ml of DIW.

Ascorbic acid, 0.01M (3 % w/v) Dissolve 2.5g L (+)-ascorbic acid,  $\text{C}_6\text{H}_8\text{O}_6$ , in 100 ml of DIW. Stored in a dark bottle and freshly prepared before every measurement.

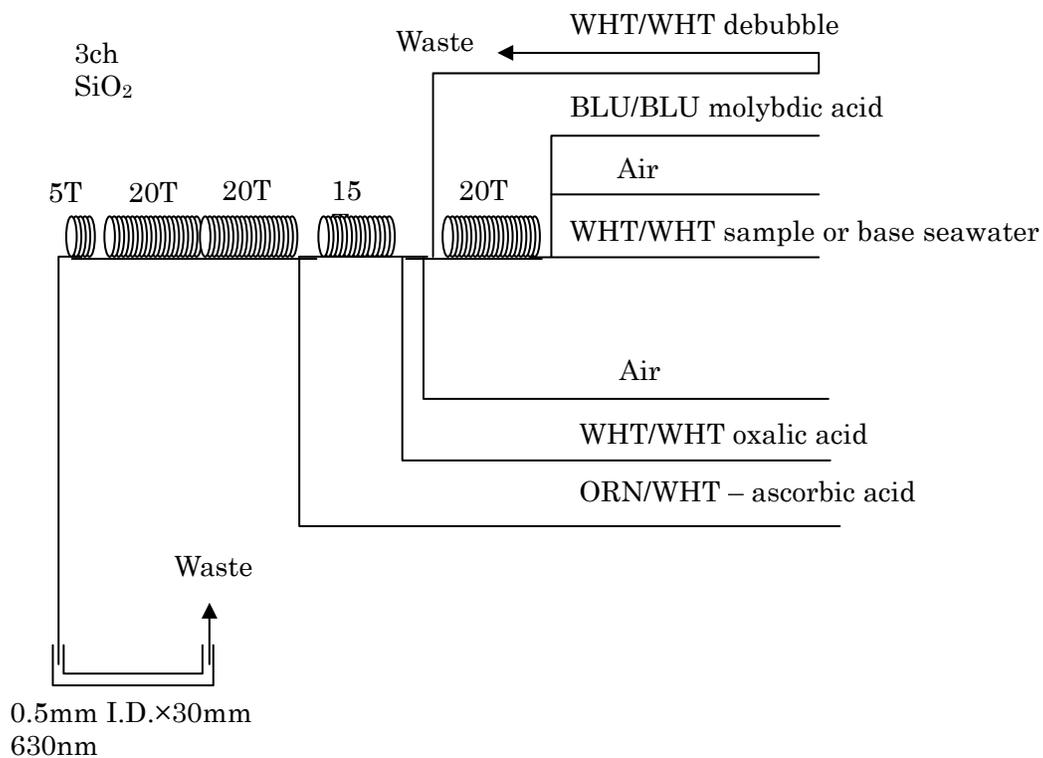


Figure 3. Flow diagram - Silicate.

## Phosphate Reagents

Stock molybdate solution, 0.03M (0.8 % w/v). Dissolve 8 g Disodium molybdate(VI) dihydrate,  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , and 0.17 g antimony potassium tartrate,  $\text{C}_8\text{H}_4\text{K}_2\text{O}_{12}\text{Sb}_2 \cdot 3\text{H}_2\text{O}$ , in 950 ml of DIW and add 50 ml concentrated  $\text{H}_2\text{SO}_4$ .

Mixed Reagent. Dissolve 0.8 g L (+)-ascorbic acid,  $\text{C}_6\text{H}_8\text{O}_6$ , in 100 ml of stock molybdate solution. After mixing, 2 ml sodium dodecyl sulphate (15 % solution in water) is added. Stored in a dark bottle and freshly prepared before every measurement.

Reagent for sample dilution. Dissolve sodium chloride,  $\text{NaCl}$ , 10 g in ca. 950 ml of DIW, add 50 ml Acetone and 4 ml concentrated  $\text{H}_2\text{SO}_4$ . After mixing, 5 ml sodium dodecyl sulphate (15 % solution in water) is added.

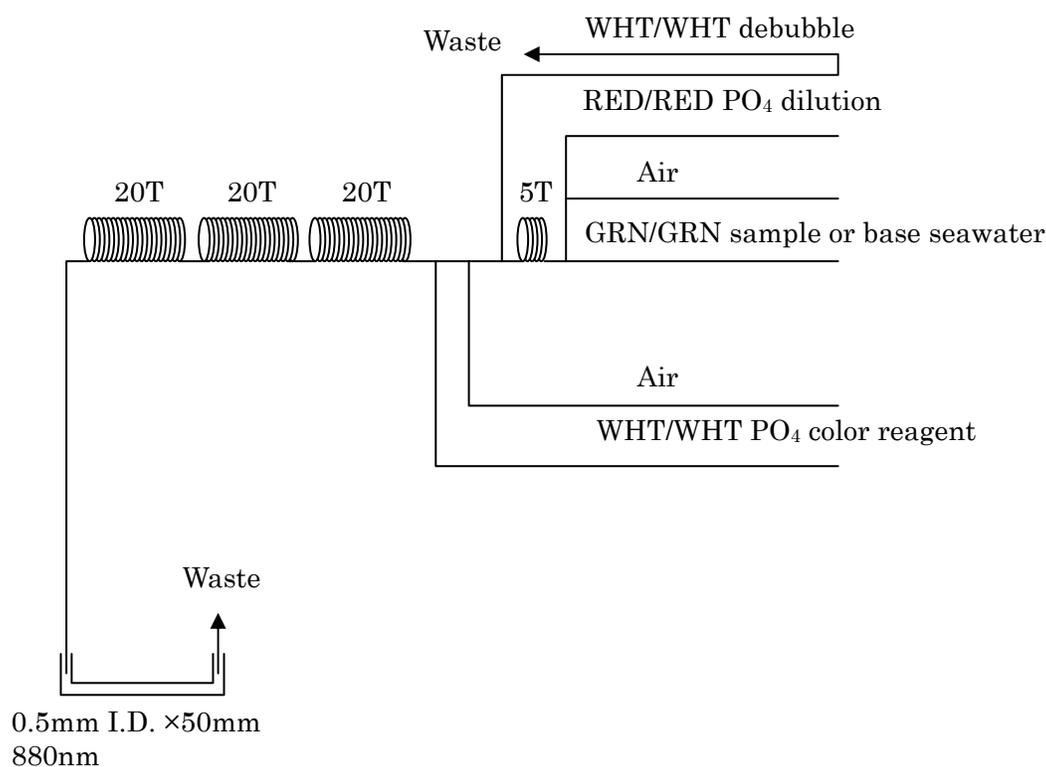


Figure 4. Flow diagram - Phosphate.

### 3.2 Sampling procedures

Sampling of nutrients followed that oxygen, trace gases and salinity. Samples were drawn into two of virgin 10 ml polyacrylates vials without sample drawing tubes. These were rinsed three times before filling and vials were capped immediately after the drawing. The vials are put into water bath at  $24 \pm 1$  deg. C in 10 minutes before use to stabilize the temperature of samples in both MR0704 and MR0706.

No transfer was made and the vials were placed directly into an auto sampler tray. Samples were analyzed after collection basically within 20 hours in MR0704 and 14 hours in MR0706.

### 3.3 Data processing

Raw data from TRAACS800 were treated as follows:

1. Check baseline shift.
2. Check the shape of each peak and positions of peak values taken, and then change the positions of peak values taken if necessary.
3. Calibration curves to get nutrients concentration were assumed second order equations.
4. Carry-over correction and baseline drift correction were applied to peak heights of each samples followed by sensitivity correction.
5. Baseline correction and sensitivity correction were done basically using liner regression.
6. Load pressure and salinity from CTD data to calculate density of seawater and convert data from  $\mu\text{M}/\text{l}$  to  $\mu\text{M}/\text{kg}$

### 3.4 Nutrients standards

#### 3.4.1 Volumetric Laboratory Ware and preparation of in-house standards

All volumetric glass- and polymethylpentene (PMP)-ware used were gravimetrically calibrated. Plastic volumetric flasks were gravimetrically calibrated at a temperature within 2-3°C of the ship's laboratory temperature (21 °C). Volumetric flasks of Class quality (Class A) are used because their nominal tolerances are 0.05 % or less over the size ranges likely to be used in this work. Class A flasks are made of borosilicate glass.

The computation of volume contained by glass flasks at various temperatures other than the calibration temperatures were done by using the coefficient of linear expansion of borosilicate crown glass.

The weights obtained in the calibration weightings were corrected for the density of water and air buoyancy.

To prevent excessive dissolution of silicate from the glass, the standard solutions were transferred to plastic bottles as quickly as possible after they are made up to volume and well mixed.

#### 3.4.2 Pipettes

All pipettes have nominal calibration tolerances of 0.1 % or better. These were gravimetrically calibrated in order to verify and improve upon this nominal tolerance, before and after the cruise.

### 3.4.3 Reagents, general considerations

Specifications:

- Nitrate standard, “potassium nitrate 99.995 suprapur” provided by Merck, CAS No. : 7757-91-1, was used.
- Phosphate standard, “potassium dihydrogen phosphate anhydrous 99.995 suprapur” provided by Merck, CAS No. : 7778-77-0, was used.
- Nitrite standard, “sodium nitrite” provided by Wako, CAS No. : 7632-00-0, was used. An assay of nitrite was determined according JIS K8019. The assays of nitrite salts were 99.1 %. We use that value to adjust the weights taken.
- Silicate standard, we use “Silicon standard solution SiO<sub>2</sub> in NaOH 0.5 mol/l CertiPUR” provided by Merck, CAS No. : 1310-73-2, of which lot number is HC623465 is used. The silicate concentration is certified by NIST-SRM3150 with the uncertainty of 0.5 %.

### 3.4.4 Ultra pure water

Ultra pure water (MilliQ water) freshly drawn was used for preparation of reagents, higher concentration standards and for measurement of reagent and system blanks.

### 3.4.5 Low-Nutrient Seawater (LNSW)

Surface water having low nutrient concentration was taken and filtered using 0.45 µm pore size membrane filter. This water is stored in 20 litre cubitainers in paper boxes. The concentrations of nutrients of this water were measured carefully in May 2007.

### 3.4.6 Concentrations of nutrients for A, B and C standards

Concentrations of nutrients for A, B and C standards are set as shown in Table 1. The C-6 standard is prepared according recipes as shown in Table 2. All volumetric laboratory tools were calibrated prior the cruise as stated above. Then the actual concentration of nutrients in each fresh standard was calculated based on the ambient, solution temperature and determined factors of volumetric lab. wares. Other standards C-1 to C-7 are RMNS solutions supplied my Technos.

**Table 1. Nominal concentrations of nutrients for A, B and C standards.**

	A	B	C-1	C-2	C-3	C-4	C-5	C-6	C-7
NO <sub>3</sub> (µM)	45000	900	BA	AY	AX	AV	BF	55	BG
NO <sub>2</sub> (µM)	4000	20	BA	AY	AX	AV	BF	1.2	BG
SiO <sub>2</sub> (µM)	36000	2880	BA	AY	AX	AV	BF	170	BG
PO <sub>4</sub> (µM)	3000	60	BA	AY	AX	AV	BF	3.6	BG

**Table 2. Working calibration standard recipes.**

<b>C Std.</b>	<b>B-1 Std.</b>	<b>B-2 Std.</b>
<b>C-6</b>	<b>30 ml</b>	<b>30 ml</b>

B-1 Std.: Mixture of nitrate, silicate and phosphate

B-2 Std.: Nitrite

### 3.4.7 Renewal of in-house standard solutions

In-house standard solutions listed above were renewed as shown in Table 3.

**Table 3. Timing of renewal of in-house standards.**

<b>NO<sub>3</sub>, NO<sub>2</sub>, SiO<sub>2</sub>, PO<sub>4</sub></b>	<b>Renewal</b>
<b>A-1 Std. (NO<sub>3</sub>)</b>	<b>maximum 1 month</b>
<b>A-2 Std. (NO<sub>2</sub>)</b>	<b>maximum 1 month</b>
<b>A-3 Std. (SiO<sub>2</sub>)</b>	<b>commercial prepared solution</b>
<b>A-4 Std. (PO<sub>4</sub>)</b>	<b>maximum 1 month</b>
<b>B-1 Std. (mixture of NO<sub>3</sub>, SiO<sub>2</sub>, PO<sub>4</sub>)</b>	<b>8 days</b>
<b>B-2 Std. (NO<sub>2</sub>)</b>	<b>8 days</b>
<b>C Std.</b>	<b>Renewal</b>
<b>C-6 Std. (mixture of B-1 and B-2 Std.)</b>	<b>24 hours</b>
<b>Reduction estimation</b>	<b>Renewal</b>
<b>D-1 Std. (7200µM NO<sub>3</sub>)</b>	<b>when A-1 Std. renewed</b>
<b>43µM NO<sub>3</sub></b>	<b>when C Std. renewed</b>
<b>47µM NO<sub>2</sub></b>	<b>when C Std. renewed</b>

## NSOP 10. PREPARATION OF CONTROL CHARTS

### 1. SCOPE AND FIELD OF APPLICATION

This procedure details the preparation and use of property ( $\bar{X}$ ) and range ( $R$ ) control charts. The  $\bar{X}$  chart is used to demonstrate whether a measurement mean is in control and the  $R$  chart is used to demonstrate whether measurement variability is in control. Such charts are basic tools for the quality assurance of analytical measurements. They can be used to document measurement uncertainty and to monitor a variety of aspects of a measurement process, such as blank levels or instrument sensitivity.

### 2. PRINCIPLE

The construction of a control chart is based on statistical principles, specifically on the normal distribution. The control limits are based on considerations of probability, so that decisions that a system is in control are supported by evidence. Similarly, the control limits can be used to warn of potential problems and reveal the need for corrective action. Control charts should be kept in real time so that such corrective action is taken promptly.

NSOP 11 provides all the necessary information to carry out the statistical calculations needed in this NSOP.

### 3. THE $\bar{X}$ CHART

Values obtained for repetitive measurements of a control sample are plotted sequentially to evaluate the stability of the measurement process (see Figure 1). Such control samples must be similar to the test samples of interest, otherwise it is not possible to draw conclusions about the performance of the system on test samples from this information.

The results from at least 12 measurements are needed to get the process underway (the temporal spread of the observations should be considered and chosen appropriately - limits can be set at the start of cruise based on experience from previous work)—are used to compute estimates of the mean and standard deviation of the data in accordance with the standard expressions given in NSOP 11.

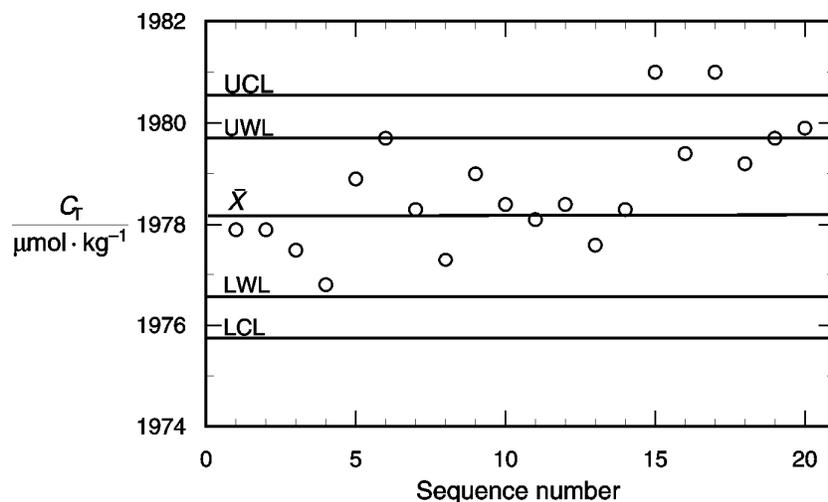


Figure 1. Example of a property control chart showing a trend in the data with time; control limits were calculated from the first 12 points. This chart indicates that the measurement process is not in control.

The central line is the mean value,  $\bar{x}$ , the control limits are based on the sample standard deviation,  $s$ :

$$\begin{array}{ll}
 \text{upper control limit} & \text{UCL} = \bar{x} + 3s, \\
 \text{upper warning limit} & \text{UWL} = \bar{x} + 2s, \\
 \text{lower warning limit} & \text{LWL} = \bar{x} - 2s, \\
 \text{lower control limit} & \text{LCL} = \bar{x} - 3s.
 \end{array}$$

When so set, approximately 95% of the plotted points should fall between the warning limits (UWL and LWL) and rarely should any fall outside the control limits (UCL and LCL).

#### 4. THE R CHART

The absolute differences ( $R$ ) of duplicate measurements are plotted sequentially to evaluate the precision of the measurement process (see Figure 2). The average range  $\bar{R}$  is related to the short-term standard deviation (or repeatability,  $s_R$ ) of the measurement process (NSOP 11). At least 12 measurements should be used to compute  $\bar{R}$ . The control limits for duplicate measurements are:

$$\begin{array}{l}
 \text{UCL} = 3.267 \bar{R}, \\
 \text{UWL} = 2.512 \bar{R}, \\
 \text{LWL} = 0, \\
 \text{LCL} = 0.
 \end{array}$$

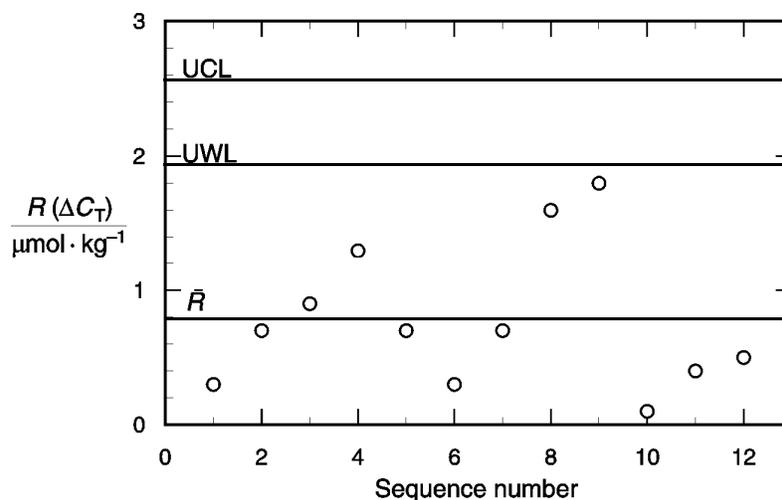


Figure 2. Example of a range control chart; control limits were calculated using all the data shown. The measurement precision is in control.

## 5. UPDATING CONTROL CHARTS

After additional control data have been accumulated—at least as much as was used originally—the control limits may be updated. A  $t$  test is made to assess whether  $\bar{x}$  for the second set of data is significantly different from that for the first (NSOP 11). If not, all the data may be used to compute a new estimate of  $\bar{x}$ , otherwise only the second set of data should be used to revise the control chart.

The value of the sample standard deviation,  $s$ , should also be calculated for the second set of data. It should be compared with the estimate from the first set of data, using the  $F$  test (NSOP 11) to decide whether to pool it with the first, or use it separately in setting new control limits.

If the values of  $R$  show no significant trends and if  $\bar{R}$  has not changed significantly, all of the values of  $R$  should be combined to obtain an updated estimate of  $\bar{R}$  from which updated control limits can be computed. Judgment of the significance of changes in  $\bar{R}$  is best decided by computing the corresponding values of the short-term standard deviation (the repeatability) and conducting an  $F$  test.

## 6. INTERPRETATION OF CONTROL CHART DATA

Points plotted on a control chart should be randomly distributed within the warning limits when the system is in a state of statistical control. If a plotted point lies outside of the warning limits, a second set of measurements should be made. If this point also lies outside the warning limits, corrective action is required and demonstrated attainment of control is necessary before measurements may be reported with confidence. Barring blunders, one point outside of the control limits is reason for corrective action. The nature of the corrective action to be taken will depend, in either case, on the kind of measurement made. If the  $X$  point is outside the limits but the  $R$  point is not, a source of bias should be sought and eliminated. If the  $R$  point is outside of limits,  $X$  probably will be as well. Sources of extraordinary random error should be sought and eliminated before any possible bias can be detected.

Control charts may be used to evaluate the uncertainty of measurement in some cases. When an appropriate control chart is maintained, a  $\bar{X}$  chart may be used to evaluate bias and to document the standard deviation of the measurement process. Then the values of  $s$  on which the control limits are based may be used in calculating confidence limits for measurement values.

## 7. REFERENCES

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- Ryan, T.P. 1989. *Statistical Methods for Quality Improvement*. John Wiley & Sons, Inc., New York, 446 pp.
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## NSOP 11. STATISTICAL TECHNIQUES USED IN QUALITY ASSESSMENT

### 1. SCOPE AND FIELD OF APPLICATION

NSOP 11, describes various statistical calculations used in quality assessment. Calculations are detailed which allow the computation of:-

- mean and standard deviation of a set of values
- standard deviation from a set of duplicate measurements
- confidence interval for a mean
- examination of the values of two means or of two standard deviations to assess if they are significantly different at some chosen level of probability
- least-squares estimates of the slope and intercept of a straight line.

### 2. PRINCIPLE

These calculations are based on statistical principles, specifically on the normal distribution. More details of the relevant statistical background are given in the bibliography.

### 3. PROCEDURE

#### 3.1 Estimation of the mean and standard deviation from a series of measurements

Given  $n$  measurements,

$$x_1, x_2, x_3, \dots, x_n,$$

the mean,  $\bar{x}$ , is given by

$$\bar{x} = \frac{1}{n} \sum_{i=1}^n x_i \quad (17)$$

and an estimate of the standard deviation,  $s$ , is given by

$$s = \left( \frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n-1} \right)^{1/2}. \quad (18)$$

#### 3.2 Estimation of the standard deviation from the difference of sets of duplicate measurements

Given  $k$  differences of duplicate measurements,

$$d_1, d_2, d_3, \dots, d_k,$$

an estimate of the standard deviation,  $s$ , is given by

$$s_R = \left( \frac{\sum_{i=1}^k d_i^2}{2k} \right)^{1/2}. \quad (19)$$

This is a measure of the short-term standard deviation, or repeatability of measurements<sup>6</sup>.

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<sup>6</sup> The International Organization for Standardization (ISO) applies two descriptions of precision: (1) the *reproducibility*, the closeness of agreement between individual results obtained with the same method but under Guideline of ocean observations Vol. 3 Chap. 2 © Michio AOYAMA 2016 G302EN:001-095

### 3.3 Confidence interval for a mean

The formula for use is

$$\bar{x} \pm \frac{ts}{\sqrt{n}} \quad (20)$$

where

$\bar{x}$  = sample mean,

$n$  = number of measurements on which the mean is based,

$s$  = estimate of the standard deviation<sup>7</sup>,

$t$  = Student's  $t$  value, *i.e.*, the probability factor for the desired confidence limit and the number of degrees of freedom associated with  $s$ . (For numerical values, see Table 1 in the Annexe to this procedure.)

### 3.4 Comparing values of two means

*Case 1.* No reason to believe that the standard deviations differ.

**Step 1:** Choose  $\alpha$ , the desired probability level (*i.e.*, the significance level) of the test.

**Step 2:** Calculate a pooled standard deviation from the two estimates to obtain a better estimate of the standard deviation:

$$s_p = \left( \frac{\nu_A s_A^2 + \nu_B s_B^2}{\nu_A + \nu_B} \right)^{1/2} \quad (21)$$

where  $\nu_A$  and  $\nu_B$  are the number of degrees of freedom associated with  $s_A$  and  $s_B$ , respectively.  $s_p$  will thus be based on  $\nu_A + \nu_B$  degrees of freedom.

**Step 3:** Calculate the uncertainty,  $U$ , of the differences

$$U = t s_p \left( \frac{1}{n_A} + \frac{1}{n_B} \right)^{1/2} \quad (22)$$

where  $t$  is the appropriate Student's  $t$  value.

**Step 4:** Compare  $\Delta = |\bar{x}_A - \bar{x}_B|$  with  $U$ . If  $\Delta \leq U$ , there is no reason to believe that the means disagree.

*Case 2.* The standard deviations differ significantly (see section 3.5).

**Step 1:** Choose  $\alpha$ , the significance level of the test.

**Step 2:** Compute the estimated variance of each mean using the individual estimates of the standard deviations,

$$V_A = s_A^2/n_A, \quad V_B = s_B^2/n_B. \quad (23)$$

**Step 3:** Compute the effective number of degrees of freedom<sup>8</sup>:

different conditions (*e.g.*, in different laboratories) and (2) the *repeatability*, the closeness of agreement between successive results obtained with the same method and under the same conditions.

<sup>7</sup> If  $\bar{x}$  and  $s$  are based on the same data set, the number of degrees of freedom,  $df = n - 1$ . However, if  $s$  is based on additional evidence, such as a system under statistical control (judged by a control chart), then the degrees of freedom on which the estimate of  $s$  is based may be used to determine  $t$ . In such a case, one can calculate a confidence interval for even a single measurement.

$$f^* = \frac{(V_A + V_B)^2}{\frac{V_A^2}{n_A + 1} + \frac{V_B^2}{n_B + 1}} - 2. \quad (24)$$

Step 4: Calculate the uncertainty,  $U$ , of the differences

$$U = t^* \sqrt{V_A + V_B} \quad (25)$$

where  $t^*$  is the effective value of  $t$  based on  $f^*$  degrees of freedom and the chosen significance level,  $\alpha$  (Table 1 in the Annex to this NSOP).

Step 5: Compare  $\Delta = |\bar{x}_A - \bar{x}_B|$  with  $U$ . If  $\Delta \leq U$ , there is no reason to believe that the means disagree.

### 3.5 Comparing estimates of a standard deviation (F test)

This test may be used to decide whether there is sufficient reason to believe that two estimates of a standard deviation are significantly different. It consists of calculating the ratio of the variances and comparing it with tabulated values. Unless the computed ratio is larger than the tabulated value, there is no reason to believe that the respective standard deviations are significantly different.

The  $F$  ratio is calculated as

$$F = \frac{s_L^2}{s_S^2} \quad (26)$$

where  $s_L$  is the larger value and  $s_S$  is the smaller of the two estimates under consideration. The critical value of  $F$  will depend on the significance level chosen and on the degrees of freedom associated with  $s_L$  and  $s_S$  (see Table 2 in the Annex to this NSOP).

### 3.6 Computation of least-squares estimates

For the linear model,

$$y_i = \beta_0 + \beta_1 x_i + \varepsilon_i \quad (27)$$

where  $x$  is essentially without error (for data with errors in  $x$  and  $y$ —see York, 1966) and the error  $\varepsilon_i$  is normally distributed with a constant variance, least-squares estimates of the coefficients,  $\beta_0$  and  $\beta_1$ , are given by the expressions

$$\beta_1 = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sum_i (x_i - \bar{x})^2}, \quad (28)$$

$$\beta_0 = \bar{y} - \beta_1 \bar{x}. \quad (29)$$

An estimate of the experimental error variance is then given by

$$s^2 = \frac{\sum_i (y_i - \beta_0 - \beta_1 x_i)^2}{n - 2} \quad (30)$$

and estimates of the standard errors of the coefficients by

$$\text{S.E.}(\beta_0) = s \left( \frac{1}{n} + \frac{\bar{x}^2}{\sum_i (x_i - \bar{x})^2} \right)^{1/2}, \quad (31)$$

<sup>8</sup> A number of expressions exist in the literature for this calculation, with some authors even arguing that such a pooling of the variances is inappropriate. The expression used here comes from Taylor (1987).

$$\text{S.E.}(\beta_1) = \frac{s}{\left(\sum_i (x_i - \bar{x})^2\right)^{1/2}}. \quad (32)$$

### 3.7 Example calculations

#### 3.7.1 Estimation of the mean and standard deviation from a series of measurements

Given the following 9 measurements:

1977.67, 1977.98, 1977.29, 1978.60, 1979.48, 1979.14, 1979.33, 1979.95, 1979.99,

the mean is 1978.83 and the standard deviation is 0.99.

#### 3.7.2 Estimation of the standard deviation from the difference of sets of duplicate measurements

Given 10 pairs of measurements:

1976.8, 1979.3;	1978.9, 1979.6;	1979.6, 1979.8;	1978.3, 1978.6;
1981.2, 1979.8;	1977.6, 1977.8;	1976.2, 1976.8;	1978.6, 1977.0;
1976.6, 1978.9;	1978.3, 1978.9		

the standard deviation calculated using

$$s_R = \left( \frac{\sum_{i=1}^k d_i^2}{2k} \right)^{1/2}$$

is 0.93.

#### 3.7.3 Confidence interval for a mean

The 95% confidence interval for the mean calculated in section 3.7.1 is

$$1978.83 \pm \frac{(2.306)(0.99)}{\sqrt{9}} = 1978.83 \pm 0.76$$

#### 3.7.4 Comparing values for two means

*Case 1.* No reason to believe that the standard deviations differ.

$$\begin{aligned} \bar{x}_A &= 1978.78, & s_A &= 0.93, & n_A &= 9 \\ \bar{x}_B &= 1981.74, & s_B &= 0.87, & n_B &= 18 \end{aligned}$$

**Step 1:** Require 95 % confidence in decision.

**Step 2:** Pooled standard deviation:

$$s_p = \left( \frac{8(0.93)^2 + 17(0.87)^2}{8+17} \right)^{1/2}$$

$$= 0.89.$$

Step 3: Calculate  $U$ :

$$U = 2.060(0.89) \left( \frac{1}{9} + \frac{1}{18} \right)^{1/2}$$

$$= 0.75.$$

Step 4: As  $\Delta$  ( $= 1981.74 - 1978.78 = 2.96$ ) is larger than  $U$ , the means disagree at the 95 % confidence level.

*Case 2.* The standard deviations differ significantly.

$$\bar{x}_A = 1978.78, \quad s_A = 0.93, \quad n_A = 9$$

$$\bar{x}_B = 1981.74, \quad s_B = 2.75, \quad n_B = 16$$

Step 1: Require 95 % confidence in decision.

Step 2: Compute the estimated variance of each mean:

$$V_A = (0.93)^2 / 9 = 0.0961$$

$$V_B = (2.75)^2 / 16 = 0.4727.$$

Step 3: Compute the effective number of degrees of freedom:

$$f^* = \left[ \frac{(0.0961 + 0.4727)^2}{(0.0961)^2 / (9 + 1) + (0.4727)^2 / (16 + 1)} \right] - 2 \approx 21.$$

Step 4: Calculate  $U$ :

$$U = 2.08(0.0961 + 0.4727)^{1/2} = 1.57.$$

Step 5: As  $\Delta$  ( $= 1981.74 - 1978.78 = 2.96$ ) is larger than  $U$ , the means disagree at the 95 % confidence level.

### 3.7.5 Comparing estimates of a standard deviation

$$\bar{x}_A = 1978.78, \quad s_A = 0.93, \quad n_A = 9$$

$$\bar{x}_B = 1975.35, \quad s_B = 1.71, \quad n_B = 12$$

Calculate  $F$ :

$$F = \frac{(1.71)^2}{(0.93)^2} = 3.38.$$

The tabulated value of  $F$ —with 8 degrees of freedom in the numerator and 11 degrees of freedom in the denominator—is 3.7. As the computed value is smaller than the tabulated value, there is no reason to believe that the two standard deviations are significantly different.

### 3.7.6 Example computation of least-squares estimates

Given 6 pairs of measurements of  $x$  and  $y$ :

0.0	1892
498.8	66537

1001.9	130818
1500.8	195216
2002.5	260068
2497.1	323456

Linear regression gives

$$\beta_0 = 2017.77,$$

$$\beta_1 = 128.765.$$

The error estimates are

$$s = 221.77,$$

$$\text{S.E.}(\beta_0) = 160.55,$$

$$\text{S.E.}(\beta_1) = 0.106.$$

#### 4. REFERENCES

- Box, G.E.P., Hunter, W.G. and Hunter, J.S. 1978. *Statistics for Experimenters. An Introduction to Design, Data Analysis, and Model Building*. John Wiley & Sons, 653 pp.
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### ANNEX NSOP 11

Table 1. Student's *t* values for 95 % and 99 % confidence intervals.

Probability level for two-sided confidence interval		
df <sup>9</sup>	95 %	99 %
1	12.706	63.657
2	4.303	9.925
3	3.182	5.841
4	2.776	4.604
5	2.571	4.032
6	2.447	3.707
7	2.365	3.499

<sup>9</sup> degrees of freedom ( $n - 1$ );

8	2.306	3.355
9	2.262	3.250
10	2.228	3.169
11	2.201	3.106
12	2.179	3.055
13	2.160	3.012
14	2.145	2.977
15	2.131	2.947
16	2.120	2.921
17	2.110	2.898
18	2.101	2.878
19	2.093	2.861
20	2.086	2.845
25	2.060	2.787
40	2.021	2.704
60	2.000	2.660
$\infty$	1.960	2.576

Table 2. Critical values for the  $F$  test for use in a two-tailed test of equality of standard deviation at 95% level of confidence.

$df_D$	$df_N$									
	1	2	4	6	8	10	15	20	30	40
1	648	800	900	937	957	969	983	993	1001	1006
2	38.5	39.0	39.2	39.3	39.4	39.4	39.4	39.4	39.5	39.5
4	12.2	10.6	9.6	9.2	9.0	8.8	8.7	8.6	8.5	8.4
6	8.8	7.3	6.2	5.8	5.6	5.5	5.3	5.2	5.1	5.0
8	7.6	6.1	5.0	4.6	4.4	4.3	4.1	4.0	3.9	3.8
10	6.9	5.5	4.5	4.1	3.8	3.7	3.5	3.4	3.3	3.3
15	6.2	4.8	3.8	3.4	3.2	3.1	2.9	2.8	2.6	2.6
20	5.9	4.5	3.5	3.1	2.9	2.8	2.6	2.5	2.4	2.3
30	5.6	4.2	3.2	2.9	2.6	2.5	2.3	2.2	2.1	2.0
40	5.4	4.0	3.1	2.7	2.5	2.4	2.2	2.1	1.9	1.9
60	5.3	3.9	3.0	2.6	2.4	2.3	2.1	1.9	1.8	1.7
12	5.2	3.8	2.9	2.5	2.3	2.2	1.9	1.8	1.7	1.6
0										
$\infty$	5.0	3.7	2.8	2.4	2.2	2.1	1.8	1.7	1.6	1.5

$df_D$  — degrees of freedom of the variance in the denominator.  $df_N$  — degrees of freedom of the variance in the numerator.

## NSOP 12. REQUIREMENTS FOR REPORTING OF NUTRIENT META-DATA

### 1. SCOPE AND FIELD OF APPLICATION

Reporting of a comprehensive meta-data set is an essential step required for the validation of data set within any data base it is entered into. A standard electronic form is being developed and will be made available to enable efficient and consistent reporting of meta-data across the global marine nutrient measurement community, an example is shown in Figure 12.1 below.

### 2. PRINCIPLE

All nutrient data collected should be accompanied by a complete meta-data set which follows the requirements set out below.

General Information: (this information is generic to all meta-data collected during a scientific cruise, and is needed to link data sets):

#### 1. Cruise information:

- Vessel (name; country; vessel ID)
- Date and Port of departure
- Date and Port of arrival
- Cruise ID (EXPOCODE)
- Name of experiment (e.g. P16 or M60/5)
- Leg
- Geographical coverage (e.g. North Atlantic; 30 °N to 50 °N and 60 °W to 10 °W)
- Number of CTD stations

#### Nutrient measurements:

#### 1. Investigator:

- Name:
- Organization:
- Address:
- Phone:
- Email:

#### 2. Variables description:

- Variable names
- Reporting units

#### Method description:

#### Record

1. Instrument: State instrumentation used for the measurements. For instance: Bran-Luebbe TrAAcs 800 autoanalyzer.
2. Method for each measured parameter, and appropriate reference. For instance: Ammonium was measured with o-phthalaldehyde (OPA) in the presence of borate buffer

solution and sodium sulfite; fluorescence measured at 460 nm, excitation at 370 nm. Method no G-327-05 Rev 3 (Seal Analytics), Kerouel and Aminot, 1997.

3. Deviations in your set-up from the reference method.
4. Modifications to the standard instrument configuration for the method
5. Settings such as the sampling/rinsing cycles, temperatures, air/nitrogen in the gas bubbles etc
6. Lab temperature (e.g. 20-24°C variable).
7. Sampling containers type (e.g. 100 ml polypropylene bottles, reused after acid clean).
8. Any preprocessing of sample (e.g filtration - record filter type and method used pressure or suction etc.)
9. Poisoning of samples?
10. Storage – method used and duration (e.g. frozen -20 °C for three months defrosted 3 days before measurement)
11. Thawing procedures if sample was frozen

#### Reagents:

1. Brands and stock information of the reagents/salts used.
2. Where the solutions prepared on the ship, or pre-made in the lab prior to cruise
3. Which medium was used for the reagents (e.g. RO water)

#### Standardization:

1. How were your stock solutions prepared (initial salts, medium)
2. Temperature of preparation of standards (This is the temperature used when converting  $\mu\text{M l}^{-1}$  to  $\mu\text{M kg}^{-1}$ )
3. Dilution sequence used to prepare working standards
4. Medium used for working standards
5. Blank measurements (medium)
6. Pipettes were used and calibration history

#### Reference material:

1. Certified reference material or certified standards used (state batch numbers, producer etc.).

#### Quantification procedures:

1. Software used for peak picking and calibration
2. Degree of equation used for calibration and zero forced through origin
3. Calibration curves/ranges (number of points used for calibration curve, concentration used for calibrants)
4. Blank corrections (Null and refractive index blank)
5. Matrix corrections (method used to quantify corrections)

#### Data quality:

1. Estimate of accuracy<sup>10</sup> and precision<sup>11</sup>.
2. State how these numbers were obtained (e.g. by measurements of X duplicates and by running X number of Certified Reference Material).
3. Proportion of samples measured in duplicate
4. Method used to round off results to the number of significant digits

#### Samples results:

1. Header file showing what was measured (variables/parameters, units);
2. Time and location of sample taken(time; latitude; longitude; station identifier)
3. Time sample was measured
4. Raw nutrient data
5. Nutrient data adjusted for tracking and RMNS results
6. Clear statement that the data are reported as  $\mu\text{M l}^{-1}$  or  $\mu\text{M kg}^{-1}$

### 3. NOTES

A significant part of the information required above is specific for the nutrient measurements. Several of these fields will be generic for a particular lab, i.e. will only have to be filled out once by each lab; variations to the standard procedures then be edited in along with specific information for each cruise such as the precision data.

Figure 1 (below) shows an example of the electronic (pdf based) meta-data reporting form that will be made available by the RMNS project for use by the global community reporting data on concentrations of nutrients in the ocean.

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<sup>10</sup> “Accuracy” is the closeness of agreement between a measured value and the true quantitative value of the measurand. It can only be quantified in situations where measurements can be made of a measurand for which an agreed value exists such as a certified reference material.

<sup>11</sup> “Precision” is the closeness of agreement of replicate measurements of the same property under specified conditions. It can be quantified by a measure such as standard deviation. Definitions follow VIM (International Vocabulary of Metrology); [http://www.bipm.org/utis/common/documents/jcgm/JCGM\\_200\\_2008.pdf](http://www.bipm.org/utis/common/documents/jcgm/JCGM_200_2008.pdf)

## Metadata for reporting on nutrient measurements Ver. 2.0



### General Information:

Data Serial \_\_\_\_\_ Vessel name \_\_\_\_\_ Cruise ID (EXPCODE) \_\_\_\_\_ Leg \_\_\_\_\_  
 Country \_\_\_\_\_ Vessel ID \_\_\_\_\_ Experiment Name \_\_\_\_\_ CTD stations Number \_\_\_\_\_  
 Date and Port of departure \_\_\_\_\_ Geographical coverage (e.g. North Atlantic; 30°N to 50°N and 60°W to 10°W) \_\_\_\_\_  
 Date and Port of arrival \_\_\_\_\_

*Significant part of the information required below is specific for the nutrient measurements. Several of these fields will be generic for a particular lab, i.e. will only have to be filled out once by each lab; variations to the standard procedures can easily be edited in.*

### Nutrients Measurements:

#### 1. Investigator

Name \_\_\_\_\_ Phone \_\_\_\_\_ Email \_\_\_\_\_  
 Organization \_\_\_\_\_  
 Address \_\_\_\_\_

#### 2. Variables description

Variable names \_\_\_\_\_  
 Reporting Units \_\_\_\_\_

#### 3. Date of measurement

date of reception \_\_\_\_\_  
 or collection of samples \_\_\_\_\_

### Method Description:

1. Instrument: State instrumentation used for the measurements. For instance: Braan-Luebbe TRAacs 800 autoanalyzer. \_\_\_\_\_
2. State settings such as the sampling/rinsing cycles, temperatures, air/nitrogen in the gas bubbles etc. \_\_\_\_\_
3. Dilution of high concentration samples \_\_\_\_\_
4. Environmental information such as lab temperature (e.g. 20-24°C variable). \_\_\_\_\_
5. Sampling containers (e.g. 100 ml polypropylene bottles, reused). \_\_\_\_\_
6. Did you filtrate your samples; if so, state details. \_\_\_\_\_
7. Storage (e.g. dark at 8°C). This includes information on samples stored for a longer time and analyzed on-shore after the cruise. T° and time of storage, standard or document reference if applicable, respect of continuous refrigeration yes/no \_\_\_\_\_
8. Poisoning of samples? \_\_\_\_\_
9. Thawing procedures if sample was frozen. \_\_\_\_\_

2. State method for each measured parameter, and appropriate reference. For instance: Ammonia was measured with o-phthalaldehyde (OPA) in the presence of borate buffer solution and sodium sulfite; fluorescence measured at 460 nm, excitation at 370 nm. Method no G-327-05 Rev 3 (Seal Analytics), Kerouel and Aminot, 1997. State any deviations in your set-up from the reference method or any modification from the standard instrument. \_\_\_\_\_

### Reagents:

1. Brands and stock information of the reagents/salts used. \_\_\_\_\_
2. Where the solutions prepared on the ship, or pre-made in the lab prior to cruise. \_\_\_\_\_
3. Which medium was used for the reagents (e.g. MilliQ, destwater). \_\_\_\_\_

### Standardization:

1. How were your stock solutions prepared (initial salts, medium), + method (volumetric, mass) \_\_\_\_\_
2. How were the stock solutions diluted to working concentrations (medium), + method (volumetric, mass) \_\_\_\_\_
3. Blank measurements (medium) (or balance (calibration, precision,...)) \_\_\_\_\_
4. Which pipettes were used? State calibration information of the pipettes. \_\_\_\_\_

### Reference Material:

1. Did you use any certified reference material or certified standards (state batch numbers, producer etc.). \_\_\_\_\_
2. Did you correct raw data before you submit your data? \_\_\_\_\_

### Quantification procedures:

1. Mathematical formula used for the calculation of concentration \_\_\_\_\_
2. Matrix corrections (method used to quantify corrections) \_\_\_\_\_
3. Calibration curves/ranges (number of points used for calibration curve, concentration used for calibrants) \_\_\_\_\_
3. Did you do carry over correction? \_\_\_\_\_
4. Did you do base line drift correction? \_\_\_\_\_
5. Blank corrections (Null and refractive index blank) \_\_\_\_\_
6. Recalculation of run? (state modifications) \_\_\_\_\_

### Data Quality:

1. Provide your best estimate of precision and accuracy. (? Cf mail) \_\_\_\_\_
2. State how these numbers were obtained (e.g. by measurements of X duplicates and by running X number of Certified Reference Material). \_\_\_\_\_
3. Number of samples/doubles measured \_\_\_\_\_
4. Detection limit and quantification limit (method used, formula for calculation or parameters used) \_\_\_\_\_
5. State uncertainty components; uncertainty calculation, confidence interval (or coverage factor) \_\_\_\_\_
6. Method used to round off results to the number of significant digits \_\_\_\_\_

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## Trace metals

○Hajime Obata (AORI, Univ. Tokyo)

For determination of trace metals in seawater, contamination is one of the most critical problems. Concentrations of most metals are very low in seawater of open ocean, but the metals are ubiquitous in the research ship and laboratories. We need to be very careful to avoid contamination at each step, collection, filtration, pretreatment, and analysis of seawater. In this chapter, clean techniques for each step are described to prevent the contamination. Clean sampling techniques are described in Vol. 2, Chap. 1, “Clean sampling”.

### 1. Filtration of seawater

We usually study “dissolved” trace metals because dissolved fraction of bioactive trace metals in seawater is considered to be available for marine microorganisms. This “dissolved fraction” is also investigated on other non-bioactive trace metals. Filtration of seawater samples is necessary to obtain the dissolved fraction of the samples, but we often suffer from contaminations during this process. To avoid the contamination, initially we have to confirm that the materials of filters and filtering apparatuses are clean enough for the target metals, and establish their cleaning method. For example, 0.2 or 0.4  $\mu\text{m}$  pore-size Nucleopore filters are frequently used to filter seawater samples for trace metal analyses, but these filters must be cleaned with diluted ultra-pure grade of acids before use. During the cleaning process, the acids remaining in the filters may cause the contamination of the seawater samples. The remaining acids should be removed thoroughly with ultra high purity water (UHPW). Filtering apparatuses, like filter holders, are usually made of Teflon, polycarbonate or polysulfon. Suitable cleaning methods for the materials should be established not to damage the apparatuses.

Capsule filters are easy to handle and frequently used for filtration. For example, many research groups in the international GEOTRACES project use 0.2  $\mu\text{m}$  pore-size polysulfon capsule filters (Acropak, PALL). To clean the filters, we fill the capsule filters with 0.1 M ultra pure hydrochloric acid for one day. The acid solution should be completely removed by passing UHPW through the capsule filters. We can effectively remove the acid solution by filling the capsule filters with UHPW for more than one day. Onboard the research vessel, one capsule filter is repeatedly used throughout the observation at several stations. In this case, to prevent the growth of bacteria inside of the capsule, the filters are preserved in the refrigerator between the stations.

### 2. Onboard analyses

For studying the marine biogeochemistry of contamination-prone trace metals, it is better to determine them onboard the ship. Onboard analyses will allow us to notice the contamination, which prevents the damage to the entire samples during the cruise. In GEOTRACES project, it is recommended to determine some contamination-prone trace metals like Fe and Zn in seawater onboard the ship. Since the clean laboratory space is limited in the research ship, we should apply the compact closed flow system for the analytical method. Several methods have already been developed for onboard analyses of trace metals in seawater (e.g. Al, Brown & Bruland, 2008. Fe: Lohan et al., 2006; Measures et al.,

1995; Obata et al., 1993. Zn: Gosnell et al., 2012; Jakuba et al., 2008; Lohan et al., 2003). Onboard analyses often enable the in situ determination of each species of trace metals in seawater. Therefore, it would be also better to determine trace metals, whose speciations are easily variable, onboard the ship.

### **3. Preservation and pre-treatments**

Some trace metals in seawater will be measured after coming back to the land-based laboratory. Especially, some metals and their isotopes will be determined by using large-sized analytical facilities, so that we have to establish the preservation methods of the samples without loss and contamination. Many trace metals are easily adsorbed onto the wall of the storing bottles at neutral to alkaline pH conditions. An optimal pH of seawater sample for preservation should be examined for each target metal in the laboratory. For most of the metals, it is better to lower the pH of the samples to less than 2 with hydrochloric acid or nitric acid. Since impurities in the acids directly cause contaminations, we need to check the blank values of the acids before use. If the samples are preserved for a long time, leachates of the target metals from the stored bottles should also be evaluated. By choosing the optimal material for the storing bottles and establishing their cleaning methods, we can preserve the seawater samples for a long time. During the production of polymers, some metals might be added to the material as catalysts. We need to examine which storing bottles are suitable for the target metals. For many trace metals, low-density polyethylene or Teflon bottles are known to be useful for storage.

On the other hand, by lowering the sample pH, the speciation of metals will be changed in seawater. In this case, we can temporally preserve the seawater samples by freezing at lower than -18 degree (e.g., Buck et al., 2012). However, we should not use dry ice for freezing because the pH of samples might be changed. It would be better to examine how long we can preserve the samples.

### **4. Analyses at land-based laboratory**

Many analytical methods have been developed to determine trace metals in seawater at land-based laboratory (Sohrin and Bruland, 2011). To determine many trace metals simultaneously, inductively coupled plasma mass spectrometry (ICP-MS) is frequently used. In the ICP-MS system, a high-temperature ICP source converts the atoms of the target metals to their ions. The produced ions are introduced to the mass spectrometer where the ions are separated and detected. The ions are separated by their mass-to-charge ratio with the quadrupole mass filter. This quadrupole mass filter can sufficiently resolve the mass-to-charge ratios of heavy elements, but this resolution is not enough to separate overlapping molecular or isobaric interferences from the isotopes of some trace metals (e.g., Fe, Cu and Zn). To obtain higher resolution, magnetic sector mass spectrometers are common in ICP-MS, which allow us to eliminate or reduce the effect of interferences.

Since seawater contains so much sea-salts, it is difficult to introduce seawater samples to ICP-MS directly. We have to separate target metals from the sea-salts before ICP-MS analyses. For this separation, several methods have been developed, such as magnesium hydroxide coprecipitation method and chelating resin preconcentration method. During these preconcentration processes, the samples are possibly contaminated, hence the processes should be performed under clean air condition or within the flow system. Moreover, the isotope dilution method, adding enriched isotope spike solution, is applied

for the precise measurement. We should select suitable methods for the target metals.

Recently, many methods have been developed by combining ICP-MS with preconcentration using chelating resin column. Among these methods, several methods, often used, are shown in the table below. To successfully determine trace metals in seawater, the analysts should have high levels of analytical skill and experience for clean sample handling. For example, we should check whether procedural blank values are low enough to measure the concentration level in oceanic water.

## 5. Reference seawater sample

Table. Determination methods of trace metals in seawater by on-line chelating resin preconcentration with ICP-MS.

References	Sohrin et al. (2008)	Milne et al. (2010)	Lee et al. (2011)	Biller and Bruland (2012)	Lagerstrom et al. (2013)	Minami et al. (2015)
Elements	Al, Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb	Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb	Fe, Cu, Cd, Pb	Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb	Mn, Fe, Co, Ni, Cu, Zn	Al, Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb
Resin	Nobias-chelate PA1	Toyopearl Chelate-650 IDA resin	NTA Superflow	Nobias-chelate PA1	Nobias-chelate PA1	Nobias-chelate PA1
ICP-MS	Quadrupole	Magnetic Sector	Quadrupole	Magnetic Sector	Magnetic Sector	Magnetic Sector
Detection Limit (nM)				Automatic	Automatic	Automatic
Fe	0.036	0.021	0.07	0.014	0.014	0.09
Cu	0.005	0.007	0.12	0.052	0.003	0.02
Pb	0.001	0.0002	0.0005	0.00056	-	0.0009
Mn	0.01	0.007	-	0.002	0.002	0.003
Zn	0.06	0.005	-	0.03	0.016	0.1

Before determination of trace metals in seawater samples, we usually examine the precision of the analytical methods. Moreover, to get the right number, it is required to analyze reference seawaters and report the results. It is better to choose and analyze reference seawaters in which the concentrations of the target metals are as the same level as those in actual samples. As reference seawaters, consensus values should be reported by several different groups with different methods. We can commercially obtain some reference seawaters, like NASS-series (National Research Council Canada). In these reference seawaters, the concentrations of trace metals are a little higher than those in Atlantic Ocean, Southern Ocean, Indian Ocean and Pacific Ocean. In the international GEOTRACES project, reference seawaters for main key parameters are widely distributed with their consensus values (e. g. SAFe, GEOTRACES seawaters) and available for Geotraces. The details of the reference seawaters are shown at the web site (<http://es.ucsc.edu/~kbruland/GeotracesSaFe/kwbGeotracesSaFe.html>). However, this distribution to the wide community is a voluntary work and the numbers of stocked seawaters are limited. Hence, the reference seawaters cannot be provided everlastingly. It is not easy to keep the reference seawaters for a long time at the same quality level. We will have to find the way to distribute the high-quality reference seawater continuously.

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## **DIC**

A Standard Operation Protocol of “Determination of total dissolved inorganic carbon in sea water” is available from the following CDIAC web site:

[http://cdiac.ornl.gov/ftp/oceans/Handbook\\_2007/sop02.pdf](http://cdiac.ornl.gov/ftp/oceans/Handbook_2007/sop02.pdf)

[http://cdiac.ornl.gov/oceans/Handbook\\_2007.html](http://cdiac.ornl.gov/oceans/Handbook_2007.html)

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## Determination of total alkalinity in sea water by spectrophotometry

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### 1. Scope

This procedure describes a method for the determination of total alkalinity in sea water by spectrophotometry. A method for the determination of total alkalinity by potentiometric titration has been described in SOP 3a and SOP 3b in Chapter 4 of Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.) 2007, “Guide to best practices for ocean CO<sub>2</sub> measurements. PICES Special Publication 3, 191 pp. ([http://cdiac.ornl.gov/oceans/Handbook\\_2007.html](http://cdiac.ornl.gov/oceans/Handbook_2007.html)). The method is suitable for assaying oceanic levels of total alkalinity (2000–2500 μmol kg<sup>-1</sup>).

### 2. Terms and definition

The total alkalinity of a sea water sample is defined as the number of moles of hydrogen ion equivalent to the excess of proton acceptors (bases formed from weak acids with a dissociation constant  $K \leq 10^{-4.5}$  at 25°C and zero ionic strength) over proton donors (acids with  $K > 10^{-4.5}$ ) in 1 kilogram of sample:

$$\begin{aligned}
 A_T = & [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B}(\text{OH})_4^-] + [\text{OH}^-] + [\text{HPO}_4^{2-}] \\
 & + 2[\text{PO}_4^{3-}] + [\text{SiO}(\text{OH})_3^-] + [\text{NH}_3] + [\text{HS}^-] + \dots \\
 & - [\text{H}^+]_F - [\text{HSO}_4^-] - [\text{HF}] - [\text{H}_3\text{PO}_4] - \dots
 \end{aligned} \tag{1}$$

Brackets represent total concentrations of these constituents in solution,  $[\text{H}^+]_F$  is the free concentration of hydrogen ion, and the ellipses represent additional minor acid or base species that are either unidentified or present in such small amounts that they can be ignored.

### 3. Principle

A known amount of sea water is placed in a container such as beaker or directly in an optical cell where it is first acidified to a pH between 3.8 and 4.2 with a known amount of hydrochloric acid. The acid titrant is made up in a sodium chloride background to approximate the ionic strength of sea water so as to maintain approximately constant activity coefficients during the measurement procedure. The solution of an indicator dye bromocresol green is also added to the sea water sample. The solution is then stirred for a period of time to allow for the escape of CO<sub>2</sub> that has evolved, and subsequently pH of the sample is measured precisely with spectrophotometric technique. Total alkalinity is computed from the volume of sample ( $V_{\text{SW}}/\text{dm}^3$ ), concentration ( $c_{\text{HCl}} / \text{mol dm}^{-3}$ ) and volume ( $V_{\text{HCl}}/\text{dm}^3$ ) of hydrochloric acid titrant added to the sample,  $[\text{H}^+]_T (=10^{-\text{pH}})$ , and the density of sea water sample when placed in the container ( $\rho_{\text{SW}}/ \text{kg dm}^{-3}$ ):

$$A_T = \frac{c_{\text{HCl}} \cdot V_{\text{HCl}} \cdot 10^6 - [\text{H}^+]_T \cdot (V_{\text{SW}} + V_{\text{HCl}}) \cdot \rho_{\text{SW}}}{V_{\text{SW}} \cdot \rho_{\text{SW}}} \quad (2)$$

It is necessary to measure the temperature of sample when it is placed into the container and when spectrophotometric measurement is made in order to compute the density of sea water sample and to correct for the effect of temperature change on spectrophotometric measurements.

#### 4. Apparatus

##### 4-1 An example of apparatus set-up

An example of apparatus set-up is shown in Figure 1. Usually, hydrochloric acid and indicator dye are added in a beaker, and spectrophotometric measurement is made in an optical cell separately. However, in an example shown here, measurements are made precisely and efficiently by adding a mixed solution of acid titrant and indicator dye to a seawater sample and making spectrophotometric measurement in the same cell in a spectrophotometric system that uses optical fibers.

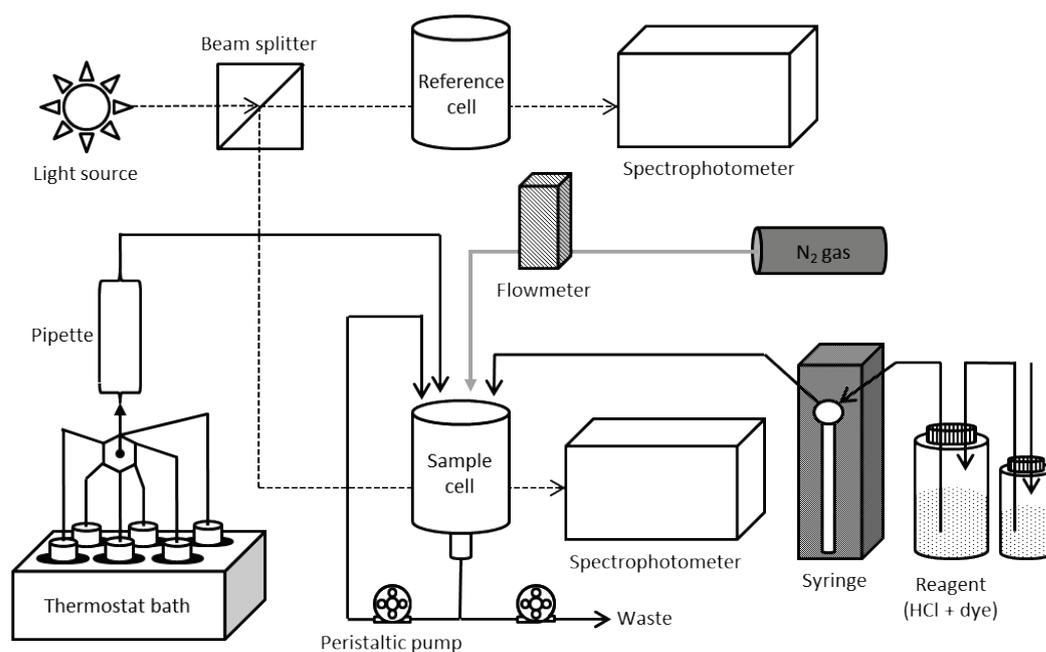


Figure 1 Schematic diagram of an apparatus to determine total alkalinity in sea water.

##### 4-2 Transferring sea water sample

Volumetric pipette ( $\sim 50 \text{ cm}^3$ ) is used to transfer sea water sample from a sample bottle into an optical cell (or beaker). In Figure 1, water-jacketed volumetric pipette with stopcocks at both ends are used to keep the sample temperature unchanged during the transfer. The pipette needs to be calibrated prior to use. In order to obtain the highest quality results with repeatability of total alkalinity analysis at around

$\pm 1 \mu\text{mol kg}^{-1}$ , it is necessary to measure the temperature of the sea water sample within the uncertainty of  $\pm 0.1^\circ\text{C}$ .

#### ***4-3 Beaker or optical cell***

Hydrochloric acid and indicator dye (it is convenient to use their mixed solution) are added to a sea water sample in a beaker or in an optical cell. It is then stirred and stripped of  $\text{CO}_2$  with a stream of  $\text{N}_2$  gas.

#### ***4-4 Addition of hydrochloric acid and indicator dye solution***

Hydrochloric acid (and indicator dye) is added to a sample seawater using a dispensing burette with its dispensing volume of  $5 \text{ cm}^3$  at its maximum. In order to obtain the highest quality results, it is necessary to use a highly reproducible burette ( $\pm 0.001 \text{ cm}^3$ ). Although such a burette is commercially available, its accuracy is typically not as good, and the burette system must be also calibrated prior to use. It is also recommended to keep the temperature of the acid unchanged.

The concentration of hydrochloric acid shifts as it is used and thereby the head space in the bottle increases. To minimize the change, the room air is introduced into the head space of the bottle after it flows through the same hydrochloric acid stored in another bottle.

#### ***4-5 Aeration of acidified sea water sample***

Cylinder of  $\text{N}_2$  gas mounted with pressure regulator and a flowmeter to indicate gas flow rate (ca.  $200 \text{ cm}^3 \text{ min}^{-1}$ ) is used to remove  $\text{CO}_2$  from the acidified sea water sample.

#### ***4-6 Spectrophotometer and optical cell***

Absorbances of the acidified seawater in an optical cell and deionized water in a reference cell are measured at wavelengths of 444nm, 616nm and 750nm with a spectrophotometer. For a better repeatability of the spectrophotometric measurements, it is necessary to use an optical cell of long path-length (8 cm or 10 cm). It is also required to control the temperature of the sample sea water in an optical cell by circulating the temperature-controlled water in the cell-holder, and measure the temperature of the sample at the time of spectrophotometric measurement by putting the temperature probe in the optical cell in the position where the probe does not disturb the light path.

#### ***4-7 Thermostat bath***

Thermostat bath is mounted with a circulation pump and capable of maintaining temperature at  $25.0 \pm 0.1^\circ\text{C}$ . This is used to control the temperature of a sea water sample transferred for measurement and that in an optical cell.

#### ***4-8 Thermometer***

Calibrated thermometer is readable to  $0.01^\circ\text{C}$ . This is used to measure the solution temperature when transferring from sampling bottle and to provide the value of solution temperature in optical cell for use

in subsequent calculations.

## 5. Reagents/Supplies

### 5-1 Nitrogen gas

Stream of N<sub>2</sub> is supplied from a gas cylinder as described in 4-5 in order to remove CO<sub>2</sub> from the sea water sample that has been acidified with hydrochloric acid.

### 5-2 Deionized water

Beaker or optical cell is washed thoroughly with deionized water each time after the measurement is finished.

### 5-3 Mixed titrant-indicator dye solution

A mixed solution of titrant and indicator dye of concentration approximately 0.05 mol dm<sup>-3</sup> in hydrochloric acid (0.05 mol dm<sup>-3</sup>), 0.65 mol dm<sup>-3</sup> in sodium chloride, and 80 μmol dm<sup>-3</sup> in bromocresol green is prepared in the following procedure.

#### 5-3-1 Instruments

- Calibrated balance to weigh 200g to within 0.01 mg.
- Oven capable of drying reagents at 600°C.
- Thermostat bath capable of controlling 25.0± 0.1°C and deep enough to immerse a 3 dm<sup>3</sup> volumetric flask to around its marked line.

#### 5-3-2 Tools

- 1×3 dm<sup>3</sup> borosilicate glass volumetric flask.
- 3×1 dm<sup>3</sup> plastic screw-cap borosilicate glass bottle with Teflon-liner cap such as those of Schott Duran<sup>R</sup>.
- 1×100 cm<sup>3</sup> capacity porcelain crucible.
- Desiccator with silica gel to store the porcelain crucible.
- 1× a few cm<sup>3</sup> capacity weighing bottle.
- 1×500 cm<sup>3</sup> measuring cylinder.
- 1×300 cm<sup>3</sup> capacity beaker.

#### 5-3-3 Reagents

- High purity primary standard grade sodium chloride (>99.98%).
- Hydrochloric acid (0.5 mol dm<sup>-3</sup>).
- Bromocresol green sodium salt.

#### 5-3-4 Procedures

*In a day before:*

- Put approximately 120g of sodium chloride in a porcelain crucible and dry it in an oven at 600°C for > 1 hour. Allow to cool it in a desiccator over silica gel and put it near the balance.
- Clean up and dry weighing bottle and beaker, and put them near the balance.

*On the day of preparation:*

- Weigh out 113.9180 g (1.95 mol) dried sodium chloride in a beaker.
- Weigh out 0.1818 g (240  $\mu$ mol) bromocresol green sodium salt in a weighing bottle.
- Pour 0.30 dm<sup>3</sup> of 0.5 mol dm<sup>-3</sup> hydrochloric acid (0.15 mol HCl) into measuring cylinder, and transfer it thoroughly into a volumetric flask by rinsing with deionized water.
- Using a funnel, transfer the weighed sodium chloride and bromocresol green to the volumetric flask. Rinse the beaker and weighing bottle into the flask to ensure quantitative transfer of the sodium chloride and bromocresol green salt into the flask.
- Pour approximately 1 dm<sup>3</sup> of deionized water into volumetric flask, shake it gently and let the solutes dissolved.
- Once sodium chloride was dissolved, pour deionized water up to below the calibration mark and shake the flask again to ensure a consistent composition.
- Immerse the flask in the thermostat bath being controlled at 25.0 $\pm$ 0.1°C, and allow the temperature of the mixed solution to reach an equilibrium value (> 20 minutes).
- Adjust the volume of solution contained in the flask to the calibration mark.
- Once it is closed, shake the flask gently to mix the solution.
- In case that the solution looks turbid because of the undissolved bromocresol green salts, filter out the undissolved salts.
- Fill 2 $\times$ 1 dm<sup>3</sup> screw-cap bottles with the solution. Store the surplus solution in another screw-cap bottle.

## 6. Procedures of the measurement

### 6-1 Outline

An analysis session, starting with clean up the burette, consists of the sequence of activities outlined in Table 1. At each stage of this procedure, compare the results obtained with the system's previous history to ensure that the method is performing according to prescribed specifications. Once the initial tests are complete, water samples can be analyzed.

Table 1. Recommended sequence of activities in an analysis session.

<b>Activity</b>
<ul style="list-style-type: none"> <li>• Clean up the pipette and burette, and calibrate them. Clean up the optical cell.</li> <li>• Set the bottle of acid titrant and indicator dye mixed solution in place. Turn on the power of thermostat bath, spectrophotometer, and so on. Wait until their performances get stabilized.</li> <li>• Rinse the burette and tubings with the acid titrant and indicator dye mixed solution.</li> <li>• Analyze two “junk” sea water samples to condition the system. Check the performance of spectrophotometer and confirm no bubbles in acid titrant and indicator dye mixed solution in the burette and tubings. If any problems are found, resolve it and analyze “junk” sea water sample again.</li> <li>• Analyze sea water reference material.</li> <li>• Analyze sea water samples.</li> <li>• Analyze seawater reference material again.</li> <li>• Calibrate the burette. Clean up the optical cell.</li> </ul>

### ***6-2 Clean up the optical cell***

It is critical to keep the optical cell always clean. If it gets stained, small bubbles tend to attach on the cell. They disturb precise spectrophotometric measurements and concurrently raise the baseline of absorbance. Optical cell is washed with brush and detergent but not with cleanser. Use of an ultrasonic cleaning machine is also recommended.

### ***6-3 Clean up and calibrate pipette and burette***

Volumetric pipette used to transfer sea water samples and volumetric burette used to deliver acid titrant to sea water sample in beaker or in optical cell are required to be kept clean and calibrated. Before and after a series of analysis, pre- and post-calibrations are required. In case that the apparatus is set on board a ship, their delivery volume should be confirmed by collecting several replicate samples that can be returned to the shore-based laboratory to be weighed. Deliver an aliquot of deionized water into a pre-weighed serum bottle using the volumetric pipette and burette. Seal the bottle and save it to be reweighed later (on return to shore).

The procedure has been described in Dickson, A.G., Sabine C. L., and Christian, J. R. (Eds.) 2007, “Guide to best practices for ocean CO<sub>2</sub> measurements. PICES Special Publication 3, 191 pp.”, chapter 4, SOP12 and SOP13 ([http://cdiac.ornl.gov/oceans/Handbook\\_2007.html](http://cdiac.ornl.gov/oceans/Handbook_2007.html)).

### ***6-4 Analyze sea water reference material and sea water sample***

#### ***6-4-1 Transfer sea water sample and measure absorbances of cell + sea water background***

Transfer a sea water sample into the optical cell, and measure absorbances at 730 nm (baseline), at

610 nm (maximum absorbance wavelength of base form of indicator dye ( $I^{2-}$ )), and at 444 nm (maximum absorbance wavelength of acid form of indicator dye ( $HI^-$ )). They are the background absorbances of the sea water sample.

#### ***6-4-2 Dispense acid titrant and indicator dye mixed solution to the sample sea water***

Transfer a known amount of sea water sample to a beaker or optical cell using a volumetric pipette and measure the sample temperature. Dispense acid titrant and indicator dye to the sample. The volume of acid titrant to be delivered is presumed from the site and depth of sampling and the salinity of the sample so that the pH of sea water sample becomes in the range between 3.8 - 4.2 after acid titrant is added and the evolved  $CO_2$  escapes from the solution. After dispensing the acid titrant and indicator dye to the sample, stir the sample and aerate it with a stream of  $N_2$ .

#### ***6-4-3 Measure absorbances of cell + sea water + dye***

Measure absorbance at the same three wavelengths as those measured in 6-4-1. The same optical cell as used to measure the background absorbances has to be used. Confirm that baseline absorbance at 730 nm agrees with that in cell + sea water background measurement within  $\pm 0.01$ . In case that the difference in the baseline exceeds 0.01, it is possible that small air bubbles attached on the cell and disturbed the spectrophotometric measurement, and the measurement should be done again after the cell is cleaned.

#### ***6-4-4 Clean up the cell***

Clean up the optical cell each time after the absorbance of the acidified sea water sample was measured.

#### ***6-4-5 Remarks***

Titrant acid in a screw-cap bottle should be replaced by another bottle of titrant acid when the head-space in the bottle exceeded half of the volume ( $500\text{ cm}^3$ ) since the concentration of hydrochloric acid is prone to change as the head-space increases. Once the acid titrant was replaced, substitute the titrant that remains in the burette and tubings with the new one. It is also critical to ensure that there are no bubbles in the burette and tubings.

It is desirable to repeat the measurement three times by changing the amount of acid titrant dispensed to the sample at least for the reference material. In case that the measurements have been properly done within the pH range of 3.8 – 4.2, the values of total alkalinity calculated will agree well irrespective of the volume of acid titrant dispensed to the sea water sample.

### ***6-5 Calculation of total alkalinity***

#### ***6-5-1 Correction of absorbance***

If the spectrophotometer that outputs the light intensity is used, users need to calculate absorbance  $A_\lambda$  from the light intensity at each of the three wavelengths for each measurement before and after delivering acid titrant and indicator dye.

$$A_{\lambda} = -\log_{10}\left(\frac{I}{I_0}\right) \quad (3)$$

In the next, subtract the absorbances measured before adding acid and dye from that after. The absorbance measured at a non-absorbing wavelength, *i.e.*, 730 nm, is used to monitor and correct for any baseline shift. This assumes that the magnitude of any observed baseline shift is identical across the visible spectrum. To do this, subtract the measured shift from the background - corrected absorbances at wavelengths 610 nm and 444 nm to obtain the final corrected absorbance value at each wavelength.

These final absorbance values, corrected for background absorbances and any observed baseline shifts, are used to calculate the absorbance ratio  $A_{616}/A_{444}$  that describes the extent of protonation of the dye.

#### 6-5-2 Calculation of the pH of the sea water + acid + dye

The pH of the sea water + acid + dye in the cell is computed from

$$\text{pH} = \text{p}K_2 + \log_{10}\left(\frac{(A_{616}/A_{444})_{25} - \varepsilon_{616}(\text{HI}^-)/\varepsilon_{444}(\text{HI}^-)}{\varepsilon_{616}(\text{I}^{2-})/\varepsilon_{444}(\text{HI}^-) - (A_{616}/A_{444})_{25} \varepsilon_{444}(\text{I}^{2-})/\varepsilon_{444}(\text{HI}^-)}\right) \quad (4)$$

The equilibrium constant  $K_2$  of the indicator dye bromocresol green at 25°C is a function of salinity and has been determined by careful laboratory measurements,

$$\text{p}K_2 = 4.2699 + (2.758 \times 10^{-3}) \cdot (35 - S) \quad (5)$$

where  $S$  denotes salinity of the sample.  $A_{616}$  and  $A_{444}$  represent final corrected absorbance values at 616 nm and 444 nm, respectively, and  $(A_{616}/A_{444})_{25}$  represents  $A_{616}/A_{444}$  ratio at sample temperature of 25°C.  $(A_{616}/A_{444})_{25}$  is calculated from Eq(6) using  $(A_{616}/A_{444})$  and sample temperature  $t$  (in °C) in optical cell when the spectrophotometric measurement is made.

$$(A_{616}/A_{444})_{25} = (A_{616}/A_{444}) [1 + 0.00907 (25 - t)] \quad (6)$$

The various extinction coefficient terms  $\varepsilon$  used in Eq(4) correspond to values measured for the specified species at wavelengths 616 nm and 444 nm, respectively (Table 2).

Table 2. Extinction coefficient ratios for bromocresol green

$\varepsilon_{616}(\text{HI}^-) / \varepsilon_{444}(\text{HI}^-)$	0.00131
$\varepsilon_{616}(\text{I}^{2-}) / \varepsilon_{444}(\text{HI}^-)$	2.3148
$\varepsilon_{444}(\text{I}^{2-}) / \varepsilon_{444}(\text{HI}^-)$	0.1299

### 6-5-3 Calculation of total alkalinity

Total alkalinity in sea water sample is calculated from Eq(2). The density of sea water is calculated from salinity that has been measured separately and temperature of sea water sample when it is transferred from the sample bottle.

In case that the mercury chloride solution was added to the sample as a bactericide, further minor corrections may need to be made to compute the total alkalinity in the original sea water sample:

$$A_T = 1.0002 A_T' \quad (7)$$

1.0002 is the coefficient when mercury chloride solution is added to the sample at the solution to sample volume ratio of 0.02/100.

### 6-5-4 Confirmation of the procedure

- In case that the spectrophotometer outputs the light intensity, it is critical that the output at 444 nm and 616 nm before and after acid – dye addition to the sea water sample is within the range that the output changes linearly with the change in the light intensity.
- pH of sea water sample is within the range of 3.8 – 4.2 when the spectrophotometric measurements is made after acid is added and CO<sub>2</sub> is removed.
- Absorbance at 730nm before and after the addition of acid and dye to a sea water sample should agree within  $\pm 0.01$ .

If any of these conditions have not been satisfied, problems have to be found and resolved, and the measurement has to be done again for the same sample. In principle, increase in the headspace air in the sampling bottle will not effect on the value of total alkalinity in sea water sample. Therefore, measurement of total alkalinity in a single bottle can be repeated if enough sea water sample to transfer to beaker or optical cell by volumetric pipette remains in the bottle.

## 7. Analysis of reference material

It is necessary that concentration of hydrochloric acid used to determine total alkalinity as a titrant has been calibrated within the uncertainty of  $\pm 0.02\%$ . However, in case that such an accurate calibration is not possible on board a ship or in a laboratory on land, circumstances may force one to use the Certified Reference Material (CRM) for dissolved inorganic carbon and total alkalinity analyses provided by Dr. A. G. Dickson in Scripps Institute of Oceanography to determine the concentration of the hydrochloric acid.

### 7-1 Procedure

Determine total alkalinity of the CRM according to the procedure described in 6.4. In order to evaluate the uncertainty due to the non-linearity in the response of spectrophotometer and due to the error in the volume of acid dispensed from a burette and so on, measurements should be repeated three times while changing the volume of acid titrant dispensed to the sample but keeping its resultant pH fall within the range of 3.8 – 4.2.

## 7-2 Calculation of the concentration of hydrochloric acid

Calculate the concentration of hydrochloric acid in the titrant:

$$c_{\text{HCl}} = \frac{\{A_{\text{T}}^{\text{CRM}} \cdot V_{\text{CRM}} + [\text{H}^+]_{\text{T}} \cdot (V_{\text{CRM}} + V_{\text{HCl}})\} \cdot \rho_{\text{CRM}}}{V_{\text{HCl}} \cdot 10^6} \quad (8)$$

$A_{\text{T}}^{\text{CRM}}$  represents the certified value of total alkalinity in the CRM.  $V_{\text{CRM}}$  represents the volume of CRM used for the analysis. The same volumetric pipette as that used for the sea water sample should be used so that  $V_{\text{CRM}}$  and  $V_{\text{SW}}$  in Eq(2) become identical.  $\rho_{\text{SW}}$  is calculated from the salinity of the CRM that has been determined and the temperature of the CRM when it is transferred from the bottle using the volumetric pipette.

## 8. Example calculations

### 8-1 Determination of the concentration of hydrochloric acid in titrant using CRM

Certified value of total alkalinity in CRM $A_{\text{T}}^{\text{CRM}}$	2224.65 $\mu\text{mol kg}^{-1}$
Salinity of CRM	33.357
Volume of pipette $V_{\text{CRM}}$	42.7495 $\text{cm}^3$
Volume of acid titrant added to the sample	2.05 $\text{cm}^3$

Measured data:

Temperature and density of CRM	25.14 °C, 1.0221 $\text{g cm}^{-3}$
Temperature of the sample at the time of spectrophotometric measurement	25.32° C

Measured light intensity and absorbance:

Wavelength	Sea water		Dye + acid + sea water		Absorbance
	Sample	Reference	Sample	Reference	
444 nm	25827	21890	18305	21863	0.1490
616 nm	36417	38894	27596	38868	0.1202
730 nm	13883	12699	13816	12679	0.0014

Result

$$A_{616}/A_{414} = \frac{0.1202 - 0.0014}{0.1490 - 0.0014} = 0.8050$$

$$(A_{616}/A_{414})_{25} = 0.8026$$

$$\text{pH}_{\text{T}} = 3.8483$$

$$[\text{H}^+]_{\text{T}} = 141.8 \mu\text{mol kg}^{-1}$$

$$c_{\text{HCl}} = 0.050577 \text{ mol dm}^{-3}$$

**8-2 Total alkalinity in sea water sample**

Salinity of sea water sample	34.424
Volume of pipette $V_{\text{SW}}$	42.7495 cm <sup>3</sup>
Concentration of hydrochloric acid $c_{\text{HCl}}$	0.050577 mol dm <sup>-3</sup>
Volume of acid titrant added to the sample	2.15 cm <sup>3</sup>

*Measured data*

Temperature and density of sea water sample	25.22 °C, 1.0228 g cm <sup>-3</sup>
Temperature of the sample at the time of spectrophotometric measurement	25.73° C

*Measured light intensity and absorbance:*

Wavelength	Sea water		Dye + acid + sea water		Absorbance
	Sample	Reference	Sample	Reference	
444 nm	25876	21904	18460	21891	0.1464
616 nm	36542	38921	25615	38912	0.1542
730 nm	13936	12734	13873	12736	0.0020

*Results*

$$A_{616}/A_{414} = \frac{0.1542-0.0020}{0.1464-0.0020} = 1.0540$$

$$(A_{616}/A_{414})_{25} = 1.0470$$

$$\text{pH}_{\text{T}} = 3.9678$$

$$[\text{H}^+]_{\text{T}} = 107.7 \mu\text{mol kg}^{-1}$$

$$A_{\text{T}} = 2373.8 \mu\text{mol kg}^{-1}$$

**9. Quality assurance****9-1 Quality of each titration data**

In order to assure the data quality of each sample, absorbance (light intensity), pH after adding acid to sample, and change in the baseline of spectrum (730nm) before and after the addition of acid and dye need to be controlled. Repeatability of analysis should be also evaluated by making replicate analyses by adding a constant volume and different volumes of acid titrant to the same sea water sample.

**9-2 Analysis of reference material**

A stable reference material should be analyzed regularly. Plot the results on a property control chart.

**9-3 Duplicate analyses**

A duplicate analysis should be made regularly on sea water sample and sea water reference material by adding a constant volume and different volumes of acid titrant. Plot the difference between each pair of analyses on a range control chart. The standard deviation expected is within 2  $\mu\text{mol kg}^{-1}$ .

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We have prepared this guideline by referring to and in a similar format to Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.) 2007, “Guide to best practices for ocean CO<sub>2</sub> measurements”. PICES Special Publication 3, 191 pp.

## pH

A Standard Operation Protocol of “Determination of the pH of sea water” is available from the following CDIAC web site:

[http://cdiac.ornl.gov/ftp/oceans/Handbook\\_2007/sop06a.pdf](http://cdiac.ornl.gov/ftp/oceans/Handbook_2007/sop06a.pdf)

[http://cdiac.ornl.gov/ftp/oceans/Handbook\\_2007/sop06b.pdf](http://cdiac.ornl.gov/ftp/oceans/Handbook_2007/sop06b.pdf)

[http://cdiac.ornl.gov/oceans/Handbook\\_2007.html](http://cdiac.ornl.gov/oceans/Handbook_2007.html)

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***p*CO<sub>2</sub>**

A Standard Operation Protocol of “Determination of  $p(\text{CO}_2)$  in air that is in equilibrium with a discrete sample of sea water” is available from the following CDIAC web site:

[http://cdiac.ornl.gov/ftp/oceans/Handbook\\_2007/sop04.pdf](http://cdiac.ornl.gov/ftp/oceans/Handbook_2007/sop04.pdf)

[http://cdiac.ornl.gov/oceans/Handbook\\_2007.html](http://cdiac.ornl.gov/oceans/Handbook_2007.html)

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# Particulate organic carbon (POC), particulate nitrogen (PN), and particulate phosphorus (PP)

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## 1. Scope and field of application

This procedure describes methods for the sampling and the analysis of particulate organic carbon (POC), particulate nitrogen (PN), and particulate phosphorus (PP) in seawater samples. For analytical methods, the high temperature combustion (HTC) method with an elemental analyzer for POC and PN, and the HTC and acid hydrolysis (HTC-AH) method and persulfate oxidation (PO) method for PP are described. Descriptions for POC and PN are based on the JGOFS protocol (Knap et al., 1996). Since many analytical methods for POC, PN, and PP have been published in past studies, analysts should select an appropriate method and establish an in-house protocol for the method based on their own situation. The HTC method for nitrogen has been adopted as the standard method for organic nitrogen analysis in research fields in Japan (Murakami and Shirai, 2015), and thus new technologies and related insights for the HTC method that can be incorporated into seawater POC and PN analysis may be published in the near future.

## 2. Definition

POC, PN, and PP in seawater are defined as carbon, nitrogen, and phosphorus in mainly non-volatile organic suspended matter derived from filtering seawater onto a filter, and expressed as moles per volume or weight of seawater. POC and PN can be analyzed simultaneously in a filter sample, and PP is analyzed using another filter sample. Samples for POC and PN are acidified prior to analysis, thus particulate inorganic carbon (PIC) is eliminated and we can measure POC. For PN, however, the acidification procedure cannot eliminate particulate inorganic nitrogen (PIN), thus we analyze PN but not particulate organic nitrogen (PON). In principle, total particulate carbon (TPC) is measured if a non-acidified sample is analyzed, therefore PIC can be estimated as the difference between TPC and POC. For PP, samples are decomposed and the liberated phosphate is analyzed, therefore particulate inorganic phosphorus (PIP) and particulate organic phosphorus (POP) cannot be differentiated and thus PP is measured.

## 3. Principles of the Analysis

For POC and PN, samples are generally combusted at temperature higher than 900 °C with oxygen. If a tin cup is used for the sample container, the combustion temperature of the sample can reach higher than 1800 °C. The combustion gas flows through a combustion column eliminating halogens, and then through a reduction column reducing nitrogen oxides to N<sub>2</sub> gas and eliminating excess oxygen. N<sub>2</sub> and CO<sub>2</sub> gases are separated with gas-chromatography or gas-specific adsorptions, and then quantified with a thermal conductivity detector. Principles of POC and PN analysis by elemental analyzer are detailed in JSAP (2008).

For PP, samples are combusted at 500 °C and then hydrolyzed by acid in the HTC-AH method or

are decomposed in persulfate solution under high temperature and high pressure conditions in the PO method. Liberated phosphate is quantified colorimetrically using the molybdenum blue method. Detailed procedures for colorimetry analysis are practically described in Oku (2002).

#### 4. Apparatus

Simultaneous analysis of POC and PN requires use of an elemental analyzer, which have been developed by various manufacturers. Various methods for combustion of the sample, separation of combustion gases, and detection of the target gas are adopted by each elemental analyzer. Currently available analyzers in Japan are reviewed in JSAP (2008).

For PP analysis, we need a hotplate, electric oven, heating device such as a hot water bath, and colorimeter for the HTC-AH method, and an autoclave and colorimeter for the PO method. For the colorimeter, a spectrophotometer for manual analysis or a nutrient autoanalyzer can be used.

#### 5. Reagents

All reagents should be of an analytical reagent grade quality unless otherwise specified.

For POC and PN analyses, concentrated HCl for the removal of PIC and acetanilide ( $C_8H_9NO$ ) for standards are required. Acetanilide is suitable as the standard for POC and PN because it has a C:N molar ratio of 8 that is a similar value to marine suspended particulate matter.

For PP analysis, sodium sulfate ( $Na_2SO_4$ ), magnesium sulfate ( $MgSO_4 \cdot 7H_2O$ ), and HCl in the HTC-AH method, and potassium persulfate ( $K_2S_2O_8$ ) (for nitrogen and phosphorus analysis) in the PO method are required to decompose the samples. For liberated phosphate analysis, ammonium heptamolybdate tetrahydrate ( $(NH_4)_6MoO_{24} \cdot 4H_2O$ ), L-ascorbic acid ( $C_6H_8O_6$ ), potassium antimony tartrate ( $K(SbO)C_4H_4O_6$ ), and potassium dihydrogen phosphate ( $KH_2PO_4$ ) (superpure grade of Merck) for standards are required.

#### 6. Sampling

If subsampling of seawater is not conducted immediately after the recovery of Niskin bottles, settling of large particles in the Niskin bottle will create a non-uniform distribution of the particles. Therefore, the Niskin bottle should be shaken before subsampling or the entire volume should be used as the sample. If needed, large zooplankton should be removed using a net e.g. by sieving through a 200  $\mu m$  mesh plankton net.

Particles in seawater are collected on binder-free glass fiber filters such as Whatman 25 mm GF/F filter. For POC and PN sampling, the filters are pre-combusted at 450 °C for 5 hours to eliminate carbon and nitrogen contamination. For PP analysis, the pre-combusted filters are washed using an acid. For this, a 10 mL aliquot of 1 N HCl is poured onto the filter attached to a filter funnel and the HCl removed by vacuum, and then the filter is thoroughly washed using Milli-Q water. After filtering the seawater for PP, rinse the filter twice with 2 mL aliquots of 0.17 M  $Na_2SO_4$  (Solórzano and Sharp, 1980). Filter samples for POC and PN, and PP are wrapped in pre-combusted (550 °C, 5 h) aluminum foil and stored at a temperature lower than -20 °C. If the filter samples are stored in a plastic container, the samples may be contaminated by carbon (Sharp, 1974). If the samples are stored in a freezer with organic

solvents or biological samples, the samples may be contaminated by carbon and nitrogen.

It is important to take a significant quantity of filter blank samples. The filter blank sample is processed by an identical procedure to the sample except filtering Milli-Q water is carried out instead of the seawater sample.

## **7. Analytical procedures**

### **7-1 POC and PN**

Stored filter samples are dried and acidified prior to the analysis. Samples are dried in a drying oven at 60 °C or in a freeze-dryer. Then, the samples are placed in a desiccator saturated with HCl fumes for 24 h. The air in the desiccator is kept saturated by leaving concentrated HCl in an open container in the desiccator, which is placed under a fume hood. Thereafter, the filter samples are placed in a vacuum desiccator with granulated NaOH to dry and remove the HCl that has detrimental effects on the subsequent elemental analysis. To vacuum the desiccator, a water-circulating aspirator should be used since a vacuum pump is not resistant to corrosion by HCl. While the filter sample can be dried in open aluminum foil that was used to store the sample in freezer, the sample has to be acidified in an acid-resistant container. The sample may be contaminated by carbon if the filter sample is placed on a plastic container (Sharp, 1974), therefore a glass container is appropriate for the acidification procedure.

Carbon and nitrogen contents of the filter sample are analyzed with an elemental analyzer following the guidelines given by the manufacturer. Reagents for the combustion column and the reduction column are sieved to remove any fine powder prior to packing into the column. To lower blank values the reagents should be pre-combusted prior to packing if applicable to the reagent. Then the reagents are packed into the column appropriately with consideration for the design of a furnace (vertical or horizontal) and the internal pressure of the columns. The analyzer should run overnight to stabilize the baseline before sample analysis. If you use a brand-new column, it is important that equilibrium conditions for adsorption-desorption between reagents and the analyte gas are achieved by processing with standard samples at the maximum concentration of the working curve several times before the start of the analysis run. Empty tin cups are used as blanks to confirm the baseline stability of the analyzer. Prior to sample analysis, a five-point working curve and three filter blank samples are analyzed. A blank and standard sample with a predetermined concentration is measured every 5–10 samples to ensure that problems have not developed. Details of the preparation, analysis run, and maintenance of an analyzer are described in JSAP (2008).

### **7-2 PP**

First, an example procedure of the HTC-AH method (Solórzano and Sharp, 1980) is described. A filter sample is placed in a glass vial with a screw cap. A two mL aliquot of 0.017 M MgSO<sub>4</sub> is poured into the vial, and the solution in the vial without the cap is dried on a hotplate. After combusting the sample in an electric oven at 500 °C for 2 h, the sample is treated in 0.2 M HCl in the vial with the cap at 80 °C for 30 min using a hot water bath. After cooling, the sample solution is filtered through a 0.22 µm pore size syringe filter. A four mL aliquot of the filtrate is added into a 50 mL volumetric flask and

make to 50 mL with Milli-Q water, and then the phosphate concentration is colorimetrically analyzed. It is important to confirm beforehand that the acid concentration of the sample solution does not inhibit color development of the molybdenum blue method. Although the sample solution may be neutralized with NaOH solution when the acid concentration is high enough to inhibit molybdenum blue color development, caution is required because excess addition of NaOH make the solution alkaline and then generates precipitation that adsorb and remove the phosphate from the sample solution. This may cause a serious underestimate or no detection of phosphate in the sample solution.

Second, an example procedure of the PO method is described. A filter sample is placed in potassium persulfate solution in an airtight container, and treated under high temperature and high pressure using an autoclave. After cooling, the sample solution is filtered through a 0.22  $\mu\text{m}$  pore size syringe filter. Since a high concentration of potassium persulfate inhibits color development of molybdenum blue method, the concentration is reduced to less than 2 %. Finally the phosphate concentration is measured colorimetrically with the molybdenum blue method. Although the PO method of Parsons et al. (1984) or equivalent has been widely used, it has been reported that the PO method may underestimate the PP concentration compared to the HTC-AH method. However, Suzumura (2008) shows that an increase in potassium persulfate concentration of the previous PO method under 120 °C for 30 min conditions can generate the same PP concentration to the HTC-AH method. They conclude that the improved PO method is a simpler procedure and a less time-consuming method than the HTC-AH method.

Regardless of the PP decomposition method, liberated phosphate is colorimetrically quantified with the molybdenum blue method of Parsons et al. (1984) for a single solution method or Hansen and Koroleff (1999) for a double solution method. Since the color development of molybdenum blue can be inhibited due to the combination of the decomposition method and colorimetry method, prior examinations to confirm a normal color development are necessary. Similarly, a manual and an automated analysis can generate a different color development, so it is important that the phosphate concentration in the sample solution has to be calculated with a working curve using standards in the same matrix to the sample solution.

## 8. Factors for errors

POC, PN, and PP have to be analyzed to a high precision using a small amount of sample, and therefore factors leading to errors should be eliminated as much as possible. A tin cup for the POC and PN sample container should be washed with ethanol and acetone to reduce absolute values and variability of blanks. If any volume of air is contained in a sample container with a sample filter, nitrogen in the air also is detected with the nitrogen derived from the sample and thus a cause of contamination (1  $\mu\text{L}$  of air is equivalent to 1  $\mu\text{g}$  of nitrogen). Although the organic and inorganic carbon and nitrogen dissolved in an approximately 0.1 mL aliquot of the seawater that is retained on a GF/F filter as a residue of filtered seawater does not affect the carbon and nitrogen values derived from the sample itself (Sharp, 1974), it has not been determined whether adsorption of dissolved matter in the sample seawater to the GF/F filter through filtering the sample seawater has a significant impact on the sample analysis value. Karl et al. (1998) and Morán et al. (1999) reported that a significant amount of dissolved organic carbon (DOC) produced during an incubation for the measurement of primary production with  $^{14}\text{C}$  method is

adsorbed onto a GF/F filter. Moran et al. (1999) reported that adsorption of DOC onto a GF/F filter is a cause of a 2–4 times higher value for several liters of filtered POC sample derived from a Niskin bottle than for several hundred liters filtered POC sample derived from an in situ pump. Wang et al. (2011) corrected their POC concentrations by subtracting 2  $\mu\text{mol}$  of carbon from the detected value of a filter sample to cancel out the effect of DOC adsorption. Adsorption of dissolved matter onto a GF/F filter can also cause a positive error for nitrogen and phosphorus, but the extent to which nitrogen and phosphorus adsorb onto a filter has not been clarified.

Furthermore, the utilization of a reference material (RM) is recommended to manage the quality of analytical results and to establish comparability to the results reported by different analysts. For example, an in-house secondary standard (plankton sample) for POC and PN analysis and apple leaves (NIST 1515) for PP analysis are used as a check standard in the Hawaii Ocean Time-series Program (<http://hahana.soest.hawaii.edu/hot/methods/results.html>). In Japan, National Institute for Environmental Studies (NIES) distributes RMs for environment measurements (<http://www.nies.go.jp/labo/crm/index.html>). In their list, water hyacinth (*Eichhornia crassipes*) (NIES CRM No. 29) has a certified value for phosphorus and a reference value for carbon and nitrogen, and thus this can be used as a RM for POC, PN, and PP analysis. A dataset for carbon, nitrogen, and phosphorus in particulate organic matter in the global ocean has recently been established by Martiny et al. (2014), and it is expected that the dataset is utilized in a wide range of research including modeling studies. More high quality data derived through better sampling and analytical protocols as reported here are needed to better understand the marine biogeochemical cycle of carbon, nitrogen, and phosphorus.

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## Biogenic silica

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### 1. Introduction

Biogenic silica (BSi) is an amorphous silica that is produced primarily by diatoms, silicoflagellates, radiolarians, and sponges. Among these organisms, planktonic diatoms are an important group and are currently responsible for 40% of primary production in the world's oceans (Nelson et al., 1995). Assimilation of dissolved silicic acid (DSi) by diatoms, dissolution of diatom frustules, and downward export and sedimentation of diatom debris are the major processes in the marine silicon cycle. To understand these processes, information regarding BSi is generally essential.

Analytical procedures for the measurement of BSi include wet-alkaline extractions, X-ray diffraction, and infrared absorption. Among them, the wet-alkaline extraction method appears to be the most versatile for the BSi measurement of suspended particulate matter (DeMaster, 1991). This method involves the alkaline extraction of BSi on filter samples and the subsequent determination of DSi using conventional colorimetry. Herein is described a modified literature procedure using NaOH for the extraction (Paasche, 1973). This procedure features a more rapid extraction and higher extraction efficiency than other wet-alkaline extraction methods (Krausse et al., 1983).

### 2. Sampling

Water samples should be collected using a silicon-free, alkaline- and acid-cleaned sampler such as a Niskin or Van Dorn sampler. Water collected in the sampler is then dispensed into a plastic bottle (and not a glass bottle) directly or using a vinyl tube. The use of silicon tubing instead of the vinyl tubing should be avoided, because silicon tubing can contaminate the BSi. The required sample volume, approximately equal to the filtration volume, is roughly several tens of milliliters to several liters for the coastal, high latitude, and equatorial upwelling regions; and several liters to several tens of liters for subtropical and equatorial warm pool regions. In practice, the required sample volume is determined based on the analytical detection limit of the colorimetry and the previously reported BSi concentration in a target region (see below).

### 3. Filtration

The sample water is accurately measured using a plastic graduated cylinder or a plastic bottle of known-volume (such as the plastic bottle used for sampling). The measured water is filtered using a plastic filtration device (with no glass components). If silicon packing is used in the filter holder, it should be replaced with a silicon-free packing, such as Teflon. There are many filters used for collecting suspended particulate matter, and a polycarbonate filter, such as a Whatman Nuclepore filter (GE Healthcare), is appropriate for BSi determination. A glass fiber filter cannot be used because it would introduce significant silicon contamination. A polycarbonate filter with a 47 or 25 mm diameter is commonly used, and its 0.4–0.8  $\mu\text{m}$  pore size is effective for collecting most diatoms. The filtration is performed using an aspirator with a gentle suction of <150–200 mmHg. After the filtration, the

particulate matter attached to the inside of the filter funnel, and the DSi adsorbed onto the filter, are washed with several tens of milliliters of low-DSi filtered seawater. The low-DSi filtered seawater is prepared by passing oligotrophic open water through a 0.2 µm pore size polycarbonate filter. The filter sample is folded and placed into a polypropylene centrifuge tube, e.g., 28 mL Oak Ridge Centrifuge Tubes, Nalgene. A tightly sealable and heat-resistant tube is preferred because it will be held at 100 °C during the extraction of BSi (see below).

#### 4. Extraction procedure

If a drying oven is available in the field, the filter sample is immediately dried at 60 °C for approximately 2 h. However, if one is unavailable in the field, the filter sample is temporarily stored at –20 °C, and after the field observation is complete the sample is dried at a land-based laboratory. BSi on the filter sample is extracted according to a procedure using a NaOH solution. First, 10 mL of 0.2 M NaOH is added to a filter-containing polypropylene centrifuge tube. At this point, the screw top of the tube should be wrapped using a Teflon seal tape to ensure a tight seal. After the tube is sealed and the filter is soaked in the NaOH solution, the sample is placed in a water bath at 100 °C for 15 min. The sample is then cooled to ambient temperature and neutralized using 10 mL of 0.2 M HCl.

#### 5. Colorimetry

BSi in the sample should be transformed into DSi after the extraction. The DSi concentration is then measured using a molybdenum blue colorimetry (see Vol. 3, Chap. 2).

During the DSi measurement, the blank and the matrix of standard solutions should be prepared using equal volumes of 0.2 M NaOH and 0.2 M HCl. A correction of filter blank is generally not required because Nuclepore filters are not contaminated with BSi. However, if other filter types are used, the absorbance of filter blank might require subtraction from the absorbance of the sample. A filter blank is prepared through the extraction and neutralization of a fresh filter that has not been exposed to sample water.

#### 6. Calculation

From the DSi concentration determined using colorimetry, the BSi concentration is calculated as follows:

$$\text{BSi } (\mu\text{M}) = \text{DSi } (\mu\text{M}) * 0.02 \text{ (L)} / \text{FV (L)}$$

where FV is the filtered volume, and 0.02 that is multiplied by DSi concentration is the total volume of 0.2 M NaOH and 0.2 M HCl used.

The FV is a critical term because it directly influences on the detection of DSi. If given a detection limit of 0.1 µM DSi and an ambient concentration of 0.01 µM BSi, the FV is calculated as follows:

$$0.01 \text{ } (\mu\text{M BSi}) = 0.1 \text{ } (\mu\text{M DSi}) * 0.02 \text{ (L)} / \text{FV (L)}$$

$$\text{FV (L)} = 0.2$$

In this case, >200 mL FV is required for the detection of BSi. Therefore, the DSi detection limit and the ambient BSi concentration should be reviewed before sampling, and an appropriate sample volume should be collected for the accurate determination of BSi.

## 7. Remarks

The wet-alkaline extraction method appears to be the most versatile, but it requires particular attention when used for samples collected from estuarine and coastal regions. In these regions, lithogenic silica (LSi) contributes significantly to the total particulate silica and its dissolution during the alkaline extraction often results in overestimation of the BSi concentration (Ragueneau and Tréguer, 1994). To determine the BSi concentration in high-LSi water, a method that corrects for the contribution of LSi to BSi should be applied (Ragueneau et al., 2005).

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# Carbon and Nitrogen Stable Isotopes in Particulate Organic Matter

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## 1. Introduction

Particulate Organic Matter (POM) in the ocean is an important food source for a variety of the marine organisms such as filter feeders and fishes. POM also functions as a reservoir of dissolved organic matter (DOM) and nutrients, which are generated during bacterial decomposition of POM, especially in oligotrophic pelagic waters. Furthermore, evaluation of the export flux of POM into the deep layers of the ocean is important for comprehending the Earth's carbon, nitrogen and phosphorus cycles.

Carbon and nitrogen stable isotopes ( $\delta^{13}\text{C}$  ·  $\delta^{15}\text{N}$ ) in POM in the euphotic layer primarily reflect the  $\delta^{13}\text{C}$  ·  $\delta^{15}\text{N}$  values of source materials, that is, dissolved inorganic carbon (DIC) and dissolved inorganic nitrogen (DIN), which are taken up by phytoplankton. POM isotopes also reflect the mixed POM carried from the other sources such as rivers. In addition to source values, the isotope values of POM reflect processes including isotope discrimination (kinetic isotope effect), such as due to prior uptake and biosynthesis of lighter isotopes by phytoplankton, and heterogenic modification such as due to bacterial decomposition of specific parts of the POM pool.

Of course we need to pay special attention to the interpretation of  $\delta^{13}\text{C}$  ·  $\delta^{15}\text{N}$  values in POM, because POM itself is an aggregation and/or mixture of different materials (i.e., phytoplankton, zooplankton and organic debris). Despite these potential complications,  $\delta^{13}\text{C}$  ·  $\delta^{15}\text{N}$  values can be an effective tool for investigating food web dynamics and material cycles (i.e., the source and pathways of the materials) in the aquatic systems, as far as we understand the characteristics of stable isotopes and interpret values in the context of other physical, chemical and biological parameters (Table 3-1, 2).

The concentration of POM is variable in space and time, and therefore, field and laboratory sample processing for POM  $\delta^{13}\text{C}$  ·  $\delta^{15}\text{N}$  can be time-consuming since we need to collect many samples to capture the spatio-temporal variation, as well as needing to adjust filtration volumes (see below). However POM  $\delta^{13}\text{C}$  ·  $\delta^{15}\text{N}$  analyses should be considered an important parameter in field observations of the ocean, now and in the future. It is also advantageous that the outsourcing of isotope analyses is available relatively cheaply, costing 2,000 to 5,000 JPY per sample, and the number of mass spectrometers in university or research institutes is increasing.

In this section, the basic protocols and devices for POM sampling, sample processing, and  $\delta^{13}\text{C}$  ·  $\delta^{15}\text{N}$  analyses are briefly explained. Because these protocols are not completely unified between researchers, previous articles dealing with  $\delta^{13}\text{C}$  ·  $\delta^{15}\text{N}$  analyses should also be consulted, depending on the conditions (e.g., Teece and Fogel, 2004; Miyajima, 2008; Ogawa et al., 2010; Ogawa et al, 2013). POM  $\delta^{13}\text{C}$  ·  $\delta^{15}\text{N}$  analyses are generally conducted using elemental analyzer-isotope ratio mass spectrometry (EA-IRMS), with POM generally defined as the particles trapped on a glass fiber filter with a pore size of 0.7 or 0.8  $\mu\text{m}$  (e.g., GF/F, Whatman). Filters with smaller pore size (e.g., GF-75, ADVANTEC) may be used in surface waters of the tropical and subtropical ocean, where the biomass of pico-plankton is not negligible. The appropriate filter pore size should be selected depending on the conditions of the target ocean, and the purpose of the observation.

Table 3-1. Factors causing variation in  $\delta^{13}\text{C}$ -POM and associated fluctuations of other parameters(※ An opposing pposite shift in  $\delta^{13}\text{C}$  values is often caused by the reverse case )

Factors causing a relative increase of $\delta^{13}\text{C}$ -POM	Probable phenomena observed simultaneously
• Increase in photosynthesis (DIC uptake)	1) Increase in irradiance, temperature, and nutrients supply 2) Increase in photosynthetic pigments (e.g., chlorophyll) 3) Increase in $\delta^{15}\text{N}$ values
Factors causing a relative decrease of $\delta^{13}\text{C}$ -POM	Probable phenomena observed simultaneously
• Supply of DIC with lighter $\delta^{13}\text{C}$ (e.g., freshwater DIC)	1) Decrease in salinity
• Contribution of terrestrial plants and freshwater plankton	1) Increase in C/N ratio (higher C/N ratio of terrestrial plants) 2) Decrease in salinity 3) Increase in organic polymers in vascular plants (e.g., lignin)

Table 3-2. Factors causing the variation of  $\delta^{15}\text{N}$ -POM and associated fluctuations of other parameters(※ An opposing opposite shift in  $\delta^{13}\text{C}$  values is often caused by the reverse case )

Factors causing a relative increase of $\delta^{15}\text{N}$ -POM	Probable phenomena observed simultaneously
• Contribution of wastewater-derived DIN with high $\delta^{15}\text{N}$	1) Decrease in salinity, Short distance from the shore
• Increase in $\delta^{15}\text{N}$ -nitrate due to denitrification	1) Decrease in dissolved oxygen 2) Increase in denitrifying bacteria (e.g., molecular approach) 3) Increase in $\delta^{18}\text{O}$ -nitrate
• Increase of photosynthesis (DIN uptake)	1) Increase in irradiance, temperature, and nutrients supply 2) Increase in photosynthetic pigments (e.g., chlorophyll) 3) Increase in $\delta^{13}\text{C}$ -DIC 4) Decrease in DIN concentration by uptake (Inverse correlation between $\delta^{15}\text{N}$ -POM & log-transformed DIN)
• Contribution of upwelling-derived $\text{NO}_3$ to primary production, compared with $\text{N}_2$ -fixation in pelagic water	1) Decrease in temperature, Increase of salinity 2) Developed vertical mixing
• Higher contribution of heterotrophs-derived organic matter with relatively heavier $\delta^{15}\text{N}$	1) Increase in heterotrophs (microscopic analysis) ( Increase of amino acid trophic level in POM) 2) Decrease in Chl/POC 3) Increase in water depth below euphotic zone
Factors causing a relative decrease of $\delta^{15}\text{N}$ -POM	Probable phenomena observed simultaneously
• Contribution of rainwater- $\text{NO}_3$ to primary production	1) Increase in $\delta^{18}\text{O}$ -nitrate 2) Decrease in salinity 3) Increase in DIN/P ratio in nutrient
• Increase in nitrogen fixation	1) Increase in nitrogen fixing bacteria 2) Increase in photosynthetic pigment, phycocerythrin
• Contribution of remineralized $\text{NH}_4$ (lower $f$ -ratio) compared with $\text{NO}_3$ from deep water in pelagic ocean	1) Increase in small-sized phytoplankton 2) Increase in nitrifying bacterial activity 3) Development of stratification

## 2. Sampling

For the analysis of  $\delta^{13}\text{C}$  •  $\delta^{15}\text{N}$  in organic matter, it is recommended to prepare samples such that they contain 60-100  $\mu\text{g}$  nitrogen in order to minimize the effect of contamination (i.e., atmospheric nitrogen, and organic matter attached to the glass fiber filter and tin capsules) on the real isotope values of the samples. The amount of carbon is not such a focus because the carbon to nitrogen ratio in natural organic matter is generally larger than 1. On the other hand, it is necessary to minimize the volume of glass fiber filter used and to maintain good analysis conditions, especially for small samples (see 3-4) because the incombustible glass fiber filters accumulate in the combustion column as debris often causing incomplete combustion of organic matter and blockage of gas passing, resulting in erroneous  $\delta^{13}\text{C}$  •  $\delta^{15}\text{N}$  values. Therefore, it is necessary to change the volume of water filtered depending on the

concentration of POM. For example, c.a. 10 L or more may be necessary over coral reefs, in the surface layers of offshore waters, and in very deep layers, compared to 2 or 10 L in the chlorophyll maximum layer or just several hundred mL for samples taken from a phytoplankton bloom in the coastal area. Increasing the amount of POM by increasing the water volume of the filtration results in an increase in filtration time. Furthermore, as POM trapped on the filter increases, it may partly fill the filter pores and then small particles less than the nominal pore size may also get trapped on the filter. We need to take care regarding the volume of water chosen for filtration to prevent an imbalance in the size of POM collected on the filter (Uematsu et al. 1978).

Seawater samples for POM  $\delta^{13}\text{C}$  ·  $\delta^{15}\text{N}$  are generally collected across water depths using a water sampler (e.g., Van Dorn sampler or Niskin water sampler equipped into RMS: Rosette Multibottle Samplers), and the water is transferred to a plastic container through a tube via a nylon net of 200 or 300 mesh size to remove large zooplankton or debris. Surface seawater can be collected by a bucket and transferred to the container with a funnel containing the nylon net. Water samples are then filtered on pre-combusted (3 hours at 450°C to remove the organic matter) glass fiber filters using an aspirator to keep suction pressure about 20–25 kPa (150–200 mmHg) to prevent larger particles from passing the filter. The entire water sample is generally introduced into the filtration system continuously using a siphon system, but it is necessary to regularly shake the container to keep an even POM concentration in suspension. At the final step of the filtration, rinse the inner wall of the funnel with filtered seawater, then wash the salt out of the filter with a few ml of the ultrapure water. Ammonium carbonate solution is also used for washing to minimize the possibility of lysis of phytoplankton cells due to the osmolar difference (e.g., Uematsu et al., 1978), but there is a risk of ammonium contamination in water samples for nutrient analysis during the sample processing. After filtration, the filter is removed from filter folder by forceps while maintaining suction pressure, and placed into pre-combusted aluminum foil or a petri dish and stored at a temperature of -20°C.

As suggested in Table 3-1, 2, there are many environmental factors causing shifts in  $\delta^{13}\text{C}$  ·  $\delta^{15}\text{N}$  values in POM. To properly interpret POM  $\delta^{13}\text{C}$  ·  $\delta^{15}\text{N}$  values in the context of environmental conditions, collection of not only basic parameters such as water temperature, salinity, chlorophyll, and dissolved oxygen, but also other data sets such as nutrients, predominant phytoplankton species, and bacteria may also be required during the field survey depending on the study purpose.

### 3. Sample Processing

Filter samples stored in the freezer are dried in a vacuum freezing oven or heating oven at about 60°C, and transferred to clean glass petri dish one by one. The filter samples are then stored in a tight box with a beaker of 20-40 ml concentrated hydrochloric acid (conc. HCl) for 1 day to remove Inorganic Carbon (e.g.,  $\text{HCO}_3^-$ ,  $\text{CaCO}_3$ ) by acid fumigation. After acidification, the petri dishes are placed in a vacuum desiccator equipped with no metal parts (or vacuumed by water aspirator) from several days to 1 week. There is the option to place granular sodium hydrate together with the samples to promote neutralization of the acid gas. At the final step, the filter is completely dried on a hotplate or in the oven for 1 day, and stored in a desiccator with silica gel.

In the next step, the marginal white portion of the filter, which contains no organic matter, is removed

using ethanol-washed forceps and scissors to minimize the amount of glass fiber filter relative to the amount of organic matter. Some researcher also slightly peel the back side of the filter for the same purpose. However, this is not recommended, because, although this method may increase the number of samples analyzed with single combustion column, carbon and nitrogen amounts are then underestimated and  $\delta^{13}\text{C} \cdot \delta^{15}\text{N}$  values can potentially deviate from the real values. Glass fiber filter samples are wrapped in a tin or silver capsule, and tightly folded into a pellet using 2 set of forceps. Generally organic matter, especially organic carbon, attaches to the surface of tin capsule. The contamination of organic matter can be minimized by soaking and washing with a clean organic solvent such as methanol or acetone. The use of disk-type smooth tin foil without wrinkles or smaller sized tin capsules decreases the amount of contaminated organic matter. Furthermore, in the case of silver capsules having a higher melting point, pre-combustion at 400-450°C effectively decreases the organic matter contamination (Ogawa et al. 2010).

To adjust the amount of organic matter to one appropriate for analysis, the filter is divided after confirmation that the samples were homogenously trapped into the filter. The relative weights of the whole filter and the filter part are measured, and used for calculation of organic matter concentration in the sample (e.g., mg-C/L, mg-N/L) based on the following equation.

POC conc. (mg-C/L) =

$$\text{POC (mg-C) in the filter part} \times \frac{\text{weight of whole filter (mg)}}{\text{weight of the filter part (mg)}} \times \frac{1}{\text{filtration volume (L)}}$$

When the amount of organic matter is large, some oxidant such as vanadium pentoxide ( $\text{V}_2\text{O}_5$ ) or cobalt oxide ( $\text{Co}_2\text{O}_3$ ) may be added with POM samples to promote complete combustion of the organic matter (Kanda et al., 1998), after checking the blank size (i.e., the contaminated organic matter in the oxidant). When tin capsules burn with a flash in the combustion column at about 1000°C, the temperature increases up to above 1800°C. Therefore, a small piece of tin-foil placed on the POM sample and wrapped together with the filter may promote complete combustion of the POM in the glass fiber filter. The sample roughly compressed with forceps can be further compacted with a handy tablet punch & dies with a pressure of 3 – 5 MPa to completely remove atmospheric nitrogen gas contained in the glass fiber. Atmospheric nitrogen remaining in the filter can cause overestimation of nitrogen contents and inaccurate estimation of the  $\delta^{15}\text{N}$  of the samples. In order to accurately evaluate the contamination of atmospheric nitrogen, it is necessary to prepare blank filter samples in the same manner as samples (see 3.4). The samples should be kept in a desiccator until analysis, but it is recommended they be analyzed as soon as possible to minimize contamination by atmospheric nitrogen.

#### 4. Sample analysis

The  $\delta^{13}\text{C} \cdot \delta^{15}\text{N}$  values of the filter samples in tin capsules are determined by EA-IRMS (Elemental Analyzer coupled to Isotope Ratio Mass Spectrometry) in the same way as the analysis of solid organic samples.

Generally  $\delta^{13}\text{C} \cdot \delta^{15}\text{N}$  values in the samples are first determined relative to the  $\delta^{13}\text{C} \cdot \delta^{15}\text{N}$  values in reference  $\text{CO}_2$  and  $\text{N}_2$  gas, respectively, and then this temporal values are converted to real values referring to the difference between the real values and measured values of standards having known

$\delta^{13}\text{C}$  ·  $\delta^{15}\text{N}$  values (Figure 3-1). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values often shift depending on the amount of carbon or nitrogen in the sample. In particular  $\delta^{15}\text{N}$  values in small samples tend to be lighter than the real values. Ideally the size of samples should be adjusted so that they contain enough nitrogen for the  $\delta^{15}\text{N}$  values to be minimally shifted, however this is often difficult for the POM samples (Fig.3-1). The mass-dependent shift in  $\delta^{15}\text{N}$  values of standards can be modelled by a regression fit, and the real stable isotope values of unknown samples then corrected based on the mass of N injected and the regression equation for the standards (Fig.3-1).

Amino acids having similar C/N ratio to that of particulate organic matter (e.g., 3.0 for alanine, 5.0 for proline, and 9.0 for phenylalanine) are often used as standards. These standard amino acids are generally secondary standards produced based on the primary international standard samples and are available through many domestic or foreign companies. The basic mechanisms of EA-IRMS, the details of machine-specific operation (e.g. based on the apparatus of Thermo Fisher Scientific Inc.), and details on the analysis of the data obtained are also available in previous reports (e.g., Brand 2004, Ogawa et al., 2013) in addition to the manuals of the product makers.

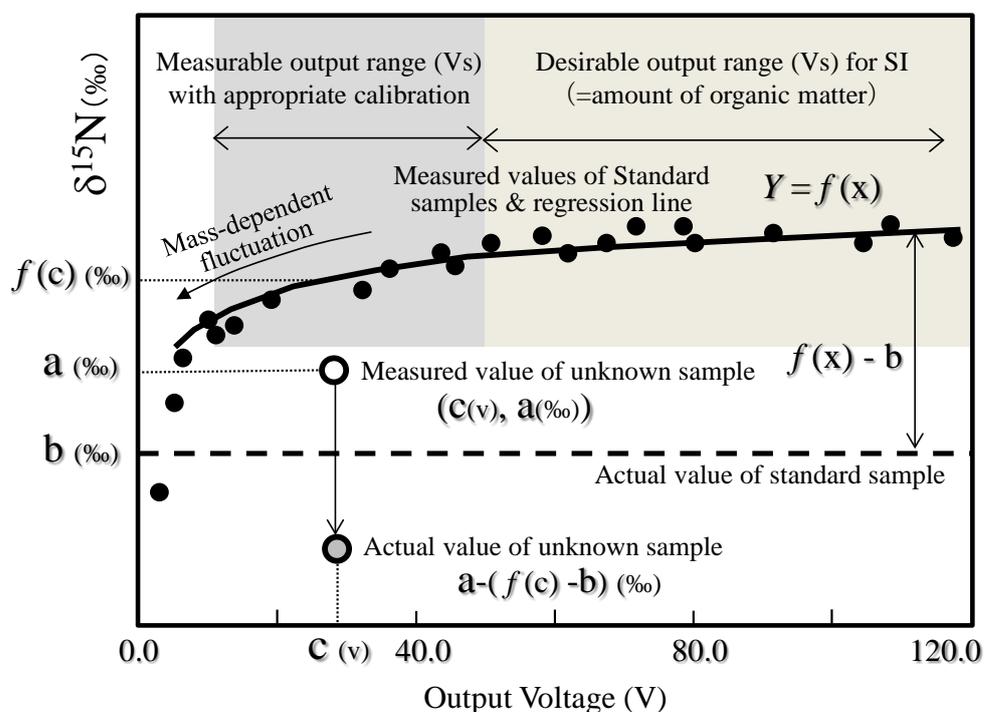


Figure 3-1. Calculation of actual stable isotope values of unknown samples based on the analyses of standards

Several points about which we should be extra careful in the analysis of filter samples having lower organic matter include: 1) to precisely measure the blank sample (i.e., tin capsule and glass fiber filter) and compensate the measured values of targeted samples by subtracting the blank size; 2) to confirm the accuracy and repeatability of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in standards having similar carbon and nitrogen content to that of targeted samples through repeated analyses; and, 3) to monitor the deterioration of the analytical conditions (e.g., clogging and contamination in the combustion column) and make a proactive decision regarding suspension of the analysis. Deterioration of the analytical conditions can be

objectively judged based on a shift of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and C/N ratios in the standards, a shift in carrier gas flow speed (ml/s) and retention time of the sample peaks, an increase the background values, and instability in repeatedly injected reference gases.

As mentioned in Section 1, nonflammable glass fiber filters accumulate as debris in the combustion column, and potentially cause incomplete combustion of organic matter and/or block the passage of the carrier gasses. These phenomena cause delays in the retention time of the sample peak and peak tailing, resulting in a shift in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values when the peaks overlap with the reference gas peak and the peak is not correctly evaluated. An inner ceramic or quartz tube placed in the combustion column may improve analytical conditions for glass fiber filter samples. Since the inner tube is easily replaced with a new tube when the symptoms of incomplete combustion are observed, many more filter samples can be analyzed using the same combustion and reduction columns (Kanda et al., 1998; Ogawa et al., 2013).

Furthermore, quartz tubes with smaller internal diameter ( $\phi$  8-10 mm) than the normal tubes with  $\phi$  14-16 mm ID decrease the dilution ratio of generated gasses ( $\text{CO}_2$  and  $\text{N}_2$ ) with carrier He gas, and are therefore useful for increasing the accuracy of analyses of samples with lower organic matter (Ogawa et al. 2010). Although the number of the samples that can be analyzed in the narrower tube is smaller than that of normal tubes, an increase in the analytical accuracy for small samples is an indispensable merit.

As introduced above, special care is needed for the analysis of filter samples. A longer time is required for these analyses to minimize interference with the reference gas peaks due to incomplete combustion and tailing peaks. Manual modification of the peak width on the chromatogram and calibration of the values based on the standards and blanks are needed for each sample analysis. Unlike the analyses of animal and plant samples, the accurate analysis of POM filter samples will be accomplished based on the accumulated experience and techniques used by each researcher.

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## Direct Counting Methods of Prokaryote and Heterotrophic Nanoflagellates by Epifluorescence Microscopy

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Prokaryotes and heterotrophic nanoflagellates (HNF) are abundant in the aquatic environment and play important roles in biogeochemical processes in the aquatic ecosystem (Konhauser, 2007; Madsen, 2008; Kirchman, 2008). A typical liter of water from the surface of a lake or ocean contains  $\sim 10^9$  prokaryotic cells and  $10^5$  HNF cells, depending on the environment (Kirchman 2012). Studying the dynamics of the abundance and activities of microbes is important because microbes are the most abundant organisms in aquatic environments and mediate many essential biogeochemical processes.

Methods for enumeration of aquatic microbes have been established and described in several papers and books (e.g., Sherr et al. 1993; Turley 1993, Caron 2001; Sherr et al. 2001). It is recommended to read those references before starting your experiments. The methods described in this chapter and in the references are not appropriate for all purposes. Users must modify the method to suit their own purpose.

This chapter is composed of the following four sections:

- 1) Sampling
- 2) Sample fixation
- 3) Staining, filtration, and producing sample slides
- 4) Sample observation and enumeration

### Procedures

#### 1. Sampling

##### *Sampling apparatus*

The sampling apparatus (bucket, bottle) should be washed and sterilized before collecting samples to avoid any biological or chemical contamination. Rinsing with the sample water is also recommended to avoid contamination. Other apparatuses for sampling (tubing, funnel) and sample bottles should also be washed and sterilized before use. Use of a pre-washed, sterilized, and disposable plastic apparatus for sampling and sample storage is convenient.

##### *Sampling*

Wear plastic laboratory gloves and use the apparatus described above during sampling to avoid contamination. Store samples in a refrigerator (dark, 4°C) immediately following sampling, and preserve the samples using a fixative as soon as possible. Long-term storage of samples without fixation may result in growth or death of microbes and grazing of HNF on prokaryotes. Freezing of samples without fixation is not recommended because the freeze-thaw process may disrupt microbial cells.

#### 2. Sample fixation

Fixation should be performed immediately following sampling. Prokaryote samples (1–20 mL) should be preserved in neutral-buffered formalin solution (final concentration, 1%) that has been passed through a 0.2  $\mu\text{m}$  filter. Samples should be refrigerated (4°C) until being subjected to filtration ( $\sim 12$  h).

HNF samples (1–100 mL) should be preserved in glutaraldehyde solution (final concentration, 1%). Samples should be refrigerated (4°C) until being subjected to filtration (~12 h). The type of fixative and its final concentration should be selected according to the objective, particularly for molecular biology techniques.

### 3. Staining, filtration, and producing sample slides

#### *Cell staining*

Both prokaryote and HNF cells must be stained with a fluorochrome for enumeration using an epifluorescence microscope because these cells do not auto-fluoresce. Cells that exhibit auto-fluorescence (those containing chlorophyll *a*, phycoerythrin, phycocyanin, bacteriochlorophyll, etc.) can be detected using an epifluorescence microscope without staining.

For prokaryotic cells, fluorochromes that bind to DNA—such as acridine orange (AO, Hobbie et al., 1977), 4',6-diamidino-2-phenylidole (DAPI, Porter and Feig, 1980), and SYBR Green I (Marie et al., 1997)—are generally used (Sherr et al. 2001). Fluorochromes that bind to protein are also used for enumeration of microbial cells. For precise determination of bacterial cell size, use of a protein-binding fluorochrome is more appropriate (Straza et al. 2009).

For HNF cells, fluorochromes that bind to DNA, such as AO and DAPI, are generally used (Sherr et al. 1993). Double-staining with a DNA- and protein-binding fluorochrome is also used for enumeration of HNF cells (Fukuda et al. 2007).

Each fluorochrome has specific excitation/emission wavelengths (Table 1). Optical filter sets for each fluorochrome must be used. The fluorochrome should be selected depending on the objectives of the experiment. DAPI is generally used for visualization of prokaryotes, whereas FITC is used for HNFs. Staining of prokaryotic cells with DAPI and of HNF cells with FITC are described below.

#### *Prokaryotic cell staining*

Add DAPI solution to the sample at a final concentration of 1  $\mu\text{g mL}^{-1}$ , followed by storage in the dark for 5–10 min. Large-volume samples (> 5 mL) should be reduced to 2 mL using a filtration funnel (to reduce the amount of DAPI required) and then stained with DAPI (final concentration, 1  $\mu\text{g mL}^{-1}$ ) in the dark (cover the funnel with aluminum foil) for 5–10 min. After staining, the remaining sample should be filtered.

#### *HNF cell staining*

Reduce the sample volume to 2 mL using the filtration funnel, and then release the vacuum. Add FITC solution to the sample at a final concentration of 0.4  $\text{mg mL}^{-1}$ , and then incubate the sample in the dark for 5–10 min. After the staining period, rinse the funnel and filter twice using a few milliliters of 0.1 M sodium carbonate buffer (pH 9.5).

#### *Sample filtration*

The filtration apparatus should be washed with 0.2 $\mu\text{m}$ -filtered (e.g., ADVANTEC, 25AS020AS) deionized water before use and between each sample.

Place a 0.45  $\mu\text{m}$  cellulose membrane backing filter (Millipore, HAWP02500) onto a 25 mm filtration bottom (note: this backing filter is to spread cells evenly on the filter; the same backing filter can be used for multiple samples; replace daily). Soak with a few drops of 0.2  $\mu\text{m}$ -filtered deionized water. Place a 0.2  $\mu\text{m}$  polycarbonate membrane filter or 0.8  $\mu\text{m}$  polycarbonate membrane filter for prokaryotes and HNF, respectively, onto the wetted backing filter. Place the filtration funnel on top of the filter. For enumeration of prokaryote cells, a filter with an effective diameter (16–25 mm) is appropriate. For enumeration of HNF cells, a filter with an effective diameter of 4 mm is appropriate (a smaller filter area provides higher concentration efficiency). Use forceps to handle filters, and try not to touch the filter area.

For prokaryotes, filter an aliquot of DAPI-stained sample through a 0.2  $\mu\text{m}$  membrane filter. For HNFs, filter an aliquot of fixed sample through a 0.8  $\mu\text{m}$  membrane filter. The sample volume required is dependent upon the cell concentration. A maximum vacuum of 80 mmHg is appropriate for filtration to avoid damage to cells and ensure their random distribution. Rinse with a few milliliters of 0.2  $\mu\text{m}$ -filtered deionized water for prokaryotes or a few milliliters of 0.1 M sodium carbonate buffer for HNF. With the vacuum on, carefully remove the membrane filter from the underlying backing filter. Proceed to the next step, “Slide preparation,” immediately.

#### *Slide preparation*

For prokaryotic cells, place the filter onto a slide and place immersion oil (~10  $\mu\text{L}$ ) on the center of the filter. Mount a new coverslip on top of the filter and gently press down until the oil moves out to the edge of the filter and forms a seal. For HNF cells, use FA mounting fluid (pH 9.0–9.6) instead of immersion oil.

## **4. Sample observation and enumeration**

### *Observation*

Prepare the optical filters appropriate for the fluorochrome (Table 1). Samples are visualized using a 100 $\times$  oil immersion objective and 10 $\times$  eyepiece. Prokaryotic cells are 0.2–2.0  $\mu\text{m}$  in length and bright blue-white in color. You may distinguish bacilli, cocci, and spirilli among prokaryotic cells. Plate 4 in Pernthaler et al. (2001) shows a representative image of a prokaryotic cell. HNF cells are 2–20  $\mu\text{m}$  in length and fit into a small quadrant of squared graticule. You may observe flagella attached to the cells. Figure 3 in Sherr et al. (1993) and Figure 7.1 in Kirchman (2012) show several representative images of HNF cells.

Table 1. Fluorochrome characteristics.

Fluorochrome	Excitation peak (nm)	Emission peak (nm)
AO	490	530, 640
DAPI	372	456
FITC	490	520
SYBR Green I	494	521
SYPRO Ruby	280	610

### *Enumeration*

Randomly choose 10 large quadrants ( $100 \times 100 \mu\text{m}$ ) and count the cells in each. Thirty to fifty cells per large quadrant are appropriate for counting. If the cell density is outwith this range, the sample volume should be adjusted appropriately. Counting errors and variations of this method are described in Kirchman (1993).

Calculate prokaryotic and HNF cell concentrations as follows:

$$\text{Cells mL}^{-1} = [\text{SC} \times \text{CF}] / \text{V},$$

where SC is the mean sample count/quadrant, CF is the effective filter area/quadrant area, and V is the volume of preserved sample (not including the fixative volume).

Biomass estimates of prokaryotes and HNF are convenient for comparing the carbon budget to other microbial groups or prokaryotes and HNF in other environments. Two methods can be used to convert cell abundance to carbon biomass. One is to convert cell volume to carbon content per cell (Norland 1993), and the other is using a conversion factor (e.g., 20 fg of C per cell, Fukuda et al., 1998). For calculation of HNF biomass, please see Chapter 6 of this guideline.

It should be noted that the fixatives and fluorochromes described here could cause personal injury and health problems. Appropriate protective equipment (e.g., protective eyewear, gloves, laboratory coat, etc.) should be worn and procedures conducted under appropriate laboratory conditions (e.g., clean bench and fume hood).

The methods described here are appropriate for current studies but may not be in the future. More appropriate fluorochromes, more efficient methods of concentrating cells, or more convenient enumeration techniques may be developed. The enumeration methods should be updated as appropriate. Cells have been enumerated by flow cytometry (described in the next section) in recent studies.

## **Materials required**

### Equipment and Supplies

Microscope: Epifluorescence microscope with a 100 W mercury lamp, a 100 $\times$  oil immersion objective, and an ocular grid of 10  $\times$  10 squares.

Filtration apparatus (for prokaryotes): apparatus fit for 25 mm  $\phi$  filters (e.g., ADVANTEC filter manifold KG25 or Hoefer Ten-Place Filtration Manifold FH225V).

Filtration apparatus (for HNF): apparatus fit for 25 mm  $\phi$  filters with a small effective filter area (e.g., ADVANTEC filter manifold KGS-04)

Also, 0.2  $\mu\text{m}$  pore size, 25 mm polycarbonate membrane filters (for prokaryotes), and 0.8  $\mu\text{m}$  pore size, 25 mm polycarbonate membrane filters (for HNF). Black-stained filters of those types are useful for reducing strong background intensity (e.g., ADVANTEC polycarbonate filter K020N025A).

Backing filter: 0.45  $\mu\text{m}$  pore-size, 25 mm cellulose membrane filter

Vacuum pump

Glass slides and cover slips

Immersion oil and FA mounting fluid (Vmrdr, Inc.)

### Solutions

DAPI stock solution, 0.5 mg mL<sup>-1</sup>. Dissolve 10 mg DAPI in 20 mL filtered distilled water. Dispense 0.5 mL aliquots of the DAPI stock solution into plastic vials and store at -20°C.

FITC staining solution

0.25 ml 0.5 M sodium carbonate buffer (pH 9.5)

1.1 mL 0.01 M potassium phosphate buffer (pH 7.2)

1.1 mL 0.85% sodium chloride

1.0 mg FITC

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## Enumeration of prokaryotes by flow cytometry

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### 1. Instrument and reagents

- A flow cytometer and its ancillaries
- Black plastic bottles of volume > 50 mL
- Micropipettes (1 mL and 10  $\mu$ L)
- Standard fluorescent bead suspension (with a diameter of  $\sim$ 1  $\mu$ m)
- SYBR Green I (or SYBR Gold) solution
- A vortex mixer

For sample fixation

- Glutaraldehyde or paraformaldehyde solution
- Disc filters (with a pore size < 0.2  $\mu$ m) and a syringe
- Microdispenser
- Cryovials and canes
- A dry shipper
- A deep freezer

To measure samples onboard, a flow cytometer with an optical system that can be readily adjusted by a user is necessary. For oceanographic requirements, an argon ion laser or a solid laser that can emit blue light is recommended, because it can excite chlorophyll, phycoerythrin and various artificial fluorescent probes including SYBR Green I and SYBR Gold. Light channels should include forward scatter (FSC), side scatter (SSC), green fluorescence (FL1) for fluorescent probes, orange fluorescence (FL2) for phycoerythrin and red fluorescence (FL3) for chlorophyll. The cytometer should be installed on a vibration isolation table in a dry laboratory, and its power should be supplied by a noise-free source. Before and after measurements, instrumental settings should be checked using standard fluorescent beads. Before sampling, plastic bottles should be soaked in detergent and rinsed well with distilled water. The bottles must be rinsed immediately after use, and air-dried. The standard bead suspension should be diluted depending on the intended use and stored in a refrigerator. The concentration should be measured using an epifluorescence microscope. SYBR Green I or SYBR Gold should be stored in a freezer after being diluted 100 times.

### 2. Sampling and measurement

Seawater can be dispensed directly into black plastic bottles or through silicon tubing from water samplers. The bottles must not be subjected to direct sunlight, to avoid temperature change. A cooler is useful to minimize the temperature change until further analysis.

Fluorescent beads are added to the sample before analysis for the following two reasons. The first is to serve as a standard for the flow rate. The flow rate of a flow cytometer is unstable onboard, except when installed with a syringe pump, which can serve a sample at a controlled rate, as it is affected by the ship's motion and engine vibration. To know an exact flow rate, a bead suspension with a known

particle concentration is added. The second is to serve as a standard of scatter and fluorescence intensities. The light intensities measured by a cytometer can be affected by machine sensitivity, configuration of optical systems, life of the laser, and run length. To compare the values among different samples, they should be normalized to those of the standard beads. The intensity of forward and side scatters is essential to estimate cell size (Ackleson and Spinrad, 1988). The concentration of the beads added must not be too low or too high, relative to the total particle concentration in the sample.

For sheath fluid, either commercially available fluid, filtered seawater (< 0.2  $\mu\text{m}$ ), or Milli-Q water can be used, although each of the three has advantages and disadvantages. Commercially available fluid can prevent adhesion of microbes to the tubing inner wall; however, it costs more than the other two alternatives. Milli-Q water is the most economical, and does not induce biofouling. Filtered seawater is advantageous in estimating cell size, because it has the same refractive index as the samples (Cucci and Sieracki 2003), although it frequently induces biofouling. Any of the three above-mentioned materials can be used as sheath fluid, depending on the purpose of the measurement. After using filtered seawater as sheath fluid, it is necessary to clean the tubing vigorously using commercially available sheath fluid or detergent diluted with Milli-Q water. Sonication treatment of sheath fluid may be effective in decreasing noise, by removing the microbubbles present in it.

For enumeration of phytoplankton, which requires no staining procedures, the appropriate volume of seawater is poured into a sample cell. The sample should be measured using FL3 as a trigger parameter, while arranging the gains so that targeted populations are properly captured on FL2-FL3 and FL3-FSC (or SSC) cytograms. The flow rate should be properly set, with a tradeoff of efficiency and sensitivity or precision. When focusing on *Prochlorococcus*, which has an extremely faint autofluorescence, large eukaryotic phytoplankton or nano-sized cyanobacteria are sometimes omitted from the detection range. When this occurs, the same sample should be measured twice using different settings. For detailed processes of categorization of different phytoplankton populations on cytograms, see Marie et al., (1999) or Sato et al., (2010).

For enumeration of heterotrophic prokaryotes, which include bacteria and archaea, fluorescent probes for nucleic acids are generally used, among which SYBR Green I (Marie et al., 1997) is the most widely used, followed by SYTO 13 or PicoGreen. SYBR Gold can be used as an alternative to them (Patel et al., 2007). When added at high concentrations, the fluorescent probe can stain unfixed marine prokaryotes efficiently for detection by flow cytometry (Kamiya et al., 2007). Ten minutes before measurement, 1 mL of seawater sample should be mixed with 10  $\mu\text{L}$  of SYBR Green I working solution and vortexed. FL1 is used for as trigger parameter. The cluster of prokaryotes that appears on an FL1-FSC (or SSC) cytogram includes the cyanobacterium *Prochlorococcus* with faint chlorophyll fluorescence; it is required to correct for it (Sieracki et al., 1995). On the cytogram, more than two clusters with different FL1 intensities are sometimes observed, which possibly reflect their ecological difference (Gasol and del Giorgio, 2000). Differentiation of the clusters becomes easier when membrane permeability is enhanced by chemical fixation (Kamiya et al., 2007).

After measurement, tubing should be rinsed repeatedly, which is followed by an appropriate shutdown procedure. Because the residual stains in the tubing may cause noise, the use of a surfactant detergent is recommended after using a staining reagent. The machine operating time should be recorded

for quality control of the laser.

### 3. Sample preservation

Owing to the limitation of space or time, samples collected for flow cytometry are sometimes preserved. Among the various preservation methods available, most include chemical fixation by aldehyde, snap freezing in liquid nitrogen, and storage at  $-80\text{ }^{\circ}\text{C}$ . Glutaraldehyde, paraformaldehyde, or a combination of the two is widely used as fixative. Since preservation efficiency depends on species composition, its physiological status and the method used (Vaulot et al., 1989; Trousselier et al., 1995; Sato et al., 2007), the optimum method applicable to every marine microbial community is yet to be established. Here, I introduce a simple preservation method using glutaraldehyde, which has high fixation efficiency.

Prior to sampling, 20% glutaraldehyde solution (for electron microscopy) should be filtered through a disc filter by using a syringe to remove particles that may cause noise. Using a micropipette, dispense the samples into cryovials (~5 mL), and then add the glutaraldehyde solution at a final concentration of 1% by using a microdispenser. Tightly close the lids, mix the solution within the cryovials by gentle shaking, and leave them still for 10 min in a refrigerator before snap-freezing in liquid nitrogen. If the dry shipper is not large enough to contain all samples, some samples can be stored in a deep freezer after snap freezing. For transportation, dry ice is recommended to maintain the lowest possible temperature. The preserved samples should be thawed in running water just before measurement. Ensure that there is no ice remaining in the samples. Refreezing of the samples is not recommended. Recently, it was reported that glutaraldehyde fixation with the addition of small amounts of surfactant can greatly improve cell preservation (Marie et al., 2014).

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## Plankton Net

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### 1. Plankton net

Plankton are drifting organisms in water bodies because they are nonmotile or poor ability to swim against a current. Most of them are tiny <20 mm, but some gelatinous plankton such as jellyfish and tunicates are larger than 100 mm with weak swimming ability. In marine ecosystems, the density is higher for smaller organisms. For small plankton (~0.2 mm), such as bacteria, archaea, phytoplankton, protozoan zooplankton, collecting water samples (milliliters to liters) and concentrating them with various methods (Vol. 4). In order to collect larger metazoan zooplankton which density is much lower, we need to concentrate organisms from much larger volume of water than water bottle sampling. Plankton nets are sampling devices of plankton that are towed thorough water and filter plankton larger than the mesh size. Fig 1 shows a NORPAC (North Pacific Standard) net and a WP-2 (UNESCO Working Party 2) net, which are widely used conical net and cylinder-conical net, respectively.

The minimum size of plankton collected with a plankton net is defined by the mesh size. The maximum size is influenced by variable factors such as tow speed and mouth opening size of the plankton net, swimming speed and visual or mechanosensory recognition ability of plankton. For the sampling of micronekton, which swim faster than zooplankton, larger nets or trawls allowing high speed tow are used (e.g., IKMT、 MOHT、 RMT, Sameoto et al., 2000; Oozeki et al., 2004). Plankton nets are also used for micro phytoplankton and microzooplankton sampling such as diatoms, radiolarians, forminiferans. In this manuscript, we use a term “zooplankton” as metazoan zooplankton which are the main target of plankton net sampling.

### 2. Filtering efficiency of plankton net and filtering rate

The choice of the type of plankton net, mesh size and towing method are depending on target zooplankton species and groups. A plankton net with larger mouth area filters more water in a certain amount of time and reduces the possibility of net avoidance of zooplankton. On the other hand, requiring larger filtering area (i.e., net length) to minimize the influence of net clogging by phytoplankton and detritus and to sustain the filtering efficiency during tow. The initial filtering efficiency (IFE) of a conical net is 75-85%, and one of conical-cylinder net, such as WP-2 (Fig. 1), reaches around 100% (Keen, 2013). IFE is decreasing with net tow due to net clogging. The decline in the filtering efficiency is more serious in the water with high abundance of chain-forming diatoms and/or sticky organisms such as *Noctiluca*. These organisms are usually abundant in the surface layer of the water column. Thus, the declining of the filtering efficiency means not only decreasing the filtering volume but also inducing the underestimation of the zooplankton abundance distributing in the surface layer and crucial impact on the quantitative sampling.

The Open Area Ratio (R) is the ratio of the filtering mesh area to the mouth area of net.

$$R = a\beta / A$$

Where, a,  $\beta$ , A mean mesh area, mesh porosity, and mouth area, respectively.

$$\beta = m^2 / (d + m)^2$$

m means mesh width, and d the diameter of mesh filament. Since the filtering efficiency declines with filtering volume by net clogging, a plankton net with large R is appropriate using in plankton rich waters. The minimum R for quantitative sampling is represented as follows (Sameoto et al, 2000),

$$\text{Green water} \quad \text{Log}_{10}(R) = 0.38 \times \text{Log}_{10}(V / A) - 0.17$$

$$\text{Blue water} \quad \text{Log}_{10}(R) = 0.37 \times \text{Log}_{10}(V / A) - 0.49$$

where V means the filtering volume of sea water ( $\text{m}^3$ ). In the case of 150-m vertical tow of a NORPAC net (Fig. 1) fitted with 335  $\mu\text{m}$  Nylal mesh ( $R=3.7$ ), the net clogging is estimated to be not serious in blue water (minimum  $R=2.1$ ). But in green water, the filtering efficiency may decline (minimum  $R = 4.5$ ). Tranter and Smith (1968) recommended  $R>5$  for a plankton net fitted with mesh $>0.33$  mm, and  $R>9$  for mesh $<0.33$  mm (Keen, 2013). In Japan, short conical nets have been used because of easy-handling on small ship and also low product price. The users should be careful these low R nets are easy clogging and inappropriate for quantitative sampling in green water.

In order to determine the filtering volume of sea water during net tow, a flow meter is mounted at net mouth as confronted with towing direction. For a plankton net with bridles in front of the net, the flow meter should be set deviated from the mouth center to minimize the interfere of bridles on the flow field (e.g., in quarter of the diameter). The flow meters should be calibrated at calm sea surface condition. Using the plankton net frame without net or a calibration frame, towing more than 7 times to obtain the revolution of the flow meter in a certain distance towed at 100 % filtering efficiency. The revolution per distance can be changed by strong impact such as hitting to the hull or falling on the deck. The flow meter should be rinsed with fresh water to remove salt after use.

### 3. Plankton net sampling and sample handling

It has been developed various plankton nets to collect zooplankton (Wiebe and Benfield, 2003). The towing methods of plankton nets which widely used in Japan are summarized in Hasumoto (2006).

Towing methods are categorized as: 1) vertical tow, 2) oblique tow, 3) multi-layer sampling with opening-closing nets, 4) neuston sampling. In this manuscript, describing the sampling procedure with vertical tow and sample handling at first, then other towing methods and devices.

#### 3-1 Vertical tow

Vertical tow of a plankton net is widely used sampling method which enable to estimate zooplankton abundance and biomass per area in relatively short sampling time. This method has been widely used for various monitoring projects. Because of relatively low filtering volume, it is inadequate for examining the abundance for minor species.

In the case of using a NORPAC net, a 10-30 kg lead weight is used as a sinker. To avoid twisting bridles and/or rope, use swivel to connect with wire and the weight (Fig. 1). When the net is at the sea surface, make zero-correction of the wire length meter. To prevent the generation of kink of wire, the

pay out speed of the wire is to be adjusted to keep the wire tension, especially when the net is at the sea surface. In the calm sea condition, the pay out speed may reach  $1 \text{ m s}^{-1}$ .

When the wire length is approaching to the target depth, measure the wire angle and pay extra length (Table 1) out to reach the net to the target depth. Hauling speed is generally  $0.5\text{-}1.0 \text{ m s}^{-1}$ . It has been observed that zooplankton specimens are taken in poorer condition at higher towing speed. Also, inducing escapement through the mesh due to the high filtration pressure. The decline of the filtering efficiency at high speed towing is more serious for conical net than cylinder-conical net. At the sea surface, it should be careful that the plankton net can be damaged by wind and swell under the rough sea surface condition.

Since some plankton are caught in mesh or stick on due to the mucus, wash the plankton net carefully using an appropriate flow of sea water from the outside of the net to remove plankton. Clogging near the cod end is usually more serious. The cod end is taken off and the obtained samples are transferred into a sampling bottle or jar (see 4). Record the revolution and ID of the flow meter.

In order to collect live zooplankton for experimental studies or obtain less damaged specimen, it is recommended to tow slowly ( $<50 \text{ cm s}^{-1}$ ) and use a large cod end. It has been used several to tens liter of bottle or plastic bag (Fig. 2) for the cod end. It is effective to enlarge the bottom diameter of the plankton net to allow smooth transfer of zooplankton to the cod end. The sample is transferred without net washing to minimize the mixing of damaged plankton stuck on the mesh.

Obtained samples are appropriately processed depending on the purpose. For species enumeration and identification, fix with sodium borate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ) buffered formalin-seawater (final concentration of formaldehyde is 4 %) as soon as possible. It is recommended fixing the sample with 8 % formaldehyde in the case that plankton volume is more than 30% of the sample bottle. Ethylalcohol is used for DNA sequence. Since RNA is labiler than DNA, a zooplankton sample for RNA is kept frozen or with specific chemicals for the preservation. It is recommended to refer latest literatures for RNA preservation. For the measurement of dry weight or carbon and nitrogen weight, filter on precombusted ( $450^\circ\text{C}$ , 4 hour) glass fiber filter such as Whatman GF/A after dividing the sample using sub-sampling devices such as Folsom's splitter as necessary. Sub-sampling devices is to be set on a bench appropriately to avoid errors through the splitting process. Since some commercial products are not enough stable, determine the variability between splits at land-based laboratory before use. For dry weight measurement, rinse the filter with a small amount of distilled water to remove the salt (Ohmori, 1978). The filter is transferred to petri dish and kept in freezer until measurement. Or, the filter is folded in half, covered with combusted aluminum foil and kept in freezer. The sequential processes of freezing and thawing is required, such as for gut pigment analysis, using mesh for plankton net as a filter inducing less damage than glass fiber filter. For gut pigment analysis, the sample on the mesh is kept in freezer  $<-70^\circ\text{C}$  to prevent the degradation of pigments. For any purposes, fix or freeze the sample as soon as possible. Even for the fixation with formalin, leaving a sample more than 30 minutes in room temperature induces significant damage.

The following are potential troubles and mistakes at a plankton net towing and sample handling: 1) wire breakage due to the generation of kink, 2) net breakage by wind and swell, 3) sample loss due to the cod end breakage or fall out of clip, 4) sample loss during transferring sample from cod end, 5)

undescribed or insufficient description of sampling information. We have to be very careful to avoid the case 1) which can induce serious accident. The second case is more probable on clogged net with containing sea water at the recovery. During handling the sample, it should be careful for unexpected rolling and running sea water on deck. To avoid case 5), take care that sampling bottle is properly marked at both lid and bottle. In the case of formalin fixation, input a waterproof paper with describing the information.

### ***3-2 Oblique tow***

For an oblique tow, various nets such as WP-2 net, Bongo-net (Fig. 3), ORI net (Fig. 4) have been used. The larger filtering volume than obtained with a vertical tow allows more accurate estimation of the composition of zooplankton population and abundance. Also, reducing the influence of plankton patchiness.

To enhancing the downforce, attaching a depressor or weight (30-100 kg) with a swivel. Setting a small depth sensor or acoustic net monitor at the flame to record the towing depth. Steaming the ship at low speed and put down the net to the sea surface, and then pay the wire off and control the ship speed (1.0-2.5 knot) to make the wire angle 45°. When the net is estimated to reach the target depth from the net sensor or wire length and angle, record the wire length and time, and haul the wire at stable speed (0.2-1.0 m s<sup>-1</sup>). The net towing speed, i.e., wire speed + ship speed, should be controlled to keep the filtering efficiency high enough. Obtained samples are processed as one of vertical tow.

### ***3-3 Multi layer sampling***

In order to collect zooplankton samples from multi-layers in a single tow, plankton nets with an opening-closing system controlled by logging the depth through armored cable with conductor, such as MOCNESS, VMPS, are used. A plankton net with closing system controlled by falling messengers, such as MTD net, had used in the past. However, such a net is hard to detect the exact sampling layer, difficult to exclude the possibility of contamination at paying out the wire, and unstable performance of the closing system. The contamination from outside the target depth induces serious error to examine the vertical distribution of species and developmental stages.

There are various types in MOCNESS, VMPS and another multi-layer sampling gears, and most of them equip various sensors for environmental parameters such as temperature, salinity, phytoplankton fluorescence, etc. In general, the 1<sup>st</sup> net which is set open during downcasting is not useful for quantitative sampling.

### ***3-4 Neuston sampling***

Neuston nets are designed to collect zooplankton, fish eggs and larvae occurring very surface of the water column. A Chigyo-net (Maruchi-net) has been widely used in Japan. A Chigyo-net is towed beside the ship being the upper 1/3 of the ring above the sea surface by controlling wire length. Since it is unable to estimate exactly the filtering volume, only the biomass and abundance per towing time or distance are obtained. To eliminate the influence of ship light, shut the light down at nighttime tow.

ORI-neuston is towed by a bridle attached to one side of the net frame (Fig. 5). This makes free-open area without wire and bridle during the net tow, keeping the net far away from the ship relative to Chigyo-net and minimize the influence of bow wave. Neuston nets stacking multiple nets vertically can examine the fine vertical distribution of zooplankton within the neuston layer (Wiebe and Benfield, 2003).

#### 4. Codend

Zooplankton collected by towing a plankton net are concentrated into a cod end attached at the bottom of the net. The materials of cod end and handling procedure are variable: 1) Cod end with rubber tube at the bottom. Folding the tube and pinched with a clip at sampling. Drain sea water and plankton off by removing the clip, 2) Cod end with screw cap. The cod end is screwed in the bottom of the net. After sampling, screw off the cod end to transfer samples, 3) Canvas or mesh bag. Attach and detach from the net with a hose clamp or strings. The cod end with screw cap is made by plastic or vinyl chloride, opening windows at the side covered by mesh to drain sea water. The mesh opening at the side windows is to be the same or smaller than one of the plankton net. Since strong filtering pressure exert on the windows, check the damage and rinse fully to remove clogging after sampling.

#### 5. Maintenance

Net clogging should be carefully washed off to maintain the filtering efficiency of the net and contamination. Nets should always be checked to be sure without damaged gauze, loozend screws and ropes. Wash metal materials with fresh water to remove salt. It should be noted that metal is more susceptible to corrosion at high temperature-high humid condition. After the cruise, remove all the parts and kept in fresh water for a day to remove salt. Then dry and keep in bags.

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## NORPAC-net

(conical net)

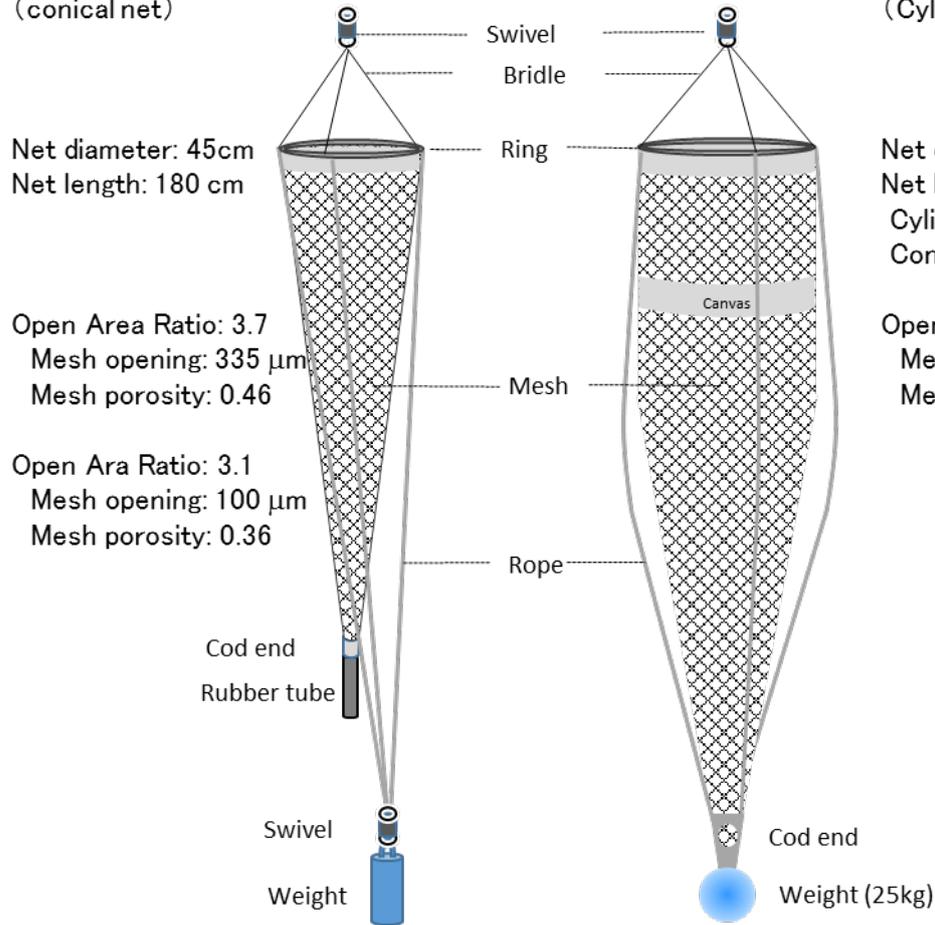


Fig. 6-1 NORPAC(North Pacific Standard) net and WP-2 (UNESCO Working Party No.2) net.



Fig. 2. A plankton net attached large plastic bag as a cod end to collect living zooplankton for incubation experiment.



Fig. 3 BONGO net (70 cm ring diameter). Photo by Dr. Y. Okazaki.



Fig. 4 ORI net (1.6 m ring diameter).



Fig. 5 Neuston net



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## Benthos

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### 1. Size categories of benthos

Benthic organisms (benthos) are organisms living on/in bottom sediments. Demersal fishes which spend most time setting on the sea bottom such as soles and eelpouts, or swimming above the bottom to feed as rattails are mostly classified into them. Benthos is conveniently divided into some size categories based on differences in sampling and observing methods. Megabenthos is a category which includes organisms large enough to be visible with the naked eye and sampled by using dragnets such as beam trawls and dredges. Organisms of smaller categories are generally sampled with bottom sediment by using bottom samplers. Among them, organisms remained on a mesh of 0.5 mm or 1 mm openings are called as macrobenthos. Organisms which pass through a mesh of 0.5 mm or 1 mm openings and remained on a mesh of 38  $\mu\text{m}$  openings and those which pass through a mesh of 38  $\mu\text{m}$  openings are meiobenthos and microbenthos, respectively.

### 2. Sampling and observing methods of megabenthos

Megabenthos has been collected by dragnets such as beam trawls and dredges for a long time. Biological dredges we use have same structures as those used during the research cruise in the Aegean Sea by Edward Forbes in 1840s, which is famous as the first systematic survey of deep-sea organisms. Biological dredges are used at sand and mud bottom at relatively shallow sites. Unlike dredges for geological studies, they collect organisms into light-weight nylon nets. Dredges of various sizes are used according to water depth and/or performance of a winch. During research cruises of R/V *Tansei-Maru* and *Shinsei-Maru*, biological dredges with a 1 m span (Fig. 1) have been used. It consists of a steel frame (1 m x 40 cm x 20 m), an inner net of 5 mm openings and an outer net of 2 cm openings. Usage of plumbic chain bridles, which swirl up mud with organisms, improves the efficiency of collecting organisms. At deep sites, a weight is attached at the codend (the end of a net) to prevent a winch cable from shrinking. Of too high tension cuts a fuse wire (6 mm thickness) between bridles and a winch cable, the dredge leaves bottom by being pulled by a life wire connecting between the winch cable and the side of the frame to protect the winch cable and the dredge. A life wire is tied and attached the frame using plastic tape not to become an obstacle. As dredges are drawn on sea bottom, they are often distorted and broken due to collision with rocks. It is better to think that dredges are consumable goods. When using a biological dredge, it is put into the sea with keeping the mouth of net upward, and lowered at a rate of 0.5 m/s from the vessel sailing at the speed through the water of 1.5 knot. Too fast lowering will loose and damage a winch cable. Grounding of the dredge is confirmed by the decrease of the tension, which is often rather difficult in the case of dredges, or the calculation based on the water depth, the angle of a winch cable, and the length of cable pay out. After grounding, vessel speed is changed to the ground speed of 1.0 knot and the winch cable of 30% of the length already pay out is additionally pay out. After then, the dredge is pulled at the ground speed of 1.0 knot for 5 min and wounded up at a rate of 0.2 m/s,

which gradually increase to 0.5 m/s. After leaving the bottom, the dredge is wound up at a rate of 1 m/s and recovered. During seining, the tension should be monitored. If the tension suddenly rises, seining is terminated and the vessel is immediately stopped. On deck, samples are taken out by opening a zipper at the side of the net. When difficult due to too much samples, it can be easily taken out by hauling up the codend using a winch. After picking out large organisms, bottom sediment is sieved with a mesh of 1 or 4 mm to select organisms. As the mouths of dredges are rather small, collected organisms are mainly infaunal mollusks, annelids, and echinoderms which are relatively small and less-swimming. A dredge is not a quantifiable sampler. As deep-sea animals are patchy distributed, a small difference in sampling points often results in a large difference in amount and composition of collected animals. So, it is difficult to estimate their density or spatial and seasonal changes based on few dredge samples. For biological sampling on rocky bottom where biological dredge and trawl cannot be used, dredges for sampling rocks such as a cylindrical dredge are available. They are, however, seldom used to collect animals as rocks badly damaged specimens in the dredges.



Figure 1 A biological dredge

For sampling larger benthic organisms and demersal fish with high swimming performance, trawls are used. During cruises of R/V *Tansei-Maru* and *Shinsei-Maru*, beam trawls with a 2 m span or a 3 m span (Fig. 2) have been used and those with a 4 m span have been used for R/V *Hakuho-Maru* cruises. A beam trawl consists of two steel poles called as beams, two lateral frames called as heads, and a duplex net. The width of the mouth of the net is fixed by beams. During seining, the frame of a trawl slides on the sea bottom as a sledge and collect sediments near the bottom surface and animals swimming above the bottom. As a biological dredge, a fuse wire and a life wire are used, although the latter is connected to the codend so that the trawl is wound up backwards abandoning sediments if the fuse wire is cut. At the great depth such as 3,000 m, plumbic chains inserted between a winch cable and a trawl or weight(s) attached to a frame and/or codend stabilizes the system in the sea for reliable landing. The variation of beam trawls contain a Sigsbee-Agassiz type beam trawl, which is not distinguished between upper and lower sides, Ocean Research Institute type beam trawl, which is distinguished between upper and lower sides, and so on. Beam trawls are operated in similar manner to biological dredges with the exception that the vessel speed at time when a trawl is put into the sea is 2 knot through the water, a trawl is lowered at a rate of 1 m/s, and the sampling is continued for 30 min. At the great depth, the winch cable is pay out 70% more after grounding. If the tension rapidly decreases or does not increase

during seining, the trawl is suspected to be away from the bottom. In that case, the winch cable is pay out additionally to see how it goes for a while. To collect demersal fish mainly, fast seining around the ground speed of 1.5 knot, and slow seining around the ground speed of 0.5 knot to collect invertebrates are recommended. In the latter case, attachment of a plumbic chain along the underside of the mouth of a net increases sampling efficiency of infauna as it sinks into the bottom sediments. As sampling area of trawls can be calculated based on the width of the net, the seining speed, and the range between grounding and leaving times, the density of organisms can be estimated. The value, however, tends to be underestimated as trawls do not necessary continue to touch the bottom during seining. In order to solve this problem, devises for real-time monitoring of status of trawls were developed (Hasumoto, 2006). An otter trawl is a net sampler used mainly by research vessels of fishery institutes and training ships of fishery high schools and colleges. They are suitable for collecting a large amount of fish and large crustaceans. Two steal boards named otter boards, which are set at both sides of a net, spread the mouth of the net when they receive water flow from the front. Although otter trawls can collect more samples, their quantitativty is inferior to beam trawls. As trawls have no mechanisms to close nets during rising, the by-catch of pelagic organisms cannot be prevented and they need be selected after sampling.



Figure 2 Beam trawls with a 2 m span (left) and a 3 m span (right)

A deep-sea camera system takes photographs of deep-sea bottom. It can obtain data of organisms inhabiting rocky bottom which cannot be sampled by trawls but can record only relatively large epifauna (organisms living on the bottom surface). Using camera(s) and a strobe contained in pressure resistant vessel(s), the system takes pictures at preprogrammed time intervals. Stereographs taken by two cameras enable to determine sizes of organisms. A pinger is turned on just before the start of the operation and the system is lowered at a rate of 1 m/s from drifting vessel. With approaching sea bottom, the lowering rate is decreased and the system is kept about 2m above the bottom. Signals from the pinger are received to monitor the distance between the system and the bottom. After photographing, the system is wound up at a rate of 1 m/s. Using deep-sea cameras attached to mooring systems with a disconnection devise, temporal changes of deep-sea bottom and gathering of large organisms around baits have been observed.

### 3. Sampling methods of macrobenthos

Macrobenthos, which is smaller than megabenthos, is generally sampled quantitatively with bottom sediment by using bottom samplers. During cruises of R/V *Tanasei-Maru* and *Hakuho-Maru*, 1/10 m<sup>2</sup> (Fig. 3) and 1/4 m<sup>2</sup> Box corer (USNEL-type spade corer) have been mainly used, respectively. The former can collect bottom sediment of surface area of 1/10 m<sup>2</sup> and the latter can collect bottom sediment of surface area of 1/4 m<sup>2</sup>, respectively. Both types of box corers can be used by R/V *Shinsei-Maru*. Box corers are samplers which cut off bottom sediment and sea water above it as they are in a rectangular steel box. A box is set in opened state in deck. The box corer is lowered at a rate of 1 m/s from the vessel which holds fixed point. When landing, the box is inserted into the bottom and a spade is unclamped to turn to close the box when it is pulled out. In addition, water ports are also closed to prevent bottom water from flowing out from the box. After confirming the box corer lands by a rapid decrease of the tension, payout of the winch cable is immediately stopped and the cable is wound up at a rate of 0.5 m/s. In the most cases, the tension rapidly increases just before the box corer leaves the bottom, which decreases to the ordinary level soon. Then, it is wound up at a rate of 1 m/s and recovered. On deck, the box is removed using a dedicated jacking dolly and bottom sediment is taken out from the upper side of the box. As a box full of mud is quite heavy, special attention is required especially on a rolling vessel. If necessary, subcores are inserted into sediment in the box to batch off subsamples. For an analysis of macrobenthos, the sediment is sieved with a mesh of 0.5 mm or 1 mm openings. As it is very difficult to select all macrobenthos by the naked eyes, samples remained on the mesh are fixed by adding neutralized formalin (final 10% in volume). In a laboratory, they are washed and sorted using a stereoscopic microscope. Macrobenthic organisms are preserved in 70% ethanol. Organisms of megabenthos-size are rarely collected by the box corer and organisms heavier than 1 g in wet weight are usually removed from data in order to prevent their presence from having a big influence on density estimation. As mentioned below, recently multiple corers, which show superior quantitativity to box corers, became popular. However, box corers, which can cover a larger sampling area, are still a excellent bottom sampler for studies in macrobenthos.



Figure 3 A 1/10 m<sup>2</sup> Box corer

Before the development of a box corer, grab samplers such as a Smith-McIntyre (SM) bottom sampler and an OKEAN grab were used to collect macrobenthos from research vessels. The latter is even

now used for sampling by small ships. Bottom sediments collected by grab samplers are not uniform in depth as those by box corers and they blow surface sediments when landing, both of which result in the underestimation of density of organisms. Comparison among data obtained using samplers of different types should be conducted carefully. Grab samplers contain a Ekman-Birge grab, which is so small that it can be managed only by human power and used for sampling a small amount of bottom sediment from piers and small ships.

In order to collect benthic-pelagic organisms, that is, animals swimming right over the sea bottom, epibenthic sleds are used. Plankton nets within a stilet frame filter sea water of the near-bottom layer without touching the bottom, which results in damage of nets by inflow of bottom sediments. Some models can mechanically open nets only when the sled is landing for preventing the by-catch of pelagic organisms and can equip video cameras (Brenke, 2005). So-called a “Manbiki (shoplifting)” net, that is, plankton nets set within a trawl net (Fig. 2, right) are also effective to collect benthic-pelagic species.

#### **4. Sampling methods of meio- and microbenthos**

Not so much as grab samplers, even box corers blow surface sediment with minute organisms at landing. On the surface of the deep-sea bottom, amorphous materials containing fresh organic matter accumulate to form an extremely unstable layer called as a fluffy layer in some season. Within that layer, bacteria and unicellular eukaryotes propagate themselves. Thus, sampling loss of box corers cannot be ignored in studies of smaller organisms than macrobenthos. To resolve this problem, a multiple corer was developed by increasing quantitativity instead of reduction in sampling area (Fig. 4). It is now used as a standard sampler of meio- and microbenthos. Disturbance of the bottom surface is minimized by landing with “legs” grinding at remote positions from cylindrical acrylic cores for the sediment collection and inserting these tubes into the bottom very slowly. To prevent the multiple corer from leaving the bottom before the insertion completes, a winch cable is pay out a bit after landing. Then, a quarter rope put between the winch cable and the multiple corer looses for the protection of the winch cable. A vinyl tape is wound around the quarter rope and shackles at the both ends to avoid entwining the corer. If bottom sediment is too soft and it overflows from the top of tubes, weights are removed from the corer to lighten it. On deck, cores are set in opened state. The corer is lowered at a rate of 1 m/s from the vessel which holds fixed point. After the corer stands still 50 m above the bottom for 3 min to stabilize the cable, the cable is pulled out again at a rate of 0.3 m/s (0.5 m/s if there is a strong swell). At the great depth where the change of tension at landing is easily overlooked, a pinger is attached to cable 50m above the corer to monitor the altitude of the corer. After landing, the winch cable is additionally (2-3m) pulled out. The winch cable is wound up at a rate of 0.3 m/s 0.5-1 min later. As the case of the box corers, the tension rapidly increases just before the corer leaves the bottom. When the tension decreases to the ordinary level, the winding rate is increased to 1 m/s. When landing, arms attached to lids of cores are released and cores are sealed when they are pulled out from the bottom. On deck, cores are removed, both ends of each core are capped, and they are kept standing up. As soft sediment easily flows out from cores, it had better be quickly held with a large spatula. After sampling, the inner part of the corer and its mechanical parts such as a trigger and arms should be closely washed using fresh water. If gravels are caught within the trigger, it results in a failure in operation. After sampling bottom

sediment containing gravels, trigger had better be released on deck to wash them away. In studies of meibenthos, core samples are often pushed from the lower side and sliced in parallel with the sea bottom. In the near bottom layer with high biomass, sediment sample is sliced at small intervals (0.5-1 mm) while intervals are wide in the deeper layer. Tools for pushing sediment out from cores have been devised by researchers. Sliced sediment samples are fixed with neutralized formalin containing Rose Bengal, which stains living organisms, and preserved. In laboratory, macrobenthos are removed from samples using a mesh of 0.5 mm or 1 mm openings and a fraction remained on a mesh of 38  $\mu$ m openings are washed. Organisms are picked up under a stereoscopic microscope and preserved in 70% ethanol. When too much organisms are collected, they can be divided equally using a plankton divider. Samples for studies of microbenthos are treated according to purposes suitably. Although multiple corers can obtain 3 to 8 core samples simultaneously, they are not independent. To estimate deviation of data, it is desired to conduct repeated sampling.



Figure 4 A multiple corer

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## $p\text{CO}_2$

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This procedure describes a method for the determination of the partial pressure of carbon dioxide ( $p\text{CO}_2$ ) in air that is in equilibrium with a flowing stream of sea water, e.g., that obtained by pumping surface sea water from the bow of a ship for underway analysis.  $p\text{CO}_2$  is proportional to a concentration of  $\text{CO}_2(\text{aq})$  in sea water following Henry's Law. Comparing with  $p\text{CO}_2$  in ambient air, we can know a condition of sea water in whether sea water is supersaturated or undersaturated in  $p\text{CO}_2$ .

### 1. Definition

A concentration of  $\text{CO}_2$  ( $x\text{CO}_2$ ) represents the mole fraction in dry air in unit of ppm. To evaluate the air-sea exchange,  $x\text{CO}_2$  is converted into the partial pressure of carbon dioxide ( $p\text{CO}_2$ ) in unit of  $\mu\text{atm}$  in consideration of saturated vapor pressure of water. When  $p\text{CO}_2$  in the surface sea water is lower than that in the ambient air,  $\text{CO}_2$  is absorbed from air to sea. On the other hand, when  $p\text{CO}_2$  in the surface sea water is higher,  $\text{CO}_2$  outgasses from sea to air.

### 2. Principle

$\text{CO}_2$  gas has the property of absorbing infrared radiation (IR). Because the amount of IR absorption is dependent on  $x\text{CO}_2$ ,  $x\text{CO}_2$  in sample air is determined with comparison with a series of calibration standards analyzed as part of the sampling sequence.

$x\text{CO}_2$  in a surface sea water is measured in the equilibrated gas phase with sea water. A fixed volume of air is equilibrated with a stream of sea water that flows through an equilibrator. As the volume of sea water that flows through the equilibrator is essentially infinite compared to the volume of air, the  $\text{CO}_2$  content of the air adjusts to equilibrium with the sea water without altering the  $\text{CO}_2$  content of the sea water appreciably.

### 3. Apparatus

The apparatus described here is intended to serve as an example of a commonly used system (Figure 1). It is based on a standardized design that is currently used on over a dozen ships at the time of writing. Some of the details of flow rates and timing are “tuned” to this system and may need to be adjusted for systems with different components.

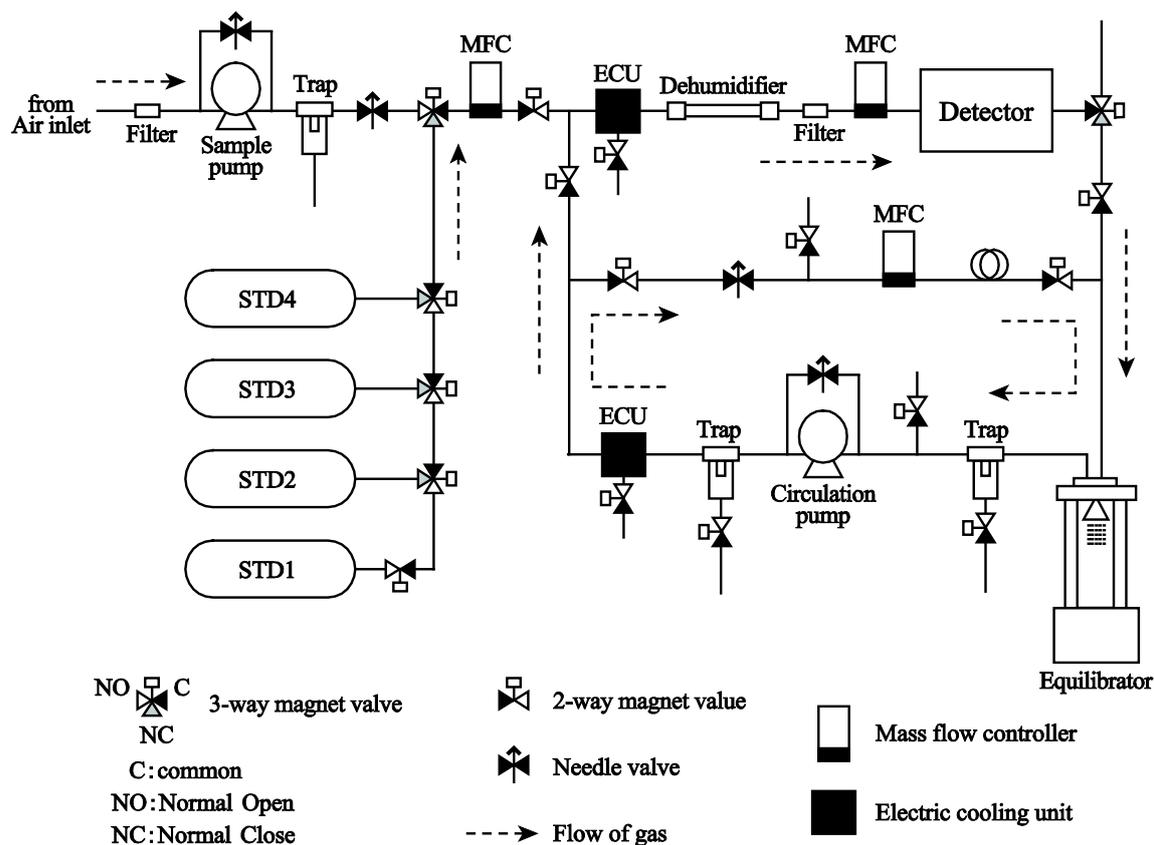


Figure1 Schematic showing the layout of the analytical system described here.

### 3-1 Analyzer

For measurement of  $x\text{CO}_2$ , a non-dispersive infra-red (NDIR) analyzer is commonly used. For operation on a ship, it is essential that the instrument not be sensitive to motion or vibration. To make a measurement under the same condition, it is necessary to keep changes in a room temperature and pressure small.

The main components of NDIR are an infrared source, a sample cell, a reference cell, and an infrared detector. The IR light is directed through the sample and reference cells towards the detector. The gas in the cells causes absorption of specific wavelengths. The attenuation of these wavelengths is measured by the detector to determine the gas concentration.

### 3-2 Equilibrator

The most common type of equilibrator involves a chamber where sea water is exposed to a headspace of air maintained at the ambient atmospheric pressure. Rapid exchange of  $\text{CO}_2$  is facilitated by enhancing the surface area of water exposed to the headspace air. (e.g., raining sea water droplets through the headspace into a sea water reservoir at the bottom of the equilibrator).

The water flow rate is  $2\text{--}5 \text{ L min}^{-1}$  and can be dispersed using a standard gardening spray head. During sampling, the headspace air is pumped through a dryer and the infrared detector. After passing

through the detector, the equilibrated air is returned to the headspace to minimize the need for replacement air from the vent. It is important to measure temperature of water in the equilibrator at all times. It is also recommended to measure pressure in the equilibrator.

### ***3-3 Thermometer in the equilibrator***

Because  $p\text{CO}_2$  is very sensitive to temperature, the temperature of water in the equilibrator is monitored continuously using a platinum resistance thermometer (regularly calibrated against a mercury thermometer traceable to NIST).

### ***3-4 Lines for samples and standard gases***

#### ***3-4-1 Outside air intake***

A tube is run from the measurement system to a location where uncontaminated outside air can be sampled. If the measurement system is located on board a ship, this line will typically be led to the bow of the ship. The air is continually pumped at a rate of 2–3 L min<sup>-1</sup> to ensure constant flushing of the tubing.

#### ***3-4-2 Lines and connectors***

For lines, materials which does not absorb and penetrate CO<sub>2</sub> is suitable. Stainless lines are normally used between standard gases and system because the gases are typically dry air. For other lines, Teflon® PFA is suitable because it is confirmed through translucent lines whether the lines have a trouble or not.

### ***3-5 Drying system***

It is desirable to dry all of the air streams going to the detector. Drying the air eliminates the possibility of condensation in the tubing leading to the analyzer; it also improves the sensitivity and the accuracy of the infrared analysis as it eliminates the need to correct for the pressure broadening of the CO<sub>2</sub> band resulting from the presence of water. Also, the calibration gases are typically dry air, so it is preferable to also analyze the unknown samples under the same dry air conditions.

### ***3-6 System control***

Since the system is intended to operate in “underway” mode, there should be a program for autonomously controlling valve switching, monitoring flow rates and logging the necessary data. This program will require a microcomputer as well as digital and analog interface boards for controlling valves, mass flow controller/meter and necessary sensors.

## **4. Reagents**

### ***4-1 Compressed gases***

The standards should be mixtures of CO<sub>2</sub> in natural air (i.e., containing N<sub>2</sub>, O<sub>2</sub>, and trace gases) that bracket the expected concentrations of the samples as closely as possible (typically 250–450 ppm for open ocean applications). CO<sub>2</sub> concentrations have been found to be most stable in aluminum cylinders.

The gases must be calibrated to better than the desired accuracy of the final measurements (i.e., typically beyond the accuracy offered by commercial gas suppliers) and should be traceable to the WMO Mole Fraction Scale for CO<sub>2</sub>.

Because the infrared detector has a non-linear response to changing CO<sub>2</sub> concentrations, it is recommended that multiple standards (3–6) be analyzed for the most accurate characterization of the response curve with an accuracy of  $\pm 1 \mu\text{atm}$ . For the measurement with an accuracy of  $\pm 2 \mu\text{atm}$ , calibration needs at least 2 standards. It is also necessary to run the reference gas (pure nitrogen gas or a known standard gas) through the sample cell.

#### ***4-2 Drying agents***

Chemical drying agents are the most reliable way to ensure that the sample gas is dry. Even if condensing systems or drying tubes are used to primarily dry the gas, many systems also run the sample gas through a chemical drying agent as a final confirmation that the sample is dry. Some common drying agents are magnesium perchlorate or silica gel.

### **5. Water Sample**

As this procedure is for underway measurements, the sampling is done as part of the analysis. The important features are to ensure that the equilibrator samples uncontaminated surface sea water. As the  $p\text{CO}_2$  is dependent on the water temperature, it is important that the water in the equilibrator be as close to sea surface temperature as possible. One should strive to set the system up in such a way that the difference in the temperature observed in the equilibrator and that observed in the surrounding sea water is less than 0.5°C. This is achieved by using a high flow rate of sea water to reduce the extent of the inevitable warming or cooling that occurs during passage from the water intake to the equilibrator. It is important to record the water intake temperature and salinity, e.g., using a thermosalinograph system as well as the equilibrator temperature. The sea water temperature should be monitored with a sensor in the equilibrator and a sensor at the ship's sea water intake (outboard of any pumps or flow restrictors) so any temperature differences can be accounted for in the data reduction sequence.

### **6. Procedure**

#### ***6-1 Introduction***

The sequence of analyses outlined below is designed to measure both the marine air and the equilibrator in a cycle together with the calibration gases. The exact sequence is not critical and can be optimized for the particular location and desired objectives of the study. In general, the frequency of analysis is determined by the length-scale of the phenomena that are being observed (compared to the ship's speed), and by the desire to conserve calibration gases.

#### ***6-2 Measurement condition***

Data quality is subject to a measurement condition. Here, classical conditions are shown.

• Flow rate of standard gas	500 ml min <sup>-1</sup>
• Flow rate of marine air sample	500 ml min <sup>-1</sup>
• Flow rate of reference gas	50 ml min <sup>-1</sup>
• Flow rate of sea water in equilibrator	5 l min <sup>-1</sup>
• Number of standard gases	4
• Measurement time of standard gas	5 min
• Measurement time of marine air	5 min
• Measurement time of sea water	10 min
• Equilibration time with sea water in equilibrator	10 min

### 6-3 Setting of measurement time

Measurement time is fixed as the total time of following step.

1. Time to take the output of NDIR to be steady.
2. Time the output is steady.
3. Time to take pressure in a sample cell of NDIR to be atmospheric open after flow of gas stops (about 20 seconds).
4. Measuring time (about 1 minute).

### 6-4 Sequence of measurement

A full set of standards should be run every 2.5 to 3 hours. Once the system has been calibrated, it alternates between marine air and equilibrated air readings. In the open ocean, sea water  $p\text{CO}_2$  generally has much larger variations than the marine air, so systems usually collect 5 to 10 times more equilibrator readings than marine air readings.

## 7. Calculation of $p\text{CO}_2$

### 7-1 Calculation principle

The partial pressure of  $\text{CO}_2$  in sea water,  $p\text{CO}_2$ , in unit of  $\mu\text{atm}$  is calculated using expression:

$$p\text{CO}_2 = x\text{CO}_2 \cdot (P - p\text{H}_2\text{O}) \quad (1)$$

where  $P$  is atmospheric pressure.  $p\text{H}_2\text{O}$  denotes saturated vapor pressure of water in unit of  $\mu\text{atm}$  and is calculated using the following equation (Weiss and Price, 1980):

$$p\text{H}_2\text{O} = \exp(a + b \cdot (100/T) + c \cdot \ln(T/100) + d \cdot S) \quad (2)$$

$$a = 24.4543$$

$$b = -67.4509$$

$$c = -4.8489$$

$$d = -0.000544$$

$$T = 273.15 + t$$

where  $S$  and  $t$  are salinity and temperature in Celsius, respectively.

Sea water undergoes some change in water temperature during passage from the water intake to the equilibrator. As  $p\text{CO}_2$  is dependent on temperature,  $p\text{CO}_2$  measured in the equilibrator is appropriate to the temperature of the water in the equilibrator (see section 1-7-2-5).

## 7-2 Calculation procedure

### 7-2-1 Correction of temperature and salinity

- (i) Correction of temperature in the equilibrator,  $T_{\text{eq}}$   
Correct instrumental error of thermometer in the equilibrator by calibration line acquired in the cruise or before cruise.
- (ii) Correction of water intake temperature,  $T_{\text{sea}}$   
If need, correct instrumental error of thermometer at the ship's sea water intake. This correction is achieved by comparison with CTD data acquired at the same depth with the intake.
- (iii) Correction of salinity of thermosalinograph  
Correct salinity of thermosalinograph by comparison with data of salinity measured by other instrument, such as AUTOSAL.

### 7-2-2 Calculation of analyzed data in $x\text{CO}_{2\text{air}}$ and $x\text{CO}_{2\text{eq}}$

It is necessary to interpolate the output of NDIR on calibration gases so as to infer the appropriate calibration function at the exact time of the measurement of a sample of air (either atmospheric or from the equilibrator). For each measurements of the sample, the calibration curve between the time-interpolated output of NDIR and concentration of calibration gases is determined. The concentrations in marine air ( $x\text{CO}_{2\text{air}}$ ) and the equilibrator air ( $x\text{CO}_{2\text{eq}}$ ) are calculated from the calibration curve.

### 7-2-3 Calculation of $p\text{CO}_{2\text{air}}$

Saturated vapor pressure of water ( $p\text{H}_2\text{O}_{\text{sea}}$ ) is calculated by sea surface temperature,  $T_{\text{sea}}$ , and salinity of thermosalinograph from equation (2). The partial pressure of marine air at surface ( $p\text{CO}_{2\text{air}}$ ) is calculated with atmospheric pressure,  $p\text{H}_2\text{O}_{\text{sea}}$ , and  $x\text{CO}_{2\text{air}}$  from equation (1).

### 7-2-4 Calculation of $p\text{CO}_{2\text{eq}}$ in the equilibrator

Saturated vapor pressure of water ( $p\text{H}_2\text{O}_{\text{eq}}$ ) is calculated by sea water temperature in the equilibrator,  $T_{\text{eq}}$ , and salinity of thermosalinograph from equation (2). The partial pressure of sea water in the equilibrator ( $p\text{CO}_{2\text{eq}}$ ) is calculated with atmospheric pressure,  $p\text{H}_2\text{O}_{\text{eq}}$ , and  $x\text{CO}_{2\text{eq}}$  from equation (1).

### 7-2-5 Correction of $p\text{CO}_{2\text{eq}}$ to $p\text{CO}_{2\text{sea}}$

The  $p\text{CO}_{2\text{eq}}$  is appropriate to the temperature of the water in the equilibrator ( $T_{\text{eq}}$ ). To use this as information about gas exchange at the sea surface, it is necessary to correct the value obtained to the measured sea surface temperature ( $T_{\text{sea}}$ ).

Some relationship have been proposed to correct change in temperature. For example, the Takahashi et al. (1993) proposed the following empirical equation:

$$p\text{CO}_2\text{sea} = p\text{CO}_2\text{eq} \cdot \exp[0.0423 \cdot (T_{\text{sea}} - T_{\text{eq}})] \quad (3).$$

It assumed that the dependence of  $p\text{CO}_2$  on temperature is about 4% regardless of ocean. If need, it is better to apply more precise relationship to correct the change in temperature, such as Copin-Montegut (1988, 1989).

#### *7-2-6. Calculation of $x\text{CO}_2\text{sea}$ in sea surface water*

The concentration of  $\text{CO}_2$  of sea surface water ( $x\text{CO}_2\text{sea}$ ) is calculated with atmospheric pressure,  $p\text{H}_2\text{Osea}$ , and  $p\text{CO}_2\text{sea}$  from equation (1).

#### **Reference**

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## Acoustic Doppler Current Profilers

○Shinya KOUKETSU (Japan Agency for Marine-earth Science and Technology)

Shipboard acoustic Doppler current profilers (SADCPs) mounted on the hulls of sea-going vessels have been widely used to monitor ocean currents. Downward-looking SADCPs transmit sound pulses, measure the Doppler shifts of the reflected pulses, and then calculate the velocity of the ocean water relative to the vessel. SADCPs with working frequencies of 38 kHz, 75 kHz, and 150 kHz have been available from Teledyne RD Instruments (TRDI) since 2000. The lower frequency instruments can measure currents in deeper layers, but vertical resolution declines. According to the TRDI manual, the maximum depths of measurement and typical cell lengths for TRDI SADCPs are respectively 1000 m and 24 m for the 38 kHz SADCP, 700 m and 16 m for the 75 kHz SADCP, and 400 m and 8 m for the 150 kHz SADCP [1]. The choice of instrument should depend on the range of water depths in the research region. However, the maximum depth of current measurements in the field depends on local seawater properties and may be shallower than the published maximum depth.

This guideline provides summary descriptions of data processing and field operations that are presented in greater detail in the Global Ocean Ship-Based Hydrographic Investigations Program (GO-SHIP) manual [2]. Instrument specifications, which are also provided in the GO-SHIP manual, are omitted here, but the operating principles of the SADCP are briefly described. More detailed information about instrument configurations and applicable software should be obtained from the GO-SHIP manual or manufacturer's manuals.

### 1. Measurement principles

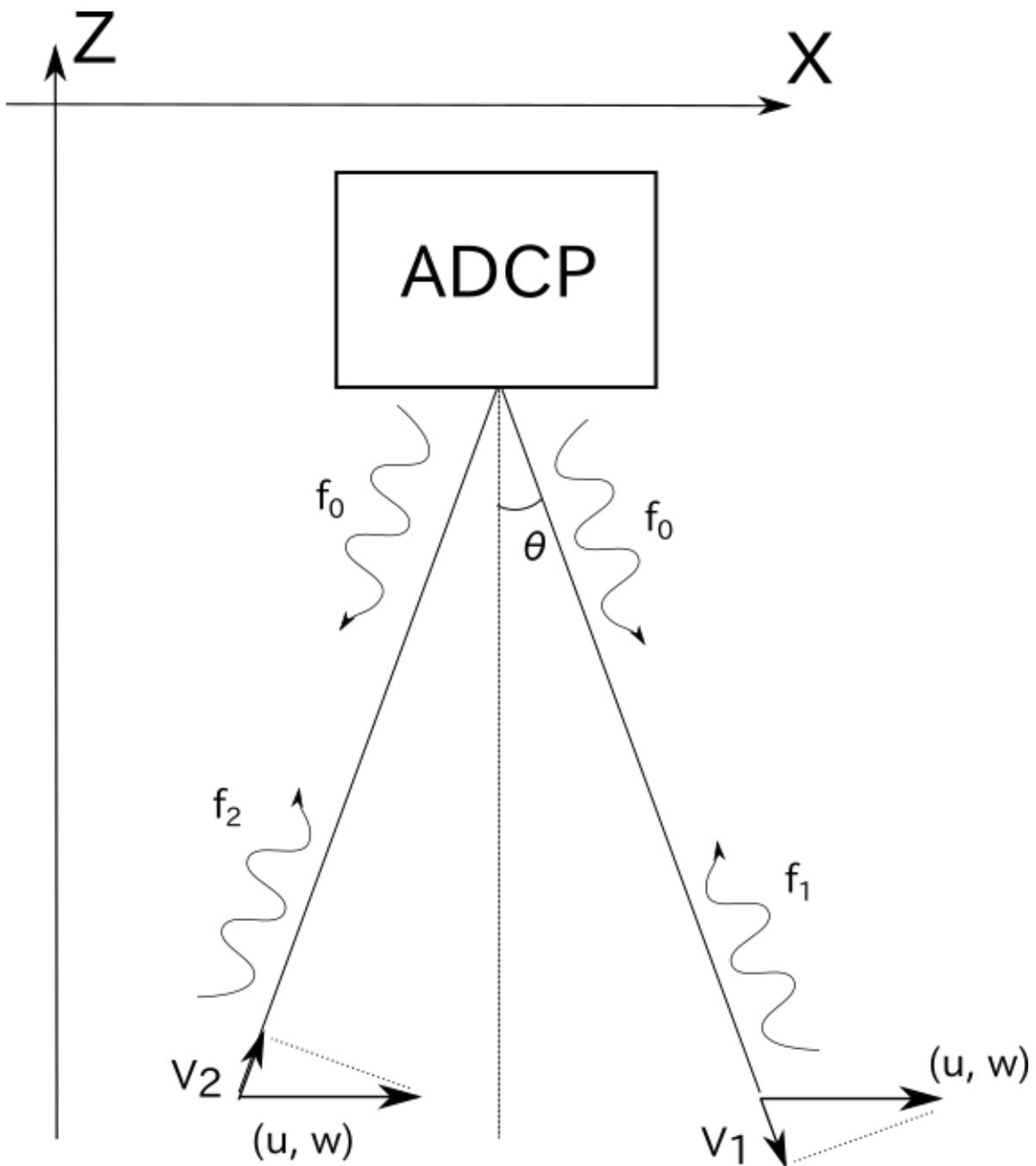


Figure 1 Schematic of acoustic Doppler current profiler (ADCP) observation in the  $x$ - $z$  plane.  $f_0$  is the transmitted pulse frequency and  $f_1$  and  $f_2$  are reflected pulse frequencies.  $V_1$  and  $V_2$  are measured velocities in the direction of the pulses.  $(u, w)$  is the current in the  $x$ - $z$  plane.

The pulses transmitted by the SADCPC are refracted at density stratification boundaries and partially reflected by particles (e.g., plankton, particulate organic matter). Currents and current depths can be calculated from the Doppler shifts and periods between transmitted and reflected pulses.

If a transducer transmits a pulse of frequency  $f_0$  and the pulse is reflected by a particle moving at velocity  $V_1$ , the reflected pulse frequency ( $f_1$ ) is

$$f_1 = f_0 \frac{c+V_1}{c-V_1}$$

Here, assuming  $V_1 \ll C$ ,

$$V_1 = \frac{f_1 - f_0}{2f_0} C.$$

Thus, the velocity of the current relative to the moving SADCPC can be calculated from the Doppler shift ( $f_1 - f_0$ ). As one transducer can obtain a velocity in only one direction (transmitted pulse direction), more than three differently oriented transducers are needed to obtain a three-dimensional velocity. For a SADCPC equipped with two transducers oriented at  $\pm\theta^\circ$  from the vertical in the x-z plane (Fig. 1) and two transducers oriented at  $\pm\varphi^\circ$  from the vertical in the y-z plane, the velocities ( $V_1, V_2, V_3, V_4$ ) observed by each transducer are

$$V_1 = w \cos\theta + u \sin\theta$$

$$V_2 = w \cos\theta - u \sin\theta$$

$$V_3 = w \cos\varphi + v \sin\varphi$$

$$V_4 = w \cos\varphi - v \sin\varphi$$

From the above equations, we obtain the three-dimensional velocity ( $u, v, w$ ) relative to the moving vessel. Although it is assumed that all four transducers measure components of the velocity of the same current, measurements can be disturbed by small-scale current fluctuations, fish, and instrument noise. Thus, the differences between the vertical velocities ( $w$ ) estimated from different transducer pairs (e.g.,  $V_1$  and  $V_2$  versus  $V_3$  and  $V_4$  pairs) are generally used as the “velocity error”.

## 2. Conversion of relative velocity to true velocity

The relative velocity measured by the SADCPC should be converted to earth coordinate velocity by using ship heading data (gyro compass or GPS compass data). This conversion can generally be done using software (e.g., [3] and [4]) provided by the instrument manufacturers.

To obtain the true ocean current velocity, ship velocity must be accounted for. If the water depth is shallow enough to obtain bottom track data (recorded by the SADCPC), the bottom velocity can be used as the ship velocity. Otherwise, ship velocity can be determined from GPS data.

## 3. Correction and evaluation of accuracy

Data obtained from individual pings are strongly disturbed by noise, and ship velocities based on GPS data at intervals of a few minutes are not very accurate. Thus, estimates of ocean current velocities are normally averaged over 5–10 minutes, which suppresses the influence of noise. Errors can be evaluated on the basis of the standard deviation of the time-averaged results. However, it is important to note that there will be large uncertainties in current velocity estimates based on data acquired during periods of ship acceleration.

Corrections are needed for the difference of the orientations of the SADCPC and gyro sensor (“arrangement angle”), which can cause considerable biases between true ship velocity (usually much faster than currents) and SADCPC measurements including ship velocity. For any one cruise, comparison of directions and magnitudes of the ship velocities obtained by SADCPC bottom track with those obtained

from GPS data allows estimation of a correction angle and amplitude coefficient for that cruise [5]. The relationships between bottom-track velocity ( $u_b, v_b$ ) and ship velocity ( $u_s, v_s$ ) are as follows:

$$\begin{aligned} u_s &= -\beta (u_b \cos \alpha - v_b \sin \alpha) \\ v_s &= -\beta (u_b \sin \alpha + v_b \cos \alpha), \end{aligned}$$

where  $\alpha$  is the arrangement angle and  $\beta$  is the amplitude coefficient. Note that the negative signs on the right-hand side of these equations indicate the direction opposite to the direction of the bottom velocity of the ship. Using these relationships and bottom-track data obtained while cruising, the arrangement angle ( $\alpha$ ) and amplitude coefficient ( $\beta$ ) are obtained as follows.

$$\begin{aligned} \tan \alpha &= \frac{\langle u_b v_s - v_b u_s \rangle}{\langle u_b u_s + v_b v_s \rangle} \\ \beta &= -\frac{\langle u_b v_s + v_b u_s \rangle}{\langle u_b^2 + v_b^2 \rangle \cos \alpha}, \end{aligned}$$

where  $\langle \dots \rangle$  indicates the ensemble average during the cruise.

Current velocity is corrected to account for the local velocity of sound in water ( $C_{real}$ , dependent on water temperature and salinity) by using, for example, TEOS-10 software [6] as follows:

$$V_{corrected} = V_{adcp} \frac{C_{real}}{C_{adcp}},$$

where  $V_{adcp}$  and  $C_{adcp}$  are raw estimated current velocity and sound velocity recorded at the SADCP, respectively. Note that vertical stratification of the water column does not affect horizontal velocities, and that this correction is not needed if a phased array system is used (e.g., [7]).

The corresponding water depth correction is done according to the following equation:

$$L_{corrected} = L_{adcp} \frac{C_{real}}{C_{adcp}},$$

where  $L_{corrected}$  and  $L_{adcp}$  are corrected and measured water depth, respectively. If the vertical profile of  $C_{real}$  is available, water depths can be obtained by integration of  $C_{real}$ .

#### 4. Data required for post-cruise processing

In areas where the water depth is shallow enough to use bottom tracking, the bottom-tracking data are generally used for correction of velocity data and for evaluation of instrument noise. However, the use of bottom-track recording can reduce the accuracy of the velocity data, so bottom tracking should be turned off when working far from the coast.

The following data are needed for post-cruise data processing.

- ADCP raw data
- GPS ship location data
- Heading data based on the ship gyro (or ADCP)

All data are collected and archived by using software provided by the equipment manufacturer (e.g., [4] and [8]). If the GPS and gyro data collected by the vessel's navigation systems are more accurate than the data collected by the ADCP, they should also be archived. Although the software provided by the manufacturer generally generates 5 or 10 minutes averaged data, the raw data (for every ping) should also be archived for future use. Time average data can be simply generated after the cruise by using standard PC software (e.g., [3] and [4]).

Available salinity and temperature measurements (ex. by a Conductivity Temperature Depth profiler) should also be archived for use in sound velocity corrections.

## References

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## **Weather Observations**

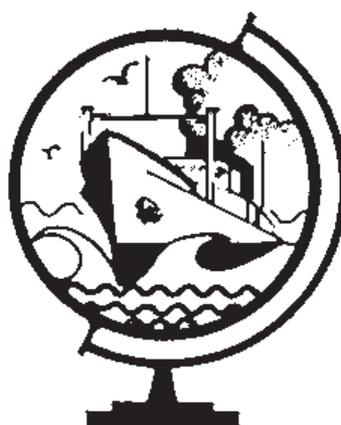
○Toshiya NAKANO (Japan Meteorological Agency)

Meteorology operational services depend on a wide-range of international co-operation. Weather observations by a ship not only benefit its own safety but that of other ships through resultant weather forecasts and storm warnings issued using such weather reports gathered by meteorological services. The Japan Meteorological Agency (JMA), the national meteorological service of Japan, requests individual ships equipped with radio communication facilities to make meteorological observations (weather observations on board ships) and report the data by radio and mail. This chapter, “Guide to Weather Observations for Ships“, was published in 2015 by JMA.



# Guide to Weather Observations for Ships

Third Edition



March, 2015

Global Environment and Marine Department  
Japan Meteorological Agency



## CONTENTS

CHAPTER 1	General Description .....	1
CHAPTER 2	Observation of Atmospheric Pressure .....	6
CHAPTER 3	Observation of Air Temperature and Dew-point Temperature .....	9
CHAPTER 4	Observation of Wind .....	16
CHAPTER 5	Observation of Cloud .....	29
CHAPTER 6	Observation of Visibility .....	49
CHAPTER 7	Observation of Atmospheric Phenomena and Weather .....	50
CHAPTER 8	Observation of Sea Surface Temperature .....	56
CHAPTER 9	Observation of Ocean Waves .....	57
CHAPTER 10	Observation of Sea Ice .....	61
CHAPTER 11	Observation of Ice Accretion .....	69
CHAPTER 12	Port Meteorological Officer (PMO) .....	71
Appendix 1	List of supplies for ship weather observations and reports .....	A-1
Appendix 2	Table for saturated vapor pressure .....	A-3



## CHAPTER 1 General Description

### 1.1 Introduction

The navigation of ships is affected by weather; consequently, methods of weather observation have developed along with those of navigation. In fact, weather forecasting might have originated to facilitate the safe navigation of ships. The commencement of storm warnings in France (1856) followed an incident where allied fleets of the UK and France, gathering off Sevastopol during the Crimean War, were suddenly enveloped by a hurricane-force storm which wrecked several vessels (14 November 1854).

Weather observations by a ship not only benefit its own safety but that of other ships through resultant weather forecasts and storm warnings issued using such weather reports gathered by meteorological services. These forecasts and warnings also help prevent and reduce risks of meteorological disasters affecting industries, transport systems, livelihoods and various other activities on land.

Furthermore, weather reports of ships on the oceans where fewer reports are available play a valuable role in monitoring global-scale climate change, which is an increasingly important issue for mankind affecting global warming.

The Japan Meteorological Agency (JMA), the national meteorological service of Japan, emphasizes the importance of ship weather observations. Meteorology operational services depend on a wide-range of international co-operation. The World Meteorological Organization (WMO), one of the specialized agencies of the United Nations, promotes meteorological observation on board ships under the umbrella "International Convention for the Safety of Life at Sea (SOLAS)" (1974). WMO has established internationally common methods of observation including codes and procedures to report and exchange data among members of WMO.

The WMO Voluntary Observing Ship (VOS) scheme is an international program in which ships are recruited to make and report meteorological observations.

Under this scheme, JMA requests individual ships equipped with radio communication facilities to make meteorological observations (weather observations on board ships) and report the data by radio and mail.

This Guide explains how to make marine meteorological observations and other related matters. In addition to this guide, JMA provides VOSs with the following materials free of charge:

- Guide to Ships' Weather Reports
- Ships' Weather Code Card
- Table for Finding the Dew-point
- JMA Cloud Plate
- Beaufort Scale of Wind Force
- OBSJMA (software to make weather reports)
- OBSJMA Operation Manual
- Ship's Weather Observation Field Note for OBSJMA (see Fig. 1.1)
- Marine Meteorological Logbook

- Envelope to send logbooks, floppy disks or CD-Rs (postage free within Japan)  
(see Appendix 1)

## 1.2 Careful practice

### 1.2.1 Accurate observations

In order to obtain reliable data, it is important for mariners to perform meteorological observations carefully and rigorously. An inaccurate reported observation may result in an erroneous forecast, whereas one that is valid and reliable could shed light on obscure and complex meteorological phenomena. It is also necessary to ensure that observations are not affected by motion or vibration of the ship, exhaust of gases or liquids, or sea spray. Needless to say, neglecting a scheduled observation should be avoided.

It is worthwhile matching observed values to the context of surrounding meteorological and ocean conditions. For example, change in atmospheric pressure. If a tropical cyclone (e.g. typhoon) or an extra-tropical cyclone is approaching, "wind speed" will increase. If a front is approaching, "direction of wind" will change and "wind waves" will be higher. Being mindful of these factors will reduce errors in reading measures and help determine malfunctioning instruments.

Observed results should be recorded directly in the Marine Meteorological Logbook. As for items requiring calculations (see the corresponding chapters for details), it is recommended to record observed and calculated values on paper at time of determination before making an entry in the logbook:

- Atmospheric pressure; correct to sea level (Chap.2)
- Dew-point temperature (Chap. 3)
  - \* dew-point hygrometer, no calculation needed
- Wind speed/direction; observed (apparent) values, correct to true wind (Chap.4)
- Weather (present and past); change during previous 3 or 6 hours required, change should be recorded at any time (Chap.7)

Do not enter any value from memory. Always ensure observation procedures, calculations, and radio weather messages are correct.

Software to make electric logbooks including OBSJMA can assist in accurate weather observations and reports.

**Ship's Weather Observation Field Note**

Observation Time and Place					Non-instrumental Observations				
Year	Month	Day	Hour	(JST)	Direct	Wind Wave		Swell 1	Swell 2
			Hour	(UTC)		(dir)	(dir)	(dir)	(dir)
Latitude (S, L, L, L, L)		Longitude (E, L, L, L, L)		Ship Direct. (Dg)	Speed (vs)	Period			
				Hours average	Hours average				
N S	E W				Height				
					(Hw)	m	(Hw)	m	(Hw)
					Visibility (V/V)	Present Weather (ww)	Past Weather		
					m		W1	W2	
Instrumental Observations									
Air Pressure (PPPP)		Temperature and Humidity			Cloud				
		by Dry- and Wet-bulb							
		Dry-bulb Temp. (T)	Wet-bulb Temp. (Tw)						
hPa					Total Cloud Amount (N)	Upper (CH)	Type		Height (h)
Pressure Tendency									
Type (s)	Amount (ppp)	by the Dew-point Hygrometer			/10	Middle (CM)			m
		Temperature (T)	Dew-Point Temp. (Td)		Lowest Cloud Amount (hc)	Lower (CL)			m
					/10				
True Wind		Apparent Wind			Ice Accretion on Ships				
Direct (dd)		Direct			Cause (ic)	Thickness (EaE)	Rate (Ra)		
Speed (ff)		Speed					cm		
Sea Surface Temperature (T <sub>s</sub> )		Ship's Heading	Sea Ice						
		Ship's Speed	kt	Concentration (cs)	Stage of Development (Su)	Land Origin (ls)			
		Ship's Course							
Remarks		Relative Humidity	Bearing of Ice Edge (D)		Trend over 3hours (z)				
		%							

**Weather Message**

INDICATOR	CALL SIGN	YYGG	99	00	10	20	30	40	50	60	70	80	90	100
<b>B B X</b>			<b>9 9</b>											<b>4</b>
Nddff	(00ff)	1snTTT	2snTatTs	4PPPP	5pppp									
7wwW1W2	8NcC1CwC1	9GGgg	222Dvs	0sT=T <sub>s</sub>	1P <sub>sw</sub> P <sub>sw</sub> H <sub>sw</sub> H <sub>sw</sub>									
<b>7</b>	<b>8</b>	<b>9</b>	<b>2 2 2</b>	<b>0</b>	<b>1</b>									
2PaPaH <sub>sw</sub> H <sub>sw</sub>	3d <sub>w</sub> d <sub>w</sub> 1d <sub>w</sub> 2d <sub>w</sub> 2	4P <sub>w</sub> 1P <sub>w</sub> 1H <sub>w</sub> 1	5P <sub>w</sub> 2P <sub>w</sub> 2H <sub>w</sub> 2	6tE <sub>s</sub> E <sub>s</sub> R <sub>s</sub>	8s <sub>w</sub> T <sub>s</sub> T <sub>s</sub>									
<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>8</b>									
ICE cSbDz: include in PLANE LANGUAGE when special phenomena are observed.														
<b>I C E</b>														
(via INMARSAT B) .....														

Remarks: The dark shaded groups are mandatory for a weather message.

Fig. 1.1 Ship's Weather Observation Field Note for OBSJMA

### 1.2.2 Non-instrumental observations

Non-instrumental observations including those of weather, cloud, and visibility are as important as those with instruments in analysis of meteorological phenomena to forecast the movement and scale of tropical or extra-tropical cyclones. As few research vessels conduct ocean wave observations with instruments, non-instrumental observations from merchant ships are indispensable for the analysis and forecast of ocean waves. The accuracy of non-instrumental observations relies upon the personal judgment and experience of the observer. A fundamental requirement of modern meteorology is that observations follow the same procedures all over the world for reliable comparison. Therefore, it is very important to follow the instructions and procedures indicated in this Guide or other materials mentioned in 1.1.

### 1.2.3 Reporting schedule

JMA requests Japanese ships navigating in area (1) in Fig. 1.2 to report their observations at 00, 03, 06, 09, 12, 15, 18 and 21 Universal Time Coordinated (UTC). Ships in area (2) are requested to report observations at 00, 06, 12 and 18 UTC. Weather reports from VOSs are requested to be conducted four times a day at 00, 06, 12 and 18 UTC even outside of the above areas and reported to JMA.

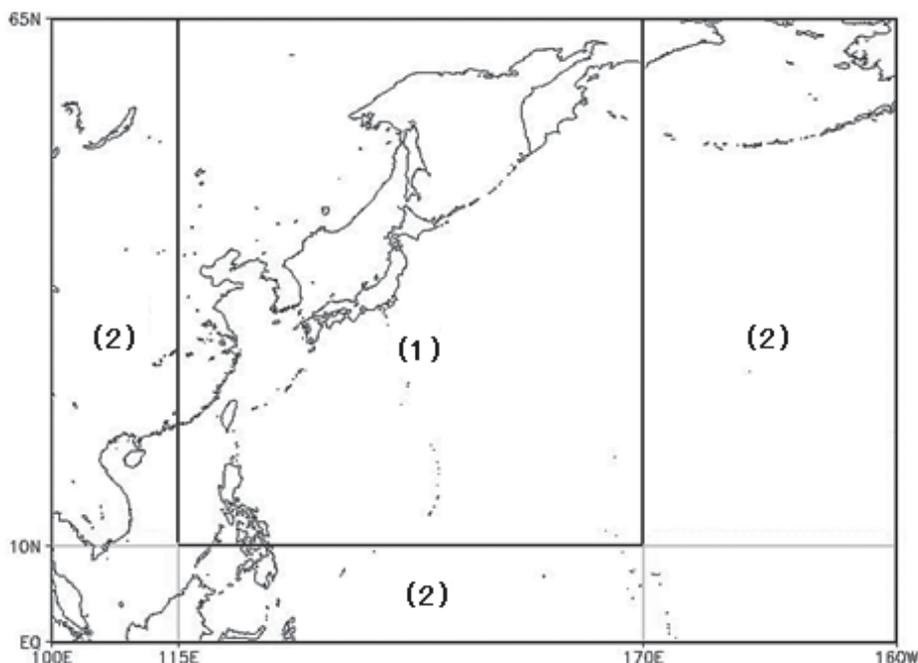


Fig. 1.2 The areas where ships are requested to report weather observations by JMA

#### 1.2.4 Sequence of observations

Although different observations cannot be conducted simultaneously, they should be conducted as close to the reporting time as possible. Non-instrumental observations should be made consecutively rather than instrumental and non-instrumental alternately, since eyes need time to accommodate to the sight over the sea (especially at night). Instrumental observations requiring the use of a light should be made after non-instrumental ones, so that dark adaptation of eyes is not impaired.

With the above remarks in mind, the following sequence of observations is recommended:

- 1) Observation of sea surface temperature (in case of bucket measurement)
- 2) Non-instrumental observations
  - Horizontal visibility
  - Cloud (amount, type, height, etc.)
  - Ocean waves (wind waves, swell)
  - Wind direction and speed (in case of observation by Beaufort scale)
- 3) Instrumental observations
  - Wind direction and speed (in case of observation by anemometer)
  - Air and wet-bulb temperatures (in case of wet-bulb thermometer) or air temperature and dew-point (in case of dew-point hygrometer)
  - Sea surface temperature (in case of intake measurement or hull-attached thermometer)
  - Atmospheric pressure to be measured exactly on time
- 4) Calculation and conversion to codes

- 5) Entry in Marine Meteorological Logbook
- 6) Preparation of Radio Weather Message
- 7) Completion and transmission of weather report

Present and past weather should be observed as necessary (see 1.2.1). Software to make weather reports including OBSJMA can assist in the above procedures 4) - 6).

### 1.3 Utilization of observed results

JMA receives weather reports from ships directly through Radio Weather Messages and/or later through Marine Meteorological Logbooks mailed from Japanese ports.

Observations reported through Radio Weather Messages are immediately utilized mainly to produce weather and ocean wave forecasts/warnings, and numerical weather and ocean wave prediction models. Observations on board ships are valuable to augment the sparse data available over the oceans. Though meteorological satellites provide observations, these data are insufficient and require corrections based upon observations provided by ships.

Marine meteorological observation data received by JMA as Marine Meteorological Logbooks are archived electronically after being checked for quality, and exchanged internationally to assemble the global database of marine meteorology. These data are being utilized for such activities as monitoring global climate change, development or improvement of numerical climate models and more.

## CHAPTER 2 Observation of Atmospheric Pressure

It is widely known from weather maps that atmospheric pressure is the most basic measure used to describe the state of the atmosphere. The distribution or time change of atmospheric pressure is an important indicator of change of weather and/or wind.

### 2.1 Definition and units

Hecto-pascal (hPa) is the standard unit of atmospheric pressure. Other units include mb, mmHg and inchHg as below:

$$1 \text{ hPa} = 100 \text{ Pa} = 1 \text{ mb}$$

$$1 \text{ mmHg} = 1.33322 \text{ hPa}$$

$$1 \text{ inchHg} = 33.8639 \text{ hPa}$$

A detailed list converting mmHg to hPa is provided in "Guide to Ships' Weather Reports" issued by JMA.

### 2.2 Measuring instruments and installation conditions

The marine aneroid barometer measures atmospheric pressure. The marine aneroid barograph records barometric tendencies (e.g. rate of change). The resonator digital barometer, as described later, also records atmospheric pressure and barometric tendencies.

When installing these instruments the following conditions should be considered:

- 1) Avoid exposure to direct sunlight.
- 2) Choose sites with minimum temperature change.
- 3) Minimize effects of vibration and shock caused by engine and/or ocean waves, with insulation material such as sponge rubber.
- 4) The marine aneroid barometer is normally installed horizontally. If fixed on a vertical wall, ensure its indicated value differs no more than 0.5 hPa from that of normal installation.

### 2.3 Aneroid barometer

The aneroid barometer works on the principle that its capsule made of metal expands or contracts in response to change in atmospheric pressure. A high quality precision marine aneroid barometer (see Fig. 2.1) is recommended for weather observations by ships.

Before reading the atmospheric pressure, tap the glass face with your finger so that the needle vibrates slightly. Then focus your best eye above the needle and read the scale. Only use one eye to avoid parallax error. If you see a reflection of your eye in the glass exactly at the same horizontal position as the needle, your eye is correctly located for observation. If your barometer has a mirror on the scale plate, you can accurately adjust your eye position.

Among high ocean waves, ships undergo large rolling and pitching resulting in displacement and vertical acceleration causing the barometer needle to show horizontal oscillations. In this case, make several readings noting the maximum and minimum values on

the scale and take the average value as the observed value.

Observation of the atmospheric pressure should be made exactly on the observation hour and the pressure should be recorded to within 0.1 hPa.

## 2.4 Electronic barometers

Two types of electronic barometers are commonly used for weather observations. One is a resonator digital barometer and the other an electric capacitance barometer. The former works on the principle that the frequency of its sensor varies with atmospheric pressure. The latter works on the principle that its electric capacitance varies with atmospheric pressure. Electronic barometers do not indicate current atmospheric pressure but an averaged value over a short period. Thus, accurate observations may be made during rolling and pitching. In addition, since these barometers display digitized values inter- and intra-observer bias is avoided.

## 2.5 Correction for instrumental error

The indicated value of a barometer differs from the true value to some extent. This intrinsic difference is known as “instrumental error” and should be corrected to obtain the station value (see 2.6) of atmospheric pressure. If the correction value for instrumental error is known from a barometer inspection, the station pressure is obtained by adding the value to the indicated value. Barometers should be inspected every 6 months by Port Meteorological Officers (PMOs) to maintain their accuracy (Chap.12).

## 2.6 Correction to sea level

The atmospheric pressure at mean sea level may be derived from the observed value of the instrument installed at a height above mean sea level (referred to as station pressure). This derivation is called “correction to mean sea level”. Correction depends not only on the height at which the instrument is installed but on station pressure\* and temperature. See “Guide to Ships’ Weather Reports” for a detailed correction table.

The height of the barometer above mean sea level should be determined when a ship is fully loaded and unloaded. If the ship draught level varies by + or - 1 m around the average level, the height of the barometer above the average draught level may be taken as its height for the correction.

Given a station pressure of 997.4 hPa, height of barometer above mean sea level of 6 m, and temperature 13°C, the correction value will be 0.7 hPa according to the table mentioned above. And the corrected value is obtained as follows:

$$\begin{array}{rccccccc}
 997.4 & & + & & 0.7 & = & 998.1 \text{ hPa} \\
 \text{(station pressure)} & & & & \text{(correction)} & & \text{(pressure at mean sea level)}
 \end{array}$$

## 2.7 Barograph

A barograph is used to check observed values of pressure and record barometric tendencies.

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\* Station pressure is defined as the pressure at barometer level.

Figure 2.2 shows an aneroid barograph, one of several available types of barographs. The barograph paper, on which a continuous record of the pressure is made, is the barogram. Barograms must be renewed at intervals (from 1 day to 7 days, depending on the instrument). It is recommended that a barograph be installed to reveal whether a high, a low, a trough or a ridge is approaching or moving away indicating improving or worsening weather conditions.

As for where and how to install the barograph, refer to section 2.2.

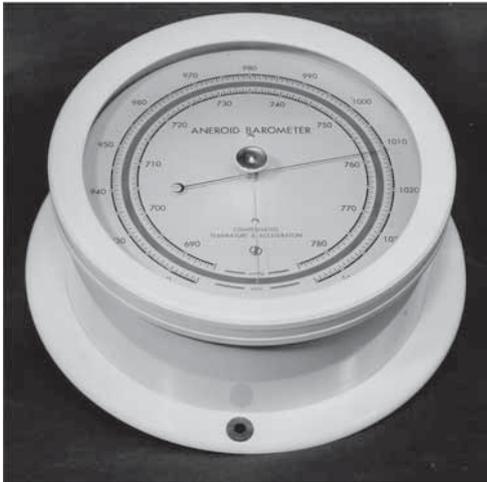


Fig. 2.1 Aneroid barometer

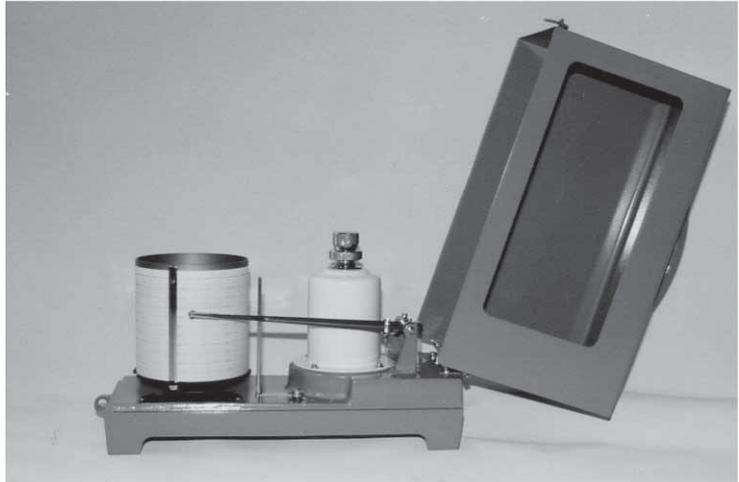


Fig. 2.2 Barograph

## CHAPTER 3 Observation of Air Temperature and Dew-point Temperature

The horizontal distribution of temperature in the atmosphere caused by latitudinal differences in incoming sunlight creates regional differences in atmospheric density giving rise to air flow (wind), clouds and precipitation. Therefore, atmospheric temperature is one of the basic elements to be observed in analyzing atmospheric conditions.

Water vapor in the atmosphere is the source of clouds and precipitation. It also transports energy in the form of latent heat through evaporation and condensation. Since water vapor absorbs and emits infrared radiation, it also affects the variation of atmospheric radiation. Therefore water vapor plays an important role in atmospheric phenomena.

### 3.1 Definition and unit

The temperature of the atmosphere is called air temperature. The partial pressure of water vapor in the atmosphere is called water vapor pressure, or vapor pressure. The maximum value of vapor pressure in the atmosphere, which is called saturated vapor pressure, depends on air temperature and whether the atmosphere faces water or ice. The ratio of vapor pressure to saturated vapor pressure at a given temperature is called relative humidity. Dew-point temperature is defined as the air temperature at which vapor pressure is equal to saturated vapor pressure at constant air pressure. Air and dew-point temperatures should be recorded as degree Celsius to the nearest 0.1.

### 3.2 Measuring instruments and installation conditions

For observations of air and dew-point temperatures, an aspiration (ventilated) psychrometer, an Assmann psychrometer, a sling psychrometer, and an electric psychrometer (combined resistance thermometer and dew-point hygrometer or electric humidity hygrometer) are commonly used (see Fig. 3.1-Fig. 3.4).

A dew-point hygrometer measures the dew-point temperature directly.

A non-ventilated psychrometer is not recommended because if there is little air movement only air near the instrument is measured, which may not represent the observation site.

An aspiration psychrometer should be screened in an instrument shelter (see Fig. 3.5).

To make accurate temperature observations, instruments should be well exposed to a fresh air stream from the sea, which has not been in contact with or passed over the ship. They should also be adequately shielded from spray, precipitation, and heat radiated by the sun, the sea and the ship itself.

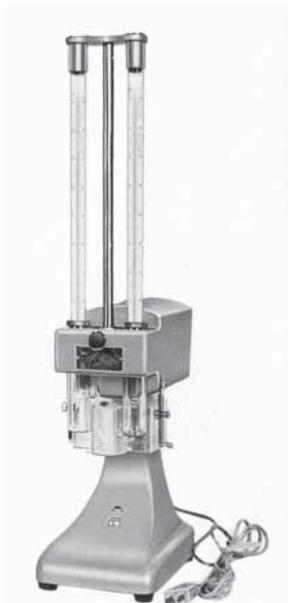


Fig. 3.1 Ventilated psychrometer

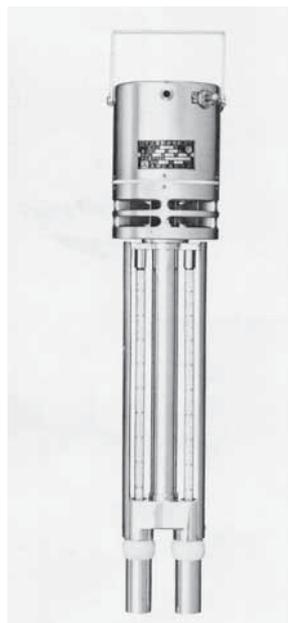


Fig. 3.2 Assmann psychrometer



Fig. 3.3 Sling psychrometer



Fig. 3.4 Electric thermometer and hygrometer housed in separate metal envelopes



Fig. 3.5 Instrument shelter called "Screen" in which a psychrometer is housed

### 3.3 Psychrometers (Dry and wet-bulb thermometers)

#### 3.3.1 Maintenance of wet-bulb thermometer

Psychrometers are instruments with both a wet-bulb and a dry-bulb thermometer. Wet-bulb temperature is measured with the wet-bulb thermometer which is a thermometer with its bulb wrapped in a wet cloth called a sleeve (or muslin wicking). The thermometer without a sleeve is called the dry-bulb thermometer. The wet-bulb thermometer is the one on the left of an aspiration psychrometer or on the outer side of a sling psychrometer. Its sleeve must be wet with the purest water available (preferably distilled). Any minerals or other impurities would change the evaporation characteristics of the water. If the sleeve becomes dirty or contaminated by sea spray, it should be replaced immediately. The sleeve should be renewed at least once a

month with normal use. The thermometer bulbs should also be kept clean. When changing a sleeve, ensure the thermometer bulbs are clean. Fig. 3.6 shows various types of wet-bulbs wrapped in sleeves.

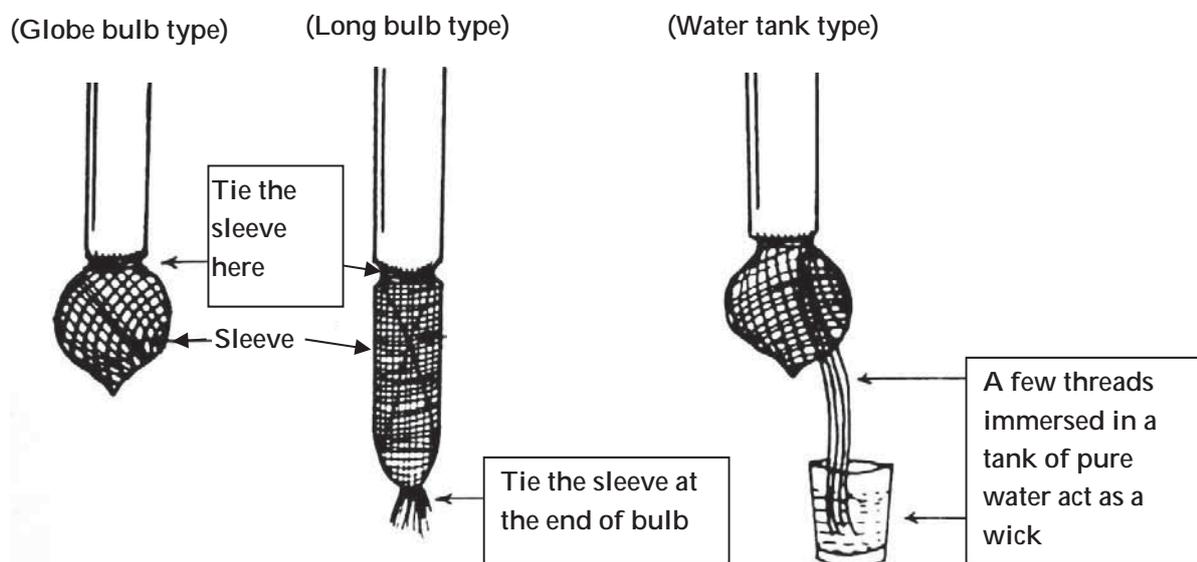


Fig. 3.6 Wet-bulbs wrapped in sleeves

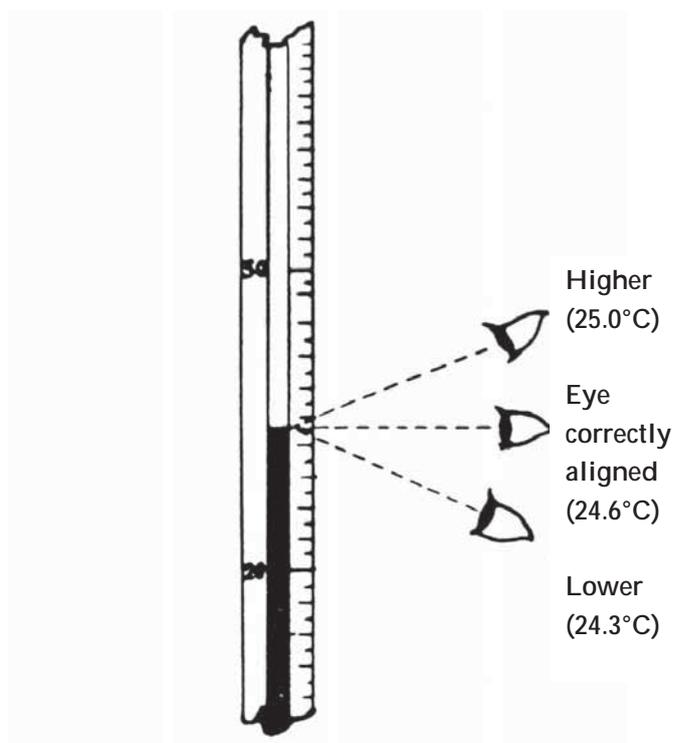


Fig. 3.7 Align eye to avoid parallax error

### 3.3.2 Observation by psychrometer

Ten minutes before observation, moisten the sleeve with pure (preferably distilled) water using a water dropper careful not to wet other parts of the psychrometer.

Hold the psychrometer out of direct sunlight, facing windward. Ensure the dry-bulb is not downwind of the wet-bulb. It takes about 5 minutes to obtain a stabilized value from a wet-bulb thermometer if the air temperature is above 0°C. But it may take 10 to 20 minutes if the wet-bulb is ice covered. Read the dry-bulb thermometer first then the wet-one taking care to avoid any influence by body heat or breath. Read the 1st decimal place of the scale first, then the higher places.

When reading a thermometer, care should be taken to align the eye with

the measurement point, otherwise there may be a risk of parallax error (see Fig. 3.7).

The minimum interval on a thermometer scale is generally  $0.5^{\circ}\text{C}$  or  $0.2^{\circ}\text{C}$ . For meteorological observations, that of  $0.2^{\circ}\text{C}$  is recommended with the measure estimated to the nearest tenth of a degree.

### 3.3.3 How to measure dew-point temperature

Unless using a dew-point hygrometer, to obtain the dew-point temperature use "Table for Finding the Dew-point" published by JMA or the table for saturated vapor pressure (see Appendix 2).

#### (1) Method using "Table for Finding the Dew-point"

The table shows dew-point temperatures under wet-bulb depression values. Wet-bulb depression is the difference between the dry-bulb temperature ( $t$ ) and the wet-bulb temperature ( $t'$ ), (wet-bulb depression:  $t-t'$ ). As both  $t-t'$  and  $t'$  are given at intervals of  $0.5^{\circ}\text{C}$  in the table, the dew-point temperature is obtained as shown in the following examples:

Example A)

Given

dry-bulb temperature ( $t$ ) =  $20.8^{\circ}\text{C}$

wet-bulb temperature ( $t'$ ) =  $19.4^{\circ}\text{C}$

$t-t' = 1.4$  and the nearest values at intervals of 0.5 for  $t'$  and  $t-t'$  are 19.5 and 1.5, respectively. In the table we find:

dew-point temperature =  $18.8^{\circ}\text{C}$  for  $t-t' = 1.5$  and  $t' = 19.5$ .

$t'$	$t-t'$			
	0.5	1.0	1.5	2.0
19.0	18.8	18.5	18.3	18.0
19.5	19.3	19.0	18.8	18.5
20.0	19.8	19.5	19.3	19.1

The table includes annotations: a box around the value 1.4 in the header row, a box around the value 19.4 in the first column, a dot in the cell (19.5, 1.5), an arrow pointing up from the dot to the value 1.4, and an arrow pointing left from the dot to the value 19.4.

Example B)

Given

$$t = 1.2^{\circ}\text{C}$$

$$t' = -2.7^{\circ}\text{C} \text{ (wet-bulb not ice covered)}$$

$t-t' = 3.9$  and the values of  $t'$  and  $t-t'$  are rounded to  $-2.5$  and  $4.0$ , respectively. In the table (for a wet-bulb not ice covered) we find:

dew-point temperature =  $-12.1^{\circ}\text{C}$  for  $t-t' = 4.0$  and  $t' = -2.5$ .

$t'$	$t-t'$			
	3.0	3.5	4.0	4.5
-3.0	-9.8	-11.3	-13.0	
-2.5	-9.0	-10.5	-12.1	
-2.0	-8.2	-9.6	-11.1	-12.8

Diagram illustrating the interpolation process for Example B. A dot is placed at the intersection of the row for  $t-t' = 4.0$  and the column for  $t' = -2.5$ . A horizontal arrow points from this dot to the left, crossing the column for  $t-t' = 3.5$ , where a vertical arrow points up to the value  $-11.3$ . A second horizontal arrow points from the dot to the left, crossing the column for  $t-t' = 3.0$ , where a vertical arrow points up to the value  $-9.8$ . A box labeled  $3.9$  is positioned above the  $t-t' = 4.0$  column, with an arrow pointing down to the dot. A box labeled  $-2.7$  is positioned to the left of the  $t' = -2.5$  row, with an arrow pointing right to the dot.

Example C)

Given

$$t = 1.2^{\circ}\text{C}$$

$$t' = -2.7^{\circ}\text{C} \text{ (wet-bulb ice covered)}$$

$t-t' = 3.9$  and the values of  $t'$  and  $t-t'$  are rounded to  $-2.5$  and  $4.0$ , respectively. In the table (for a wet-bulb ice covered) we find:

dew-point temperature =  $-11.1^{\circ}\text{C}$  for  $t-t' = 4.0$  and  $t' = -2.5$ .

$t'$	$t-t'$			
	3.0	3.5	4.0	4.5
-3.0	-9.4	-10.7	-12.1	
-2.5	-8.6	-9.8	-11.1	
-2.0	-7.7	-8.9	-10.1	-11.5

Diagram illustrating the interpolation process for Example C. A dot is placed at the intersection of the row for  $t-t' = 4.0$  and the column for  $t' = -2.5$ . A horizontal arrow points from this dot to the left, crossing the column for  $t-t' = 3.5$ , where a vertical arrow points up to the value  $-9.8$ . A second horizontal arrow points from the dot to the left, crossing the column for  $t-t' = 3.0$ , where a vertical arrow points up to the value  $-8.6$ . A box labeled  $3.9$  is positioned above the  $t-t' = 4.0$  column, with an arrow pointing down to the dot. A box labeled  $-2.7$  is positioned to the left of the  $t' = -2.5$  row, with an arrow pointing right to the dot.

## (2) Method using "Table for saturated vapor pressure (Appendix 2)"

Appendix 2 is a series of tables to determine saturated vapor pressure from air temperature. Different tables are used according to condition:

- 1) Air temperature above 0°C, wet-bulb not ice covered
- 2) Air temperature below 0°C, wet-bulb not ice covered
- 3) Air temperature below 0°C, wet-bulb ice covered.

To calculate dew-point temperature given dry-bulb temperature (t), wet-bulb temperature (t'), and sea level pressure (P) as

$$t = 19.8^{\circ}\text{C}$$

$$t' = 17.3^{\circ}\text{C}$$

$$P = 985.2 \text{ hPa}$$

saturated vapor pressure (E') for t' is 19.74 hPa from Table 1. Vapor pressure (e) is calculated as

$$e = E' - A / 755 \times (t - t') \times P$$

where A is 0.5 (wet-bulb not ice covered) or 0.44 (wet-bulb ice covered). In this case,

$$\begin{aligned} e &= 19.74 - 0.5/755 \times (19.8 - 17.3) \times 985.2 \\ &= 19.74 - 1.63 \\ &= 18.11 \text{ hPa} \end{aligned}$$

The nearest value of (e) in the table 1) is 18.06 hPa, so its corresponding dew-point temperature 15.9°C should be recorded.

### 3.4 Electric psychrometer

#### 3.4.1 Platinum resistance thermometer

The thermometer has a platinum sensor whose resistance changes with temperature change. It is housed in a ventilated cylinder to shield it from direct sunlight and precipitation, and to provide adequate ventilation. Adequate wind speed ventilation for the sensor is 6 to 8 m/s.

#### 3.4.2 Dew-point hygrometer

The sensor of the dew-point hygrometer is a heater coated with lithium chloride which absorbs water vapor from the air. Dry lithium chloride is non-conductive but with water it becomes conductive. As it absorbs water, conductivity increases and a current flows to the heater which evaporates water from the sensor until a dynamic equilibrium is reached when the water vapor pressure of the lithium chloride matches that of the atmosphere. Then, the temperature of the sensor is closely related to the dew-point temperature of the air. A hygrometer, which generates heat, and a thermometer must be housed separately. Comparison checks with a psychrometer should be conducted frequently. If the difference in dew-point temperature among them exceeds 1°C, apply new lithium chloride.

### 3.4.3 Electric humidity hygrometer

The hygrometer has a capacitance sensor including a polymer film which absorbs water. As the polymer absorbs water there is a change in capacitance which can be directly converted to the relative humidity of the surrounding atmosphere. The hygrometer is housed in a ventilated cylinder to shield it from direct sunlight and precipitation. Adequate wind speed ventilation for the sensor is about 4 m/s. Comparison checks with a psychrometer should be conducted frequently.

## CHAPTER 4 Observation of Wind

Wind is an important meteorological element, closely related to the pattern of atmospheric pressure. Wind speed is proportional to the pressure gradient while wind direction deviates about 15 degrees from the direction of isobars over the oceans in mid-latitudes. Reported observations of wind over the oceans are utilized in real time for weather analysis, forecasting and warnings, and also as statistical data for navigation of ships and aircraft.

### 4.1 Definition and units

Wind vectors indicate direction (wind direction) and magnitude (wind speed). Values averaged over 10 minutes immediately preceding the observation time should be reported. If the wind characteristics were markedly changeable showing discontinuity during the period, adopt the values averaged over the period since the discontinuity, hence less than 10 minutes..

Wind direction is the direction from which it is blowing. In marine meteorological observation, wind direction is measured as one of 36 direction codes, with the east as 09, the south as 18, the west as 27 and the north as 36. The scale of the direction code increases clockwise with an increment of 1 for each angle increment of 10 degrees. If the wind speed is 0.2 m/s (0.4 knot) or less, the wind direction should be recorded as "calm" and coded as 00 in the logbook. If wind is indeterminate, enter 99 as the direction code.

The unit for wind speed is "knot".

1 knot = 1 nautical mile/hour

= 1852 m/hour

= 0.5144 m/s

1 m/s= 1.9438 knot



Fig. 4.1 Anemometer (wind vane type)



Fig. 4.2 Indicator of wind direction

Fig. 4.3 Indicator of wind speed

## 4.2 Measuring instruments and installation conditions

A wind vane/anemometer, generally used to measure wind (Fig. 4.1), should be installed where the wind is least affected by the ship structure and it is accessible to repair in case of malfunction.

## 4.3 Measurement of wind direction and speed

With an anemograph, examine the data recorded over 10 minutes preceding the observation time. Determine the wind direction as the averaged direction at the center of the most densely dotted part in the record. Similarly determine the mean wind speed at the center of the fluctuating values of wind speed. Without an anemograph, determine the average of wind direction values shown by the indicator for about 1 minute. Regarding wind speed, discard the maximum and the minimum values and adopt the value around which fluctuations are most constant.

## 4.4 Calculation of true wind direction and speed

Unless your instrument is capable of measuring wind compensating for ship motion (true wind), the observed wind obtained on board ship during navigation is taken as apparent wind.

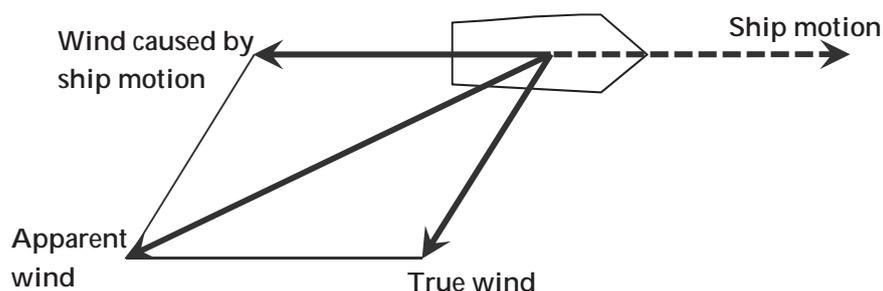


Fig. 4.4 Ship motion vector and wind vectors

True wind is the vector sum of apparent wind and ship motion. A circle graph is useful in its calculation. Plot an angle scale on the circumference in increments of 10 degrees. Then plot a speed scale on the diameter in increments of 10 knots. Example (Fig. 4.5) to find the true wind:

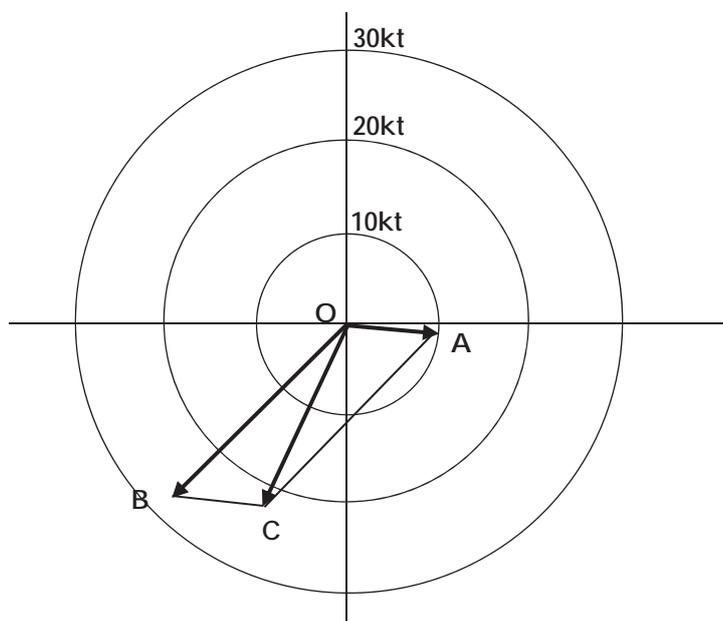


Fig. 4.5 Vectors of ship motion ( $\vec{OA}$ ), apparent wind ( $\vec{OB}$ ), and true wind ( $\vec{OC}$ )

Given

Ship motion ( $\vec{OA}$ ):

ship course, 95 degrees

ship speed, 10 knots

Apparent wind ( $\vec{OB}$ ):

wind direction, 50 degrees on the port side

wind speed, 27 knots

Calculations:

Mark point A at the angle of 95 degrees and distance of 10 knots from the center. The arrow from the center (O) to A indicates the ship motion vector.

Mark point B at the angle of 225 degrees = 95 (ship course) – 50 (apparent wind direction relative to the bow; negative in case of wind on the port side and positive on the starboard side) + 180 (to obtain the direction to which wind blows) and distance of 27 knots from the center.

The vector direction of apparent wind is the direction to which it is blowing, opposite from which it is blowing. The arrow from the center (O) to B indicates the apparent wind vector.

The true wind vector is the summation of vectors,  $\vec{OB}$  and  $\vec{OA}$ . Form a parallelogram using OA and OB to find point C. This yields  $\vec{OC}$  as the true wind vector and the length of  $\vec{OC}$  as the true wind speed (22 knots). The opposite direction of  $\vec{OC}$ , is the true wind direction (25 degrees) from which the wind blows. True wind can be also calculated using a

wind velocity scale (see Fig. 4.6), which comprises two rotatable disks and a rectangular scale fastened at the center. The lower disk is called the compass disk, and the upper the wind direction disk. The rectangular scale is called a wind speed ruler. To calculate true wind:

- 1) Turn the wind direction disk to set "0" at the direction of the ship bow (95 degrees in the above example) on the compass disk.
- 2) Turn the wind speed ruler to the direction of apparent wind (50 degrees to the left) on the wind direction disk.
- 3) Mark point "A" on the wind direction disk using the wind speed ruler to measure wind speed (27 knots) from the center, and next, mark point "C" going down from "A" at a distance of ship speed (10 knots) in parallel with vertical lines of the wind direction disk.
- 4) Turn the wind speed ruler to "C" to find the true wind speed by reading the scale of the ruler at "C" (it must be 22 knots).
- 5) The cross point of the wind speed ruler and the wind direction disk shows the true wind direction from the bow, and the cross point of the wind speed ruler and the compass disk shows the true wind direction from true north (it must be 25 degrees).

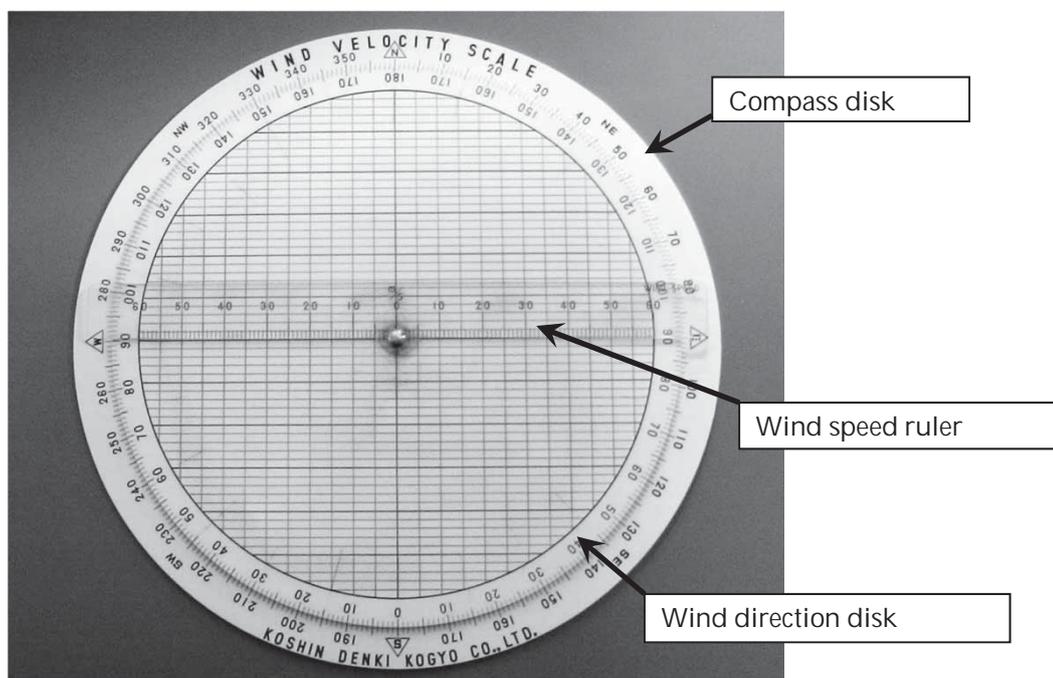


Fig. 4.6 Wind velocity scale

You can confirm the calculated true wind direction by observing wind waves, which travel in almost the same direction as the wind.

#### 4.5 Non-instrument observations

If a wind vane/anemometer is unavailable wind direction and force may be found as below.

### 4.5.1 Wind direction

True wind direction may be estimated from streaks of foam on the sea surface and/or sea (wind) wave direction with a gyro compass. Consider the following:

- 1) Observe wind waves far enough from the ship that they are not influenced by the ship.
- 2) When wind direction changes abruptly, note the direction of streaks of foam and the orientation of the crests of sea waves other than their overall shape since wave characteristics remain affected by the preceding wind for some time.
- 3) When wind is too weak to cause perceptible waves, estimate wind direction from the deck considering the ship travel direction.
- 4) Do not rely on wind waves near shore or sea ice for proper wind direction.
- 5) At night or in poor visibility (such as heavy rainfall, snow, fog etc.), estimate wind direction from smoke, tape and so on.
- 6) Although windward is preferable, observe wind leeward to avoid dazzle by the sun or its reflection from the sea surface.

### 4.5.2 Wind force

To estimate wind force from the sea surface, use the Beaufort Scale of Wind Force (see Table 4.1) and refer to photographs. The velocity equivalent in Table 4.1 is for a standard height of 10 meters above the sea surface. When using the table, heed the following:

- 1) Even while wind is strengthening, there are time lags before changes in sea surface characteristics (wave length, wave height, etc.) occur.
- 2) The scale of a wave depends on the fetch (the distance along which a wind is blowing straight) of the wind in question. This is why a wave with land on its upstream side has a different scale from an offshore wave.
- 3) If a tidal or ocean current is predominant, the sea surface does not always correspond to the wind force. Especially, unusual waves are remarkable near an ocean front.
- 4) The sea depth affects the way a wave is formed.
- 5) When a swell is prevailing, it is more difficult to discern wind waves.
- 6) At night, estimation of wind force is not easy. Since it is more difficult to recognize white caps of wind waves, slightly inflate the wind force value.
- 7) Rainfall tends to make the sea surface smoother. Severe rainfall is particularly effective. In such conditions inflate the wind force value accordingly.
- 8) The height of a wind wave depends on the difference between air and sea surface temperatures. Accordingly, adopt a smaller wind force value than estimated from wind waves when the air temperature is lower than the sea surface temperature.

Table 4.1 Beaufort scale of wind force

Beaufort number	Descriptive term	Wind speed equivalents		Specifications for observations
		m/s	knots	
0	Calm	0 - 0.2	< 1	Sea like a mirror
1	Light air	0.3 - 1.5	1 - 3	Ripples with the appearance of scales are formed, but without foam crests
2	Light breeze	1.6 - 3.3	4 - 6	Small wavelets, still short but more pronounced; crests have a glassy appearance and do not break
3	Gentle breeze	3.4 - 5.4	7 - 10	Large wavelets; crests begin to break; foam of glassy appearance; perhaps scattered white horses
4	Moderate breeze	5.5 - 7.9	11 - 16	Small waves, becoming longer; fairly frequent white horses
5	Fresh breeze	8.0 - 10.7	17 - 21	Moderate waves, taking a more pronounced long form; many white horses are formed (chance of some spray)
6	Strong breeze	10.8 - 13.8	22 - 27	Large waves begin to form; the white foam crests are more extensive everywhere (probably some spray)
7	Near gale	13.9 - 17.1	28 - 33	Sea heaps up and white foam from breaking waves begins to be blown in streaks along the direction of the wind
8	Gale	17.2 - 20.7	34 - 40	Moderately high waves of greater length; edges of crests begin to break into the spindrift; the foam is blown in well-marked streaks along the direction of the wind
9	Strong gale	20.8 - 24.4	41 - 47	High waves; dense streaks of foam along the direction of the wind; crests of waves begin to topple, tumble and roll over; spray may affect visibility
10	Storm	24.5 - 28.4	48 - 55	Very high waves with long overhanging crests; the resulting foam, in great patches, is blown in dense white streaks along the direction of the wind; on the whole, the surface of the sea takes on a white appearance; the tumbling of the sea becomes heavy and shock-like; visibility affected
11	Violent storm	28.5 - 32.6	56 - 63	Exceptionally high waves (small and medium-sized ships might be for a time lost to view behind the waves); the sea is completely covered with long white patches of foam lying along the direction of the wind; everywhere the edges of the wave crests are blown into froth; visibility affected
12	Hurricane	32.7 =<	64 =<	The air is filled with foam and spray; sea completely white with driving spray; visibility very seriously affected



**Force 0 Calm**

Wind speed < 1 knot

Sea like a mirror

**Force 1 Light air**

Wind speed 1 – 3 knots

Ripples with the appearance of scales are formed, but without foam crests

**Force 2 Light breeze**

Wind speed 4 – 6 knots

Small wavelets, still short but more pronounced; crests have a glassy appearance and do not break

**Force 3 Gentle breeze**

Wind speed 7 – 10 knots

Large wavelets; crests begin to break; foam of glassy appearance; perhaps scattered white horses

**Force 4 Moderate breeze**

Wind speed 11 – 16 knots

Small waves, becoming longer; fairly frequent white horses

**Force 5 Fresh breeze**

Wind speed 17 – 21 knots

Moderate waves, taking a more pronounced long form; many white horses are formed (chance of some spray)

**Force 6 Strong breeze**

Wind speed 22 – 27 knots

Large waves begin to form; the white foam crests are more extensive everywhere (probably some spray)

**Force 7 Near gale**

Wind speed 28 – 33 knots

Sea heaps up and white foam from breaking waves begins to be blown in streaks along the direction of the wind

**Force 8 Gale**

Wind speed 34 – 40 knots

Moderately high waves of greater length; edges of crests begin to break into the spindrift; the foam is blown in well-marked streaks along the direction of the wind

**Force 9 Strong gale**

Wind speed 41 – 47 knots

High waves; dense streaks of foam along the direction of the wind; crests of waves begin to topple, tumble and roll over; spray may affect visibility

**Force 10 Storm**

Wind speed 48 – 55 knots

Very high waves with long overhanging crests; the resulting foam, in great patches, is blown in dense white streaks along the direction of the wind; on the whole, the surface of the sea takes on a white appearance; the tumbling of the sea becomes heavy and shock-like; visibility affected

**Force 11 Violent storm**

Wind speed 56 – 63 knots

Exceptionally high waves (small and medium-sized ships might be for a time lost to view behind the waves); the sea is completely covered with long white patches of foam lying along the direction of the wind; everywhere the edges of the wave crests are blown into froth; visibility affected



**Force 12 Hurricane**

Wind speed  $\geq 64$  knots

The air is filled with foam and spray; sea completely white with driving spray; visibility very seriously affected



## CHAPTER 5 Observation of Cloud

A cloud is a hydrometeor consisting of water droplets or ice crystals, or a mixture of both, suspended in the air above land or sea. It differs from fog only in that fog is in contact with the earth's surface. Cloud observations help determine the state of the atmosphere with their distribution related to meteorological disturbances including Typhoons or extra-tropical lows. Although wide range observations by meteorological satellites have been introduced, the importance of cloud observation by ships over the oceans remains significant.

### 5.1 Observation of cloud

Observation of cloud on board ship covers:

- Amount of cloud and type of cloud
- Identification of cloud types
- Height of the base of each cloud

Observe the total amount of cloud first, then from lower cloud to higher cloud. Among the same height of clouds, groups with larger amount should be considered first.

#### 5.1.1 Cloud amount (Cloud cover)

"Total cloud amount" is the proportion of the sky occupied by clouds of all types.

In contrast, the proportion of a specified type of cloud is called "cloud amount" for that type. Observers should estimate each cloud type exclusively, as if no other type were present at different levels, taking into consideration the evolution of the sky. Total summation of the cloud amounts could then exceed the total cloud amount.

Cloud amount for any particular type is recorded as 0 if there is none present, 10 if it covers the whole sky and 1 to 9 according to its coverage. If the amount seems too small to be 1, put 0+. If it is almost 10 but a break is present, put 10-.

Care should be taken as follows:

- 1) If there is fog, haze, or other phenomena preventing observation of any cloud above, record cloud amount as unknown (sky obscured), code 9 in the logbook. If cloud can be seen through the fog, etc., cloud amount should be estimated as well as possible.
- 2) If the sun, moon or stars are visible through the fog, etc., and there is no evidence of cloud above the fog, cloud amount should be estimated as 0.
- 3) If cloud amount cannot be estimated at night, rather than make an ambiguous guess record it as unknown with the code ×.
- 4) If part of the sky is obscured by rain or snow, decide the amount assuming the cloud bringing the rain or snow covers that part of the sky.

#### 5.1.2 Cloud type (genus)

Clouds are classified into 10 principal types (genera), see 5.2. They are incessantly changing,

not necessarily appearing in typical form so care is needed to determine which types are prevailing. If uncertain, record as unknown.

### 5.1.3 Cloud height

Cloud height is the height of the cloud base above the sea measured in units of 100 m, reaching up to about 10 km. A cloud can be classified according to height as: high level cloud ( $C_H$ ), middle level cloud ( $C_M$ ) and low level cloud ( $C_L$ ). The maximum cloud height observed varies according to latitude, being higher in the tropics than in the higher latitudes. If it is difficult to discern the cloud type, consideration of its height may help.

## 5.2 Details of cloud types (genera)

Table 5.1 shows 10 types of clouds (genera) by 3 levels of height, and Table 5.2 summarizes the relationship between cloud type and precipitation.

**Table 5.1 Approximate heights for high, middle and low clouds**

Level	Cloud type (genus)	Polar Regions	Temperate Regions	Tropical Regions
High ( $C_H$ )	Cirrus (Ci)	3 - 8 km	5 - 13 km	6 - 18 km
	Cirrocumulus (Cc)			
	Cirrostratus (Cs)			
Middle ( $C_M$ )	Alto cumulus (Ac)	2 - 4 km	2 - 7 km	2 - 8 km
	Altostratus (As)			
	Nimbostratus (Ns)	As: usually found in the middle level, but often extends higher. Ns: usually found in the middle level, but often extends into the other levels.		
Low ( $C_L$ )	Stratocumulus (Sc)	below 2 km	below 2 km	below 2 km
	Stratus (St)	Cu, Cb: usually have bases in the low level, but their tops often reach into the middle and high levels.		
	Cumulus (Cu)			
	Cumulonimbus (Cb)			

Table 5.2 Hydrometeors consisting of falling particles and the cloud genera

Hydro- meteors	Genera	As	Ns	Sc	St	Cu	Cb	No cloud
Rain		○	○	○		○	○	
Drizzle					○			
Snow		○	○	○	○	○	○	
Snow grains					○			
Snow pellets				○		○	○	
Diamond dust								○
Hail							○	
Small hail							○	
Ice pellets		○	○					

\*Reference: International Cloud Atlas Vol.1 (1975)

The following descriptions of general features of each type of cloud are excerpted from "International Cloud Atlas Vol.1 (WMO-No.407, 1975)".

### 5.2.1 High level clouds (C<sub>H</sub>)

#### (1) Cirrus (Ci)

- Cirrus is defined as detached clouds in the form of white, delicate filaments or white or mostly white patches or narrow bands. These clouds have a fibrous (hair-like) appearance, or a silky sheen, or both. Cirrus is composed almost exclusively of ice crystals. Cirrus tufts with rounded tops often form in clear air.

- At all times of day, cirrus not too close to the horizon is white, in fact whiter than any other cloud in the same part of the sky. When the sun sinks below the horizon, cirrus high in the sky is yellow, then pink, red and finally grey. The color sequence is reversed at dawn.

- Cirrus clouds often evolve from virga of cirrocumulus (Cc) or altocumulus (Ac), or from the upper part of a cumulonimbus (Cb).

- Cirrus clouds are distinguished from cirrostratus (Cs) by their discontinuous structure or, if they are in patches or bands, by their small horizontal extent or the narrowness of their continuous parts.

#### (2) Cirrocumulus (Cc)

- Cirrocumulus is defined as thin, white patch, sheet or layer of cloud without shading, composed of very small elements in the form of grains, ripples, etc., merged or separate, and more or less regularly arranged; most of the elements have an apparent width of less than one degree.

- Cirrocumulus is composed almost exclusively of ice crystals; strongly supercooled water droplets may occur but are usually rapidly replaced by ice crystals.

- Cirrocumulus often forms as a result of the transformation of cirrus (Ci) or cirrostratus (Cs). Cirrocumulus may also form as the result of a decrease in size of the elements of a patch,

sheet or layer of altocumulus (Ac).

- Cirrocumulus differs from cirrus (Ci) and cirrostratus (Cs) in that it is rippled or subdivided into very small cloudlets; it may include fibrous, silky or smooth portions which, however, do not collectively constitute its greater part.

- Cirrocumulus differs from altocumulus (Ac) in that most of its elements are very small (by definition, of an apparent width less than one degree when observed at an angle of more than 30 degrees above the horizon) and without shading.

### (3) Cirrostratus (Cs)

- Cirrostratus is defined as transparent, whitish cloud veil of fibrous (hair-like) or smooth appearance, totally or partly covering the sky, and generally producing halo phenomena. Cirrostratus is composed mainly of ice crystals.

- Cirrostratus is never thick enough to prevent objects on the ground from casting shadows, at least when the sun is high above the horizon.

- Cirrostratus differs from altostratus (As) by its thinness and by the fact that it may show halo phenomena. Cirrostratus near the horizon may be mistaken for As. The slowness of the apparent movement and the slowness of the variations in optical thickness and in appearance, both characteristic of cirrostratus, give useful guidance in distinguishing this cloud from As and also from stratus (St).

- Cirrostratus differs from stratus (St) by being whitish throughout, and by the fact that it may have a fibrous appearance. Moreover cirrostratus often displays halo phenomena, whereas St does not, except occasionally at very low temperatures.

- Cirrostratus differs from a veil of haze by the fact that latter is opalescent or has a dirty yellowish to brownish color.

## 5.2.2 Middle level clouds (C<sub>M</sub>)

### (1) Altocumulus (Ac)

- Altocumulus is defined as white or grey, or both white and grey, patch, sheet or layer of cloud, generally with shading, composed of laminae, rounded masses, rolls, etc., which are sometimes partly fibrous or diffuse and which may or may not be merged; most of the regularly arranged small elements usually have an apparent width between one and five degrees.

- Altocumulus is, at least in the main, almost invariably composed of water droplets. A corona or irisation is often observed in thin parts of altocumulus.

- An altocumulus layer may sometimes be confused with altostratus (As); in case of doubt, clouds are called altocumulus if there is any evidence of the presence of laminae, rounded masses, rolls, etc.

- Altocumulus, with dark portions, may sometimes be confused with stratocumulus (Sc). If most of the regularly arranged elements have, when observed at an angle of more than 30 degrees above the horizon, an apparent width between one and five degrees, the cloud is altocumulus.

- Altocumulus in scattered tufts may be confused with small cumulus (Cu) clouds; the altocumulus tufts, however, often show fibrous trails (virga) and moreover are, in their majority, smaller than the Cu clouds.

## (2) Altostratus (As)

- Altostratus is defined as greyish or bluish cloud sheet or layer of striated, fibrous or uniform appearance, totally or partly covering the sky, and having parts thin enough to reveal the sun at least vaguely, as through ground glass. Altostratus does not show halo phenomena.

- Altostratus is composed of water droplets and ice crystals. Raindrops or snowflakes are often present in altostratus and below its base.

- Pannus clouds may be present; they occur under the altostratus in the lower turbulent layers when these are moistened by evaporation from precipitation.

- Altostratus may evolve from a thickening veil of cirrostratus (Cs); it is sometimes formed by the thinning of a layer of nimbostratus (Ns). Altostratus may also develop from an altocumulus (Ac) layer; this happens when widespread ice crystal trails (virga) fall from the latter. Sometimes, particularly in the tropics, altostratus is produced by the spreading out of the middle or upper part of cumulonimbus (Cb).

- A low, thick layer of altostratus may be distinguished from a similar layer of nimbostratus (Ns) by the presence in altostratus of thinner parts through which the sun is, or could be, vaguely revealed. Altostratus is also of a lighter grey and its under surface is usually less uniform than that of Ns. When, on moonless nights, doubt exists regarding the choice of the designation altostratus or Ns, the layer is by convention called altostratus, if no rain or snow is falling.

- Altostratus is distinguishable from stratus (St), with which it may be confused, by its ground glass effect. Furthermore, altostratus is never white, as thin St may be when observed more or less towards the sun.

## (3) Nimbostratus (Ns)

- Nimbostratus is defined as grey cloud layer, often dark, the appearance of which is rendered diffuse by more or less continuously falling rain or snow, which in most cases reaches the ground. It is thick enough throughout to blot out the sun. Low, ragged clouds frequently occur below the layer, with which they may or may not merge.

- Nimbostratus is composed of water droplets (sometimes supercooled) and raindrops, of snow crystals and snowflakes, or of a mixture of these liquid and solid particles.

- The under surface of nimbostratus is often partially or totally hidden by pannus clouds resulting from turbulence in the layers under its base, which are moistened by partial evaporation of precipitation.

- In the tropics, particularly during short lulls in the rainfall, nimbostratus can be seen breaking up into several different cloud layers, which rapidly merge again.

- Nimbostratus usually develops from thickening altostratus (As). It also sometimes forms by the spreading out of cumulonimbus (Cb).

- Nimbostratus is distinguished from thick stratus (St) by the fact that it is a dense cloud

producing rain, snow or ice pellets; the precipitation which may fall from St is in the form of drizzle, ice prisms or snow grains.

- When the observer is beneath a cloud having the appearance of a nimbostratus, but accompanied by lightning, thunder or hail, the cloud should by convention be called cumulonimbus (Cb).

### 5.2.3 Low level clouds (C<sub>L</sub>)

#### (1) Stratocumulus (Sc)

- Stratocumulus is defined as grey or whitish, or both grey and whitish, patch, sheet or layer of cloud which almost always has dark parts, composed of tessellations, rounded masses, rolls, etc., which are non-fibrous (except for virga) and which may or may not be merged; most of the regularly arranged small elements have an apparent width of more than five degrees.

- Stratocumulus is composed of water droplets. During extremely cold weather stratocumulus may produce abundant ice crystal virga which may be accompanied by a halo. When stratocumulus is not very thick, a corona or irisation is sometimes observed.

- Stratocumulus differs from cumulus (Cu) in that its elements usually occur in groups or patches and generally have flat tops; if however, stratocumulus tops are in the form of domes, they rise, unlike those of Cu, from merged bases.

- Stratocumulus is often formed by the spreading out of cumulus (Cu) or cumulonimbus (Cb).

#### (2) Stratus (St)

- Stratus is defined as generally grey cloud layer with a fairly uniform base, which may give drizzle, snow or snow grains. When the sun is visible through the cloud, its outline is clearly discernible. Sometimes stratus appears in the form of ragged patches.

- Stratus is usually composed of small water droplets; this cloud may, when very thin, produce a corona round the sun or moon. At low temperatures, stratus may consist of small ice particles. The ice cloud is usually thin and may, on rare occasions, produce halo phenomena.

- A common mode of stratus formation is the slow lifting of a fog layer, due to warming of the earth's surface or an increase in wind speed.

- Stratus fractus clouds may also form as accessory clouds (pannus) under altostratus (As), nimbostratus (Ns), cumulonimbus (Cb) and precipitating cumulus (Cu); they develop as a result of turbulence in the moistened layers under these clouds.

- Stratus fractus is distinguished from cumulus (Cu) fractus in that it is less white and less dense. Furthermore, it shows a smaller vertical development, since it owes its formation mainly to turbulence without thermal convection.

#### (3) Cumulus (Cu)

- Cumulus is defined as detached clouds, generally dense and with sharp outlines, developing vertically in the form of rising mounds, domes or towers, of which the bulging upper part often resembles a cauliflower. The sunlit parts of these clouds are mostly brilliant

white; their base is relatively dark and nearly horizontal. Sometimes cumulus is ragged.

- Cumulus is composed mainly of water droplets. Ice crystals may form in those parts of a cumulus in which the temperature is well below 0°C; they grow at the expense of evaporating supercooled water droplets, thereby transforming the cloud into cumulonimbus (Cb).

- Cumulus develops in convection currents which occur when the lapse rate in the lower layers is sufficiently steep. Such steep lapse rates result from heating of the air near the earth's surface.

- Since cumulonimbus (Cb) generally results from the development and transformation of cumulus, it is sometimes difficult to distinguish cumulus with a great vertical extent from Cb. The cloud should be named cumulus as long as the sprouting upper parts are everywhere sharply defined and no fibrous or striated texture is apparent. If it is not possible to decide on the basis of other criteria whether a cloud is to be named cumulus or Cb, it should by convention be called cumulus if it is not accompanied by lightning, thunder or hail.

#### (4) Cumulonimbus (Cb)

- Cumulonimbus is defined as heavy and dense cloud, with a considerable vertical extent, in the form of a mountain or huge towers. At least part of its upper portion is usually smooth, or fibrous or striated, and nearly always flattened; this part often spreads out in the shape of an anvil or vast plume.

- Under the base of this cloud which is often very dark, there are frequently low ragged clouds either merged with it or not, and precipitation sometimes in the form of virga.

- Cumulonimbus is composed of water droplets and, especially in its upper portion, of ice crystals. It also contains large raindrops and, often, snowflakes, snow pellets, ice pellets or hailstones. The water droplets and raindrops may be substantially supercooled.

- Cumulonimbus clouds may appear either isolated clouds or in the form of a continuous line of clouds resembling a very extensive wall. In certain cases, the upper portion of cumulonimbus clouds may be merged with altostratus (As) or nimbostratus (Ns). Cumulonimbus may also develop within the general mass of As or Ns.

- Cumulonimbus most commonly evolves from cumulus (Cu) which was formed in the normal manner. Cumulonimbus sometimes develops from altocumulus (Ac) or stratocumulus (Sc).

### 5.3 Observation of cloud states and their coding

For forecasters who eventually receive and use observers' reports, reports on what clouds are present in the sky are not so important but reports on distributions or arrangements of clouds, namely the states of clouds are much more valuable.

The state of clouds is coded not only based on 10 cloud types but by the characteristics  $C_L$  (low level cloud),  $C_M$  (middle level cloud) and  $C_H$  (high level cloud), amount, thickness, variation and combination of clouds. Procedures to define the code are shown in Figs. 5.1-5.3.

Typical photos for each code are shown in succeeding pages with explanations taken from "International Cloud Atlas Vol.1 (WMO-No.407, 1975)"

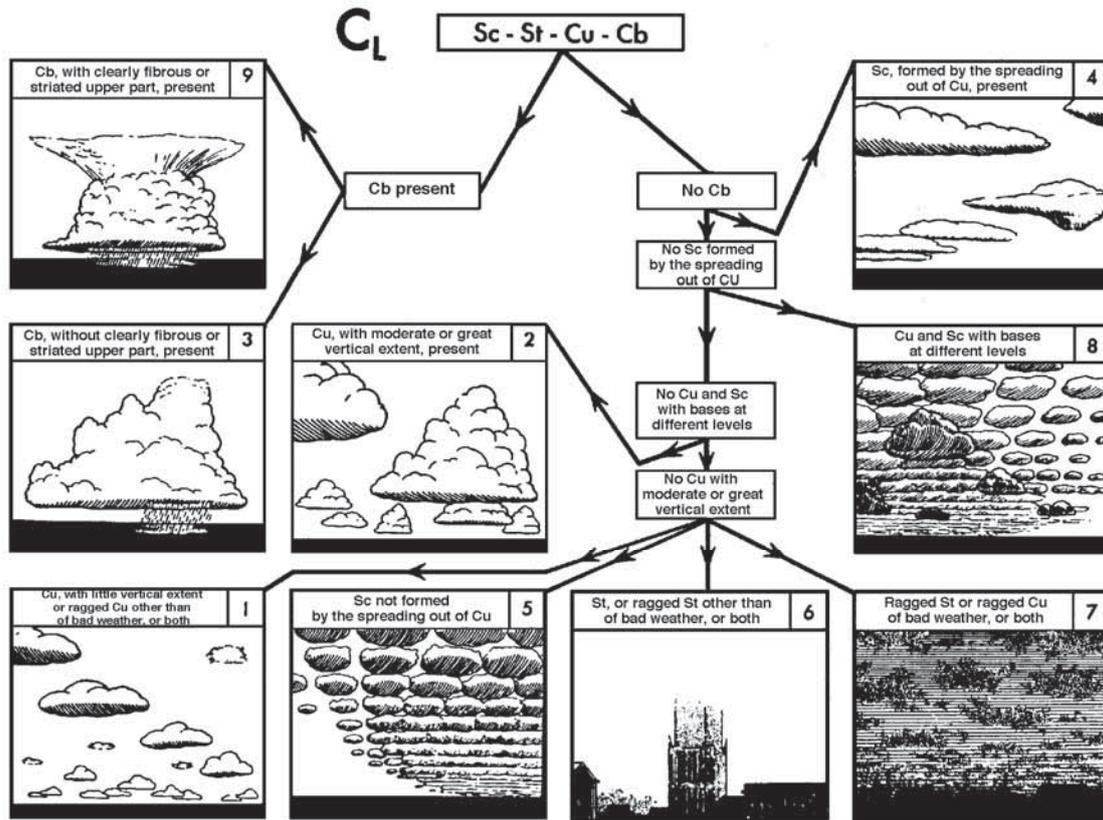


Fig. 5.1 Selection chart to define the code for CL

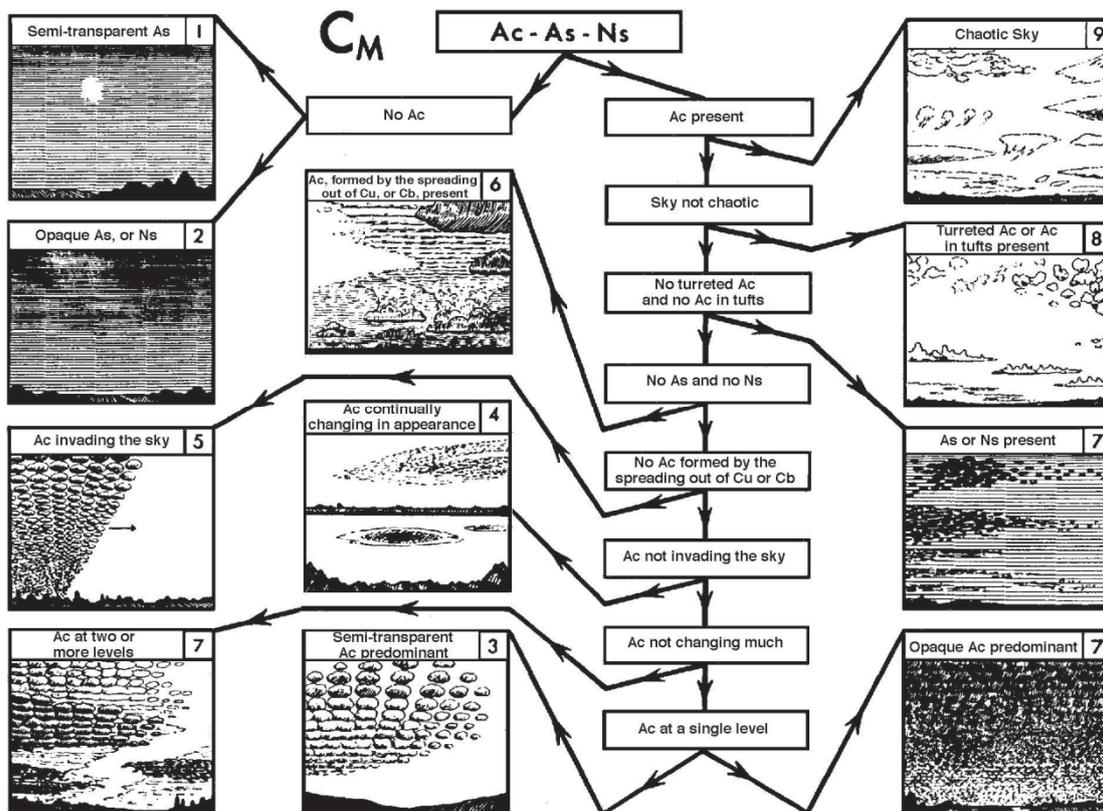


Fig. 5.2 Selection chart to define the code for CM

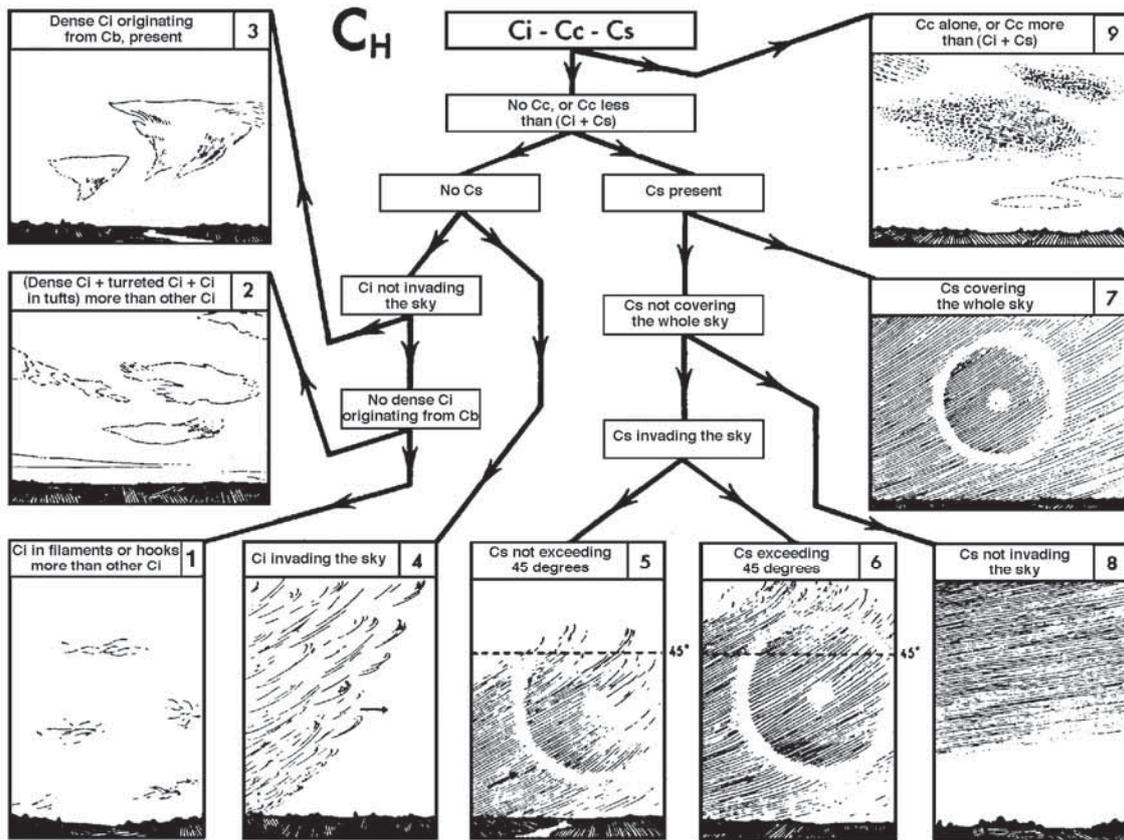


Fig. 5.3 Selection chart to define the code for C<sub>H</sub>



**C<sub>L</sub> : 1**

Cu with little vertical extent and seemingly flattened, or ragged Cu other than of bad weather, or both.

**C<sub>L</sub> : 2**

Cu of moderate or strong vertical extent, generally with protuberances in the form of domes or towers, either accompanied or not by other Cu or by Sc, all having their bases at the same level.

**C<sub>L</sub> : 3**

Cb the summits of which, at least partially, lack sharp outlines, but is neither clearly fibrous (cirriform) nor in the form of an anvil; Cu, Sc or St may also be present.



**C<sub>L</sub> : 4**

Sc formed by the spreading out of Cu; Cu may also be present.



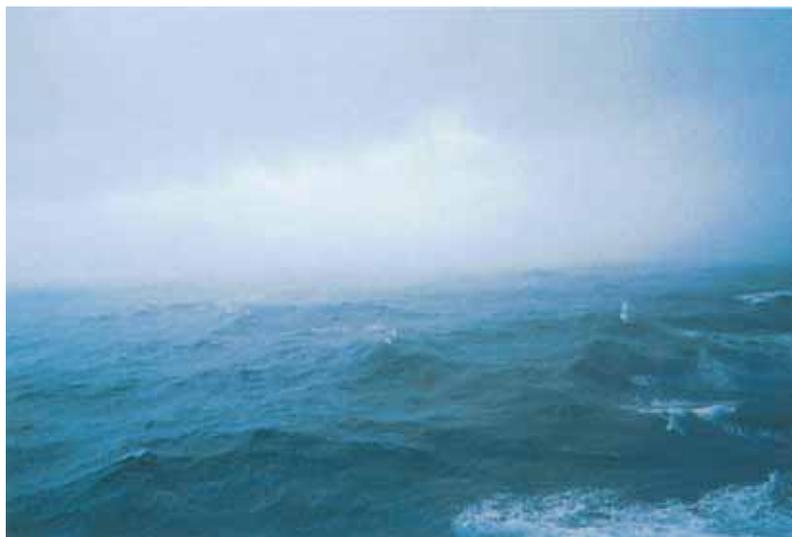
**C<sub>L</sub> : 5**

Sc not resulting from the spreading out of Cu.



**C<sub>L</sub> : 6**

St in a more or less continuous sheet or layer, or in ragged shreds, or both, but no St fractus of bad weather.

**CL : 7**

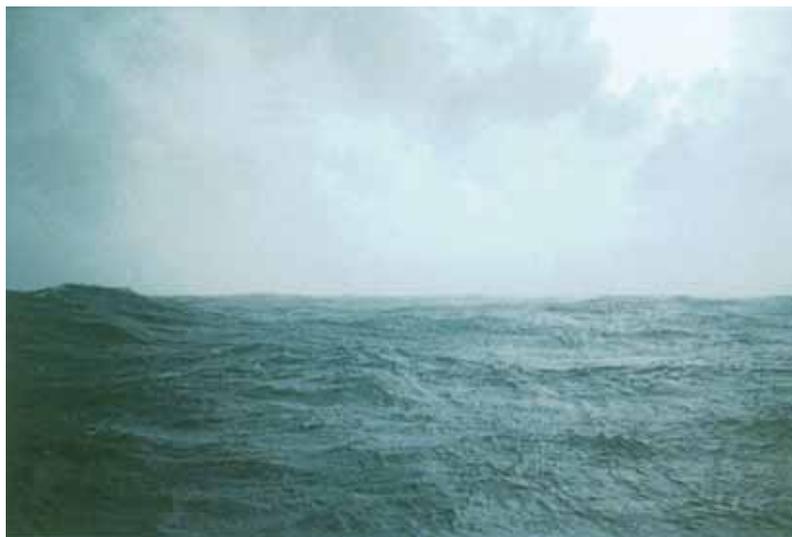
St fractus of bad weather or Cu fractus of bad weather, or both (pannus), usually below As or Ns.

**CL : 8**

Cu and Sc other than that formed from the spreading out of Cu; the base of the Cu is at a different level from that of the Sc.

**CL : 9**

Cb, the upper part of which is clearly fibrous (cirriform), often in the form of an anvil; either accompanied or not by Cb without anvil or fibrous upper part, by Cu, Sc, St or pannus.

**C<sub>M</sub> : 1**

As, the greater part of which is semi-transparent; through this part the sun or moon may be weakly visible, as through ground glass.

**C<sub>M</sub> : 2**

As, the greater part of which is sufficiently dense to hide the sun or moon, or Ns.

**C<sub>M</sub> : 3**

Ac, the greater part of which is semi-transparent; the various elements of the cloud change only slowly and are all at a single level.

**C<sub>M</sub> : 4**

Patches (often in the form of almonds or fishes) of Ac, the greater part of which is semi-transparent; the clouds occur at one or more levels and the elements are continually changing in appearance.

**C<sub>M</sub> : 5**

Semi-transparent Ac in bands, or Ac in one or more fairly continuous layers (semi-transparent or opaque), progressively invading the sky; these Ac clouds generally thicken as a whole.

**C<sub>M</sub> : 6**

Ac resulting from the spreading out of Cu (or Cb).



$C_M : 7$

Ac in two or more layers, usually opaque in places, and not progressively invading the sky; or opaque layer of Ac, not progressively invading the sky; or Ac together with As or Ns.



$C_M : 8$

Ac with sprouting in the form of small towers or battlements, or Ac having the appearance of cumuliform tufts.



$C_M : 9$

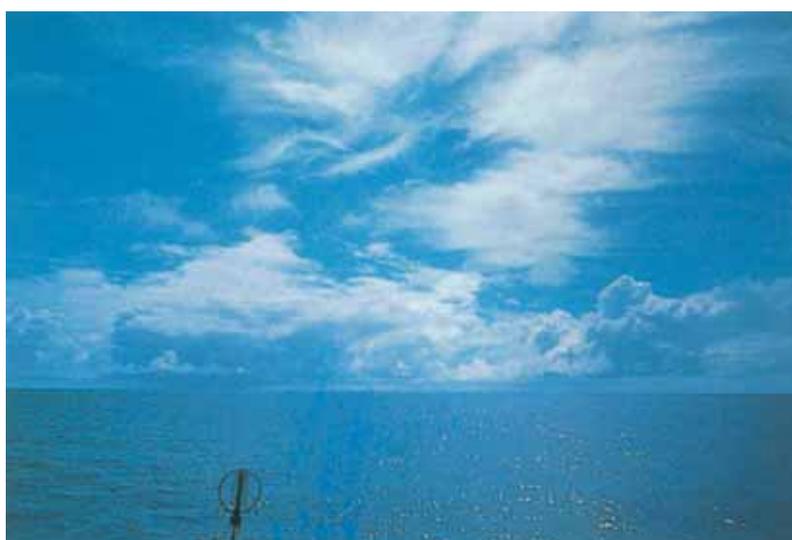
Ac of a chaotic sky, generally at several levels.

**C<sub>H</sub> : 1**

Ci in the form of filaments, strands or hooks, not progressively invading the sky.

**C<sub>H</sub> : 2**

Dense Ci, in patches or entangled sheaves, which usually do not increase and sometimes seem to be the remains of the upper part of a Cb; or Ci with sprouting in the form of small turrets or battlements, or Ci having the appearance of cumuliform tufts.

**C<sub>H</sub> : 3**

Dense Ci, often in the form of an anvil, being the remains of the upper parts of Cb.

**C<sub>H</sub> : 4**

Ci in the form of hooks or of filaments, or both, progressively invading the sky; they generally become denser as a whole.

**C<sub>H</sub> : 5**

Ci (often in bands converging towards one point or two opposite points of the horizon) and Cs, or Cs alone; in either case, they are progressively invading the sky, and generally growing denser as a whole, but the continuous veil does not reach 45 degrees above the horizon.

**C<sub>H</sub> : 6**

Ci (often in bands converging towards one point or two opposite points of the horizon) and Cs, or Cs alone; in either case, they are progressively invading the sky, and generally growing denser as a whole; the continuous veil extends more than 45 degrees above the horizon, without the sky being totally covered.



$C_H : 7$

Veil of Cs covering the celestial dome.



$C_H : 8$

Cs not progressively invading the sky and not completely covering the celestial dome.



$C_H : 9$

Cc alone, or Cc accompanied by Ci or Cs, or both, but Cc is predominant.



## CHAPTER 6 Observation of Visibility

Visibility is not only to what extent an object can be seen by the observer but a simple indicator of atmospheric stability. Visibility is decreased by material in the atmosphere including water droplets in the form of fog, rain or snow and solid particles in the form of sand dust, smoke or crystallized salt from sea water spray. Visibility is affected by refraction and diffusion of light due to differences in air density. Visibility observations are utilized not only for meteorological analysis but marine traffic and management of atmospheric pollution.

### 6.1 Definition of visibility

Visibility (horizontal visibility) is determined as the greatest distance at which a black object of suitable dimensions can be seen and identified against the sky at the horizon during daylight or at night if as bright as daytime, meaning visibility is constant regardless of observation time if the atmospheric condition is unchanged.

If visibility differs by direction, adopt the shortest distance. Visibility should be observed with the naked eye or with best corrected vision, not with a telescope, binoculars or a sextant.

See "Guide to Ships' Weather Reports" to code observations.

### 6.2 Daytime observation

If a ship, island or buoy can be seen at the limit of visibility, the bestmost precise way is to use radar to measure visibility. However, this seldom occurs so training and experience is required for visibility observation. One simple method is to use the relationship between height (h) of observation above the sea surface in meters and distance (L) to the horizon in kilometers.

$$L = 3.6\sqrt{h}$$

as shown in Table 6.1.

**Table 6.1 Height of observation above sea surface and distance to the horizon**

Height of observer eyes above sea level (m)	2	4	6	8	10	15	20	25	30	40
Distance of object on the horizon at sea (km)	5	7	9	10	11	14	16	18	20	23

For example, if the horizon cannot be seen clearly from a bridge 15 m above the sea surface but can from the deck 8 m above the sea surface, visibility is about 12 km (between 14 km and 10 km in the Table).

### 6.3 Night-time observation

Estimation of visibility at night is not easy and requires time (from 5 to 15 minutes) to adapt the eyes to the dark. Lights of other vessels, buildings on land and stars around the horizon may be used to estimate visibility.

## CHAPTER 7 Observation of Atmospheric Phenomena and Weather

Weather is a manifestation of the atmosphere at a particular time described by atmospheric phenomena. Atmospheric phenomena are generally classified into hydrometeors, lithometeors, electrometeors, and photometeors. This chapter describes these atmospheric phenomena except for photometeors, which are luminous phenomena in the atmosphere including halos, rainbows, mirages, which are unrelated to weather reports.

### 7.1 Atmospheric phenomena to be reported

#### 7.1.1 Hydrometeor

Hydrometeor is a meteor comprising liquid and/or solid water particles falling through or suspended in the atmosphere.

In the case of precipitation, report whether it is uniform (intermittent or continuous) or shower type and whether it is rain, drizzle, snow, or hail. Shower is characterized by its sudden beginning and ending, and generally by large and rapid changes of intensity. It is often short-lived and heavy, falling from convective clouds such as cumulus (Cu) and cumulonimbus (Cb). Uniform precipitation falls from stratiform clouds including altostratus (As) and nimbostratus (Ns).

Various hydrometeors and their definitions are as follows:

Rain ●, Rain Shower ⚡: Precipitation of liquid water particles, either in the form of drops of more than 0.5 mm in diameter, or of smaller widely scattered drops.

Freezing Rain ❄️: Rain drops freezing on impact with the ground or ship.

Drizzle ⚪: Fairly uniform precipitation in very fine drops of water (diameter less than 0.5 mm) very close to one another, falling from stratiform clouds.

Freezing Drizzle ❄️: Drizzle drops freezing on impact with the ground or ship.

Snow ❄️, Snow Shower ⚡: Precipitation of ice crystals, isolated or clustered, falling from clouds.

Snow Pellets ❄️: Precipitation of white and opaque ice particles, which fall from Cu and Cb clouds and are generally conical or spherical, with diameters attaining as much as 5 mm.

Snow Grains ❄️: Precipitation of very small opaque white particles of ice and which are fairly flat or elongated with diameters generally less than 1 mm. They usually fall in small quantities, mostly from stratus (St) or from fog and never in the form of a shower.

Ice Pellets ❄️: Precipitation of transparent particles of ice which are spherical or irregular, rarely conical, and which have a diameter of 5 mm or less.

Small Hail ⚡: Precipitation of translucent ice particles, which falls from Cu and Cb clouds. These particles are almost always spherical and sometimes have conical tips. Their diameter may attain or even exceed 5 mm

Hail ⚡: Precipitation of either transparent, or partially or completely opaque particles of ice (hailstones), usually spheroidal, conical or irregular in form and of diameter generally between 5 and 50 mm, which fall from Cu and Cb clouds either separately or frozen together into irregular lumps and generally accompanied by strong thunder.

Diamond Dust ⇄: Precipitation which falls from a clear sky in very small ice crystals, often so tiny that they appear to be suspended in the air.

Fog ≡: Suspension of very small, usually microscopic water droplets in the air, generally reducing horizontal visibility to less than 1 km.

Ice Fog ≡: Suspension of numerous minute ice particles in the air, reducing visibility. It appears under air temperatures below  $-30^{\circ}\text{C}$  and calm wind conditions.

Mist ≡: Suspension in the air of microscopic water droplets or wet hygroscopic particles with visibility of more than 1 km.

Spout ⌋: A phenomenon consisting of an often violent whirlwind, revealed by the presence of a cloud column or inverted cloud cone (funnel cloud), protruding from the base of a Cb, and of a “bush” composed of water droplets raised from the surface of the sea or of dust, sand or litter, raised from the ground.

Ice Accretion: Process by which a layer of ice builds up on a solid surface which is exposed to freezing precipitation or supercooled droplets.

Spray ⌘: Ensemble of water droplets torn by the wind from the surface of an extensive body of water, generally from the crests of waves, and carried up a short distance into the air.

### 7.1.2 Lithometeor

Lithometeor is meteor consisting of particles most of which are solid and dry; they are more or less suspended in the air, or lifted by the wind from the ground.

Typical lithometeors and their definitions are as follows:

Haze ∞: A suspension in the air of extremely small, dry particles which are invisible to the naked eye but numerous enough to give the sky an opalescent appearance.

Smoke ℄: A suspension in the air of small particles produced by combustion.

Dust Storm (Sand Storm) ⚡: An ensemble of particles of dust or sand energetically lifted to great heights by a strong and turbulent wind.

Dust Haze (Sand Haze) ⚡: A suspension in the air of small sand or dust particles, raised from the ground prior to the time of observation by a dust storm or sand storm.

### 7.1.3 Electrometeor

Electrometeor is a visible or audible manifestation of atmospheric electricity.

Main electrometeors and their definitions are as follows:

Thunderstorm ⚡: Sudden electrical discharges manifested by a flash of light (lightning) and a sharp or rumbling sound (thunder). Thunderstorms are associated with convective clouds (Cumulonimbus) and are, most often, accompanied by precipitation in the form of rainshowers or hail, or occasionally snow shower, snow pellets, or small hail.

Lightning ⚡: A luminous manifestation accompanying a sudden electrical discharge which takes place from or inside a cloud or, less often, from high structures on the ground or from mountains.

**Thunder T:** A sharp or rumbling sound which accompanies lightning. It is emitted by rapidly expanding gases along the channel of a lightning discharge.

## 7.2 Observation and recording of atmospheric phenomena

For observations of atmospheric phenomenon, record the following:

- 1) Time of appearance
- 2) Name of the phenomenon
- 3) State of the phenomenon
- 4) Time of ending

For state of the phenomenon, record whether the precipitation is shower or not, the level of visibility and intensity of the phenomenon, etc. Intensity is classified into three ranks for each atmospheric phenomenon as described below:

### (1) Intensity of rain

Light: Scattered drops that do not completely wet an exposed surface, regardless of duration, to a condition where individual drops are easily seen; slight spray is observed over the decks; puddles form slowly; sound on roofs ranges from slow pattering to gentle swishing; steady small streams may flow in scuppers and deck drains.

Moderate: Individual drops are not clearly identifiable; spray is observable just above deck and other hard surfaces; puddles form rapidly; sound on roofs ranges from swishing to gentle roar.

Heavy: Rain seemingly falls in sheets; individual drops are not identifiable; heavy spray to height of several inches is observed over hard surfaces; visibility is greatly reduced; sound on roofs resembles the roll of drums or distant roar.

### (2) Intensity of drizzle

Light: Visibility 1 km or more.

Moderate: Visibility more than 0.5 km but less than 1 km.

Heavy: Visibility less than 0.5 km.

### (3) Intensity of snow

Light: Visibility 1 km or more.

Moderate: Visibility more than 0.2 km but less than 1 km.

Heavy: Visibility less than 0.2 km.

### (4) Intensity of hail and ice pellets

Light: Few stones or pellets falling with little, if any, accumulation.

Moderate: Slow accumulation.

Heavy: Rapid accumulation.

### (5) Intensity of thunder, thunderstorm and lightning

Light thunder: Slightly perceived as distant thunder.

Moderate thunder: Thunder with considerably large sound so as to vibrate a window pane.

Heavy thunder: Thunder with deafening and astonishing sound with the effect of rattling windows.

Light thunderstorm: Light thunder with a flash of light.

Moderate thunderstorm: Moderate thunder with a flash of light.

Heavy thunderstorm: Heavy thunder with a flash of light.

Light lightning: Slightly perceived in the daytime and clearly visible at night.

Moderate lightning: Perceived without confrontation in the daytime and perceived from inside a lighted room.

Heavy lightning: A flash of light brightens the surrounding area in the daytime and at night everything is dazzled by the heavy flash of light.

## 7.3 Present weather and past weather

Weather should be reported by codes under present weather and past weather as described in “Ships’ Weather Code Card” and “Guide to Ship’s Weather Reports”.

Present weather is defined as the state of the atmosphere at the time of observation or within the hour (60 minutes) preceding it in some cases. Table 7.1 shows the relationship between weather phenomenon and codes of present weather, total cloud amount, and visibility.

Past weather refers to the type(s) of weather which occurred for a certain period of time prior to the observation. Table 7.2 shows the period to be covered by the past weather at each observation time. And Table 7.3 shows the past weather codes.

Table 7.1 Relationship between weather phenomenon and weather report codes

Weather phenomenon		Weather report codes			
		Present weather (ww)	Total cloud amount (N: octas)	Visibility (VV)	Remarks
Clear sky	○	00~16 18~32 36~38 40・41, 76	0, 1	≥ 94 (≥ 1 km)  (except the cases of ww = 40, 41)	
Fine	⊕		2~6		
Overcast (covered with mostly upper level cloud)	⊖		7, 8		
Overcast (covered with mostly lower or middle level cloud)	⊙				
Haze	∞	04~06	9 (unknown; obscured sky)	< 94 (< 1 km)	The criteria of either N or VV must be met.
Dust/Sand storm	☼	30~35			The criteria VV must be met.
Blowing snow	✎	38・39			
Fog	≡	42~49			
Drizzle	☂	50~57			
Rain	●	58~67, 80~82, 91, 92			
Rain and snow mixed	⚡	68・69, 83・84, 93・94			ww 93 or 94 is interpreted as hail under warm season and snow under cold season if other information is not available.
Snow	✖	70~75, 77・78, 85・86 93・94			
Small hail	△	79, 87・88, 93・94			
Hail	▲	89・90, 93・94			
Thunderstorm	⚡	17, 95~99			

Table 7.2 Period to be covered by past weather

Observation time	Period
00,06,12,18 UTC	Preceding 6 hours
03,09,15,21 UTC	Preceding 3 hours
Other	Preceding 1 hour

Table 7.3 Past weather codes

Code (W <sub>1</sub> W <sub>2</sub> )	Symbols	Description of past weather
0	○①	Cloud covering 1/2 or less of the sky throughout the appropriate period
1	○①②③	Cloud covering more than 1/2 of the sky during part of the appropriate period and covering 1/2 or less during part of the period
2	①②③	Cloud covering more than 1/2 of the sky throughout the appropriate period
3	☼	Sand storm, dust storm or blowing snow, visibility less than 1 km
4	≡≡∞	Fog or ice fog, visibility less than 1 km; or thick haze, visibility less than 2 km
5	●	Drizzle
6	●	Rain
7	×✱	Snow, or rain and snow mixed
8	▽▽▽▲	Shower(s)
9	⚡	Thunderstorm(s)

## CHAPTER 8 Observation of Sea Surface Temperature

Sea surface temperature plays an important role in the interaction between the atmosphere and the ocean. The difference between the air temperature and the sea surface temperature gives a basic measure of the vertical stability of the atmosphere and the heat exchange between the atmosphere and the ocean.

The temperature of well mixed sea water at the depth of 1 to 2 m should be measured and recorded to the nearest 0.1°C.

Sea surface temperature is normally observed by either

- 1) Measuring the temperature of the condenser intake water
- 2) Exposing an electrical thermometer to the sea-water either directly or through the hull
- 3) Measuring the temperature of the sea-water sampled with a sea-bucket

Methods 3) and 1) have been used for many years. Recently, as the speed and dimension of ships have increased, method 2) has been more widely used.

### 8.1 Intake method

The intake sea-water temperature is measured by a thermometer installed in the intake pipe. If this requires reading in cramped conditions, the observer should guard against parallax error (Fig. 3.7). The observer should be aware that when the ship has a deep draught, or there is a marked temperature gradient in the surface layer, intake temperature may differ from that nearer the sea surface. When the ship is stationary, sea-bucket temperature should be measured for comparison because the cooling water may not be circulating. Also, as temperature of the intake pipe may be influenced by heat of the engine. This method is not the best to observe sea-surface temperature. However, it is frequently used in consideration of safety and convenience.

### 8.2 Hull-attached thermometer method

The sensor in this method is mounted either externally in direct contact with the sea using a "through-the-hull" connection, or internally (the "limpet" type) attached to the inside of the hull. Such hull-attached thermometers provide a convenient and accurate means of measuring sea-surface temperature. Its readout device is installed in a cabin for observation.

### 8.3 Sea-bucket method

In this method, the sea-bucket is lowered over the side of the ship to collect sea-water which is hauled on board and measured by thermometer. The bucket should be filled and emptied several times beforehand to control for the bucket temperature. The sample should be taken near the bow, from the leeward side of the ship during navigation, from the windward side while at anchor, and well forward of all outlets. The thermometer should be read as soon as possible after it has attained the temperature of the water sample. The bucket should be stored out of direct sunlight.

## CHAPTER 9 Observation of Ocean Waves

Navigation of ships is profoundly affected by winds and waves. Consequently, accurate information on ocean waves is essential for disaster prevention and economical navigation. Recent developments in satellite technology do not diminish the importance of observations of ocean waves by ships. The data of ocean waves reported by ships contribute to the analysis and forecasting of ocean waves and statistical studies of the climatology of ocean waves.

### 9.1 Classification of ocean waves

There are thousands of waves with different wave heights and periods on the ocean. The sea surface is incessantly moving up and down. Among such waves, those caused by wind over the sea surface are called ocean waves and have periods from about 1 to 30 seconds.

Ocean waves are classified into wind waves (or sea) and swell. Wind waves and/or swell change into surfs in approaching a coast, or tidal races in colliding with tidal or ocean currents.

#### (1) Wind waves (or Sea)

Waves raised by local wind blowing at the time of observation are usually referred to as "wind waves" or "sea".

Development of wind waves depends on three characteristics of wind: wind speed, fetch (the distance along which a wind is blowing straight) and duration (the time during which a wind with an almost fixed wind direction and speed is blowing along a fetch).

#### (2) Swell

Waves not raised by the local wind blowing at the time of observation, but due either to winds blowing a distance away or winds that have ceased to blow, are referred to as "swell".

Figure 9.1 shows a schematic of wind waves (sea) with sharp crests and swell with gentle ones.

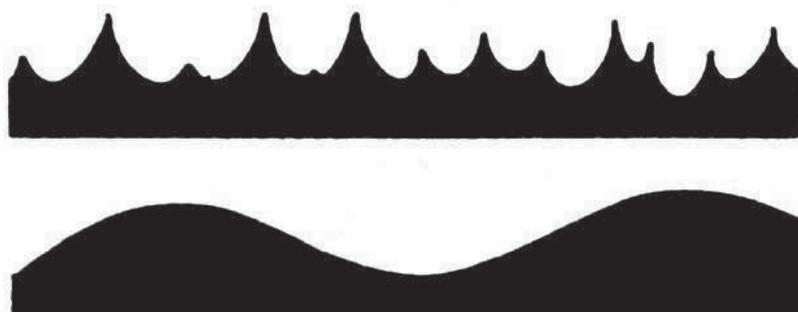


Fig. 9.1 Schematic view of wind wave (upper) and swell (lower)

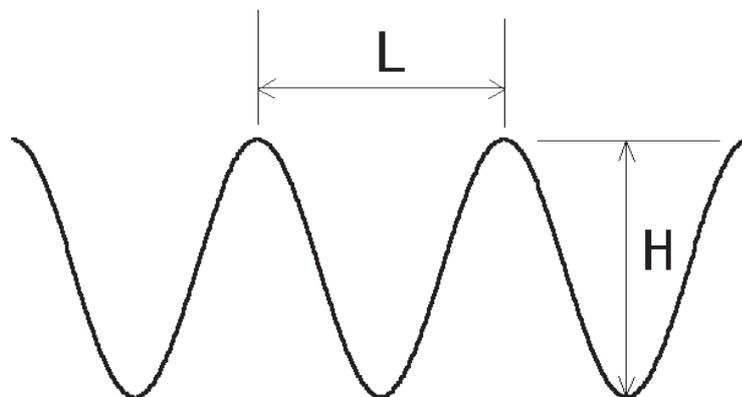


Fig. 9.2 Wave height (H) and wave length (L)

## 9.2 Characteristics of ocean waves

Major characteristics of ocean wave include,

- 1) Wave direction: the direction from which they come.
- 2) Wave period: the time between the passage of two successive wave crests past a fixed point, which is equal to wave length divided by wave speed.
- 3) Wave height: the vertical distance between trough and crest (see Fig. 9.2).
- 4) Wave length: the horizontal distance between successive crests or troughs, which is equal to wave period multiplied by wave speed (see Fig. 9.2).
- 5) Wave speed: the distance traveled by a wave in a unit of time, which is equal to wave length divided by wave period.

Speed (C; unit: m/s), length (L; unit: m) and period (T; unit: second) of a wave whose height is small compared to the ocean depth are related in theory by the following formulas:

$$C = 1.56T \quad (1)$$

$$L = 0.64C^2 = 1.56T^2 \quad (2)$$

$$T = 0.80L^{1/2} = 0.64C \quad (3)$$

Although the above formulae are not necessarily applicable to all types of waves, they are useful to roughly estimate the value of each element.

### 9.3 Non-instrument observation

Measures to be observed are wave direction, period and height with respect to each distinguishable wave among wind waves and swells traveling from various directions. Bearing in mind the distinction between wind waves (sea) and swell, observers should differentiate between recognizable waves, on the basis of the direction, appearance and period of the waves. If it is difficult to observe a measure precisely due to, for example, poor light conditions, record that measure as unknown.

#### (1) Direction

The direction (one of 36) from which waves are coming, is measured from the direction of wave trains traveling a little far from your ship with a gyrocompass. The direction of a wind wave generally coincides with that of the local wind, but keep in mind that swells traveling from different directions are often combined.

#### (2) Period

Measurement of wave period is made in the unit of second by a stopwatch. The observer notes an object floating on the water at some distance from the ship: if nothing better is available, a distinctive patch of foam can usually be found which remains identifiable for the time required for an observation.

He starts his watch when the object appears at the crest of a wave. As the crest passes on, the object disappears into the trough to reappear on the next crest. The time at which the object appears at the top of each crest is noted. The observations are continued for as long as possible; they will usually terminate when the object becomes too distant to identify, on account of the ship's motion. The longest observation period will be attained by choosing an object initially on the bow as far off as it can be clearly seen.

It is possible to estimate wave period using a formula (3) where wave length is measured based on the length of the ship.

The ratio of wave height to length is less than  $1/7$  in theory. For example, if a wave period is 3 seconds, which means its length is about 14 m from the formula (2), its height must be less than 2 m. If observed wave height is larger, the period should be underestimated in most cases.

#### (3) Height

With experience fairly reliable estimates can be made. For estimating the height of relatively low waves, the observer should take up a position as low down in the ship as possible, preferably amidships where the pitching is least, and on the side of the ship from which the waves are coming. Height is measured by comparing with the known length of the ship.

In case of relatively high wave heights, by climbing up or down some steps when the ship is in a wave trough and vertical observers should position themselves where the wave crest appears to coincide with the horizon (see Fig. 9.3 (a)). The wave height is then equal to the height of the observer's eyes above the level of the water. If the ship is rolling, care should be taken in the estimation as shown in Fig. 9.3 (b).

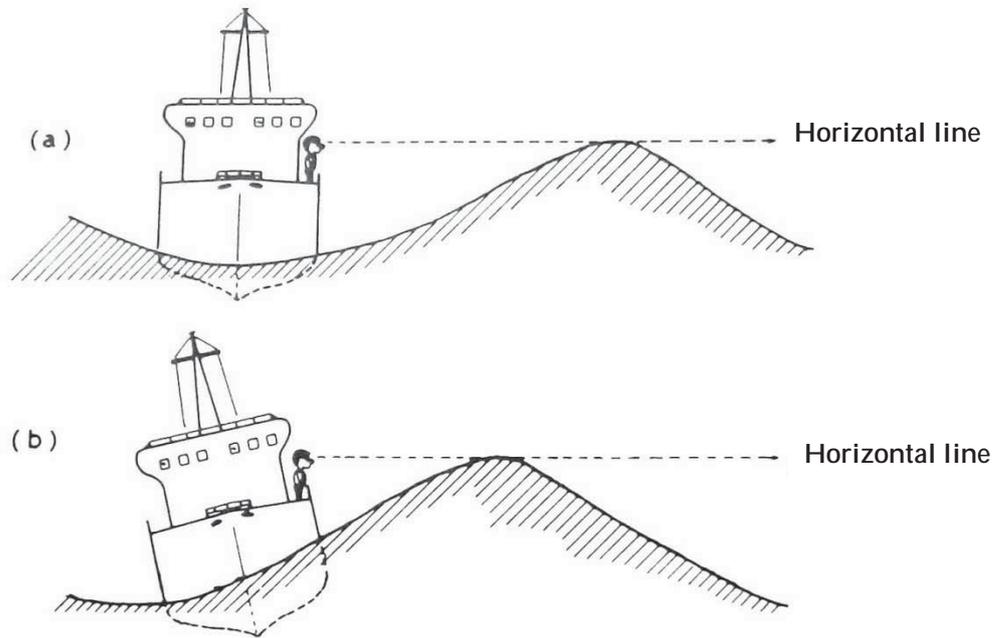


Fig. 9.3 Estimation of wave height at sea  
(a) Good example and (b) bad example

## CHAPTER 10 Observation of Sea Ice

Several forms of floating ice may be encountered at sea, including sea ice, which results from freezing of the sea surface, and icebergs which are large pieces that have detached from a glacier or an ice shelf. Other forms of ice include river ice which is encountered in harbors and estuaries where it is kept in motion by tidal streams and normally presents only a temporary hindrance to ships, and lake ice. Both icebergs and sea ice can be dangerous to ships and affect navigation. The extent of sea-ice cover may vary significantly from year to year affecting adjacent ocean areas and weather over large areas of the world. Consequently, its distribution is of considerable interest to meteorologists and oceanographers. Although knowledge of the extent of sea-ice cover has been revolutionized by satellite photography, observations from shore stations, ships and aircraft are of great importance in corroborating satellite observations.

### 10.1 Observation items

Non-instrumental observation of sea ice consists of the following items:

- Concentration or arrangement of sea ice
- Stage of development of sea ice
- Ice of land origin
- Bearing of principal ice edge
- Present ice situation and trend of conditions during past 3 hours

Observed results should be reported according to the format shown in "Guide to Ships' Weather Reports". To follow the Guide, refer to the definitions of technical terms in 10.2.

#### 10.1.1 Concentration or arrangement of sea ice

Concentration or arrangement of sea ice (referred to as "ci" in the Marine Meteorological Logbook) is expressed by code figures 0 to 9 as shown in Table 10.1. Code figures 2 to 9 are divided into two sections depending on whether sea ice concentration within the area of observation is more or less uniform (code figures 2 to 5) or not (6 to 9). Concentration is the ratio of the amount of sea surface covered by ice to the whole area and expressed in tenths or phrases such as "open ice" and "close ice" (see 10.2.2 (1) for details).

Table 10.1 Concentration or arrangement of sea ice

<i>ci</i>	Concentration or arrangement of sea ice	
0	No sea ice in sight	
1	Ship in open lead more than 1.0 nautical mile wide, or ship in fast ice with boundary beyond limit of visibility	
2	Sea ice present in concentrations less than 3/10 concentration, open water or very open pack ice	Sea ice concentration is uniform in the observation area
3	4/10 to 6/10, open pack ice	
4	7/10 to 8/10, close pack ice	
5	9/10 or more but not 10/10, very close pack ice	Ship in ice or within 0.5 nautical mile of ice edge
6	Strips and patches of pack ice with open water between	
7	Strips and patches of close or very close pack ice with areas of lesser concentration between	
8	Fast ice with open water, very open or open pack ice to seaward of the ice boundary	
9	Fast ice with close or very close pack ice to seaward of the ice boundary	Sea ice concentration is not uniform in the observation area
×	Unable to report, because of darkness, lack of visibility, or because ship is more than 0.5 nautical mile away from ice edge	

### 10.1.2 Stage of development of sea ice

Code figures for this item (referred to as “*Si*”) are determined based on the stage of development of sea ice and its thickness as shown in Table 10.2. The ice surface color is helpful to distinguish its stage of development such as Nilas, Young ice or First-year ice (see 10.2.1 (1) for details).

Table 10.2 Stage of development

<i>Si</i>	Stage of development
0	New ice only (frazil ice, grease ice, slush, shuga)
1	Nilas or ice rind, less than 10 cm thick
2	Young ice (grey ice, grey-white ice), 10-30 cm thick
3	Predominantly new and/or young ice with some first-year ice
4	Predominantly thin first-year ice with some new and/or young ice
5	All thin first-year ice (30-70 cm thick)
6	Predominantly medium first-year ice (70-120 cm thick) and thick first-year ice (>120 cm thick) with some thinner (younger) first-year ice
7	All medium first-year ice and thick first-year ice
8	Predominantly medium first-year ice and thick first-year ice with some old ice (usually more than 2 m thick)
9	Predominantly old ice
×	Unable to report, because of darkness, lack of visibility or because only ice of land origin is visible or because ship is more than 0.5 nautical mile away from ice edge

### 10.1.3 Ice of land origin

The number of types of visible ice formed over land such as growlers, bergy bits and icebergs (see 10.2.1 (5) for definition) are reported for this item (referred to as “*bi*”) and corresponding code figures are shown in Table 10.3.

Table 10.3 Ice of land origin

<i>bi</i>	Ice of land origin
0	No ice of land origin
1	1-5 icebergs, no growlers or bergy bits
2	6-10 icebergs, no growlers or bergy bits
3	11-20 icebergs, no growlers or bergy bits
4	Up to and including 10 growlers and bergy bits -- no icebergs
5	More than 10 growlers and bergy bits -- no icebergs
6	1-5 icebergs, with growlers and bergy bits
7	6-10 icebergs, with growlers and bergy bits
8	11-20 icebergs, with growlers and bergy bits
9	More than 20 icebergs, with growlers and bergy bits -- a major hazard to navigation
×	Unable to report, because of darkness, lack of visibility or because only sea ice is visible

### 10.1.4 Bearing of closest part of the principal ice edge

This item (referred to as “*Di*”) reports the direction (eight points of compass) of the closest part of the ice edge from the ship as shown in Table 10.4.

Table 10.4 True bearing of principal ice edge

<i>Di</i>	True Bearing of principal ice edge
0	Ship in shore or flaw lead
1	Principal ice edge towards North-East
2	Principal ice edge towards East
3	Principal ice edge towards South-East
4	Principal ice edge towards South
5	Principal ice edge towards South-West
6	Principal ice edge towards West
7	Principal ice edge towards North-West
8	Principal ice edge towards North
9	Not determined (ship in ice)
×	Unable to report, because of darkness, lack of visibility or because only ice of land origin is visible

### 10.1.5 Present ice situation and trend of conditions over preceding 3 hours

This item (referred to as “*zi*”) represents how sea ice is affecting navigation of the reporting ship. The present status of sea ice (e.g. penetrability) and its change over the preceding 3 hours before the observation should be reported based on Table 10.5.

Table 10.5 Present ice situation and trend of conditions over preceding 3 hours

<i>zi</i>	Present ice situation and trend of conditions over preceding 3 hours
0	Ship in open water with floating ice in sight
1	Ship in easily penetrable ice; conditions improving
2	Ship in easily penetrable ice; conditions not changing
3	Ship in easily penetrable ice; conditions worsening
4	Ship in ice difficult to penetrate; conditions improving
5	Ship in ice difficult to penetrate; conditions not changing
6	Ice forming and floes freezing together
7	Ice under slight pressure
8	Ice under moderate or severe pressure
9	Ship beset
×	Unable to report, because of darkness or lack of visibility

} Ship in ice

} Ship in ice difficult to penetrate and conditions worsening

## 10.2 Terms of sea ice forms

The definitions of terms in this section are from WMO Meteorological codes “WMO Sea-ice Nomenclature (WMO-No. 259 Edition 1970-2014).”

### 10.2.1 Classification of sea ice forms

Sea ice form can be classified by its developing or melting stage, and as *fast ice* or *drift ice*. *Ice of land* origin is also distinguished from others.

**(1) Development stage**

- (a) **New ice:** A general term for recently formed ice which includes *frazil ice*, *grease ice*, *slush* and *shuga*. These types of ice are composed of ice crystals which are only weakly frozen together (if at all) and have a definite form only while they are afloat.

Frazil ice: Fine spicules or plates of ice, suspended in water.

Grease ice: A later stage of freezing than *frazil ice* when the crystals have coagulated to form a soupy layer on the surface. *Grease ice* reflects little light, giving the sea a matt appearance.

Slush: Snow which is saturated and mixed with water on land or ice surfaces, or as a viscous floating mass in water after a heavy snowfall.

Shuga: An accumulation of spongy white ice lumps, a few centimeters across; they are formed from *grease ice* or *slush* and sometimes from *anchor ice* rising to the surface.

- (b) **Nilas:** A thin elastic crust of ice, easily bending on waves and swell and under pressure, thrusting in a pattern of interlocking "fingers" (finger rafting). Has a matt surface and is up to 10 cm in thickness. May be subdivided into *dark nilas* and *light nilas*.

Dark nilas: *Nilas* which is under 5 cm in thickness and is very dark in color.

Light nilas: *Nilas* which is more than 5 cm in thickness and rather lighter in color than *dark nilas*.

Ice rind: A brittle shiny crust of ice formed on a quiet surface by direct freezing or from *grease ice*, usually in water of low salinity. Thickness to about 5 cm. Easily broken by wind or swell, commonly breaking in rectangular pieces.

- (c) **Pancake ice (see drift ice)**

- (d) **Young ice:** Ice in the transition stage between *nilas* and *first-year ice*, 10-30 cm in thickness. May be subdivided into *grey ice* and *grey-white ice*.

Grey ice: *Young ice* 10-15 cm thick. Less elastic than *nilas* and breaks on swell. Usually rafts under pressure.

Grey-white ice: *Young ice* 15-30 cm thick. Under pressure more likely to ridge than to raft.

- (e) **First-year ice:** Sea ice of not more than one winter's growth, developing from *young ice*, thickness 30 cm - 2 m. May be subdivided into *thin first-year ice (white ice)*, *medium first-year ice (white ice first stage)* and *thick first-year ice (white ice second stage)*.

Thin first-year ice (white ice): *First-year ice* 30-70 cm thick.

Medium first-year ice (white ice first stage): *First-year ice* 70-120 cm thick.

Thick first-year ice (white ice second stage): *First-year ice* over 120 cm thick.

- (f) **Old ice:** Sea ice which has survived at least one summer's melt; typical thickness up to 3 m or more. Most topographic features are smoother than those of *first-year ice*. May be subdivided into residual, second-year ice and multi-year ice.

**(2) Melting stage**

- (a) **Puddle:** Accumulation on ice of melt-water, mainly due to melting snow, but in the advanced stages due to melting ice. The initial stage consists of patches of melted snow.

- (b) **Thaw holes:** Vertical holes in sea ice formed when surface *puddles* melt through to the underlying water.
- (c) **Dried ice:** Sea ice on the surface where melt-water has disappeared by draining through cracks and *thaw holes*. During the period of drying, the surface whitens.
- (d) **Rotten ice:** Sea ice which has become honeycombed and is in an advanced stage of disintegration.
- (e) **Flooded ice:** Sea ice, which has been flooded by melt-water or river water and is heavily loaded by water and wet snow.

### (3) Fast ice

Sea ice which forms and remains fast along the coast, where it is attached to the shore, to an ice wall, to an ice front, between shoals or grounded/*icebergs*. Vertical fluctuations may be observed during changes of sea-level. *Fast ice* may be formed *in situ* from sea water or by freezing of *floating ice* of any age to the shore which may extend from a few meters to several hundred kilometers from the coast. *Fast ice* may be more than one year old and may then be prefixed with the appropriate age category (*old*, second-year, or multi-year). If it is thicker than about 2 m above sea-level it is called an *ice shelf*.

- (a) **Young coastal ice:** The initial stage of *fast ice* formation consisting of *nilas* or *young ice*, with a width varying from a few meters up to 100-200 m from the shorelines.
- (b) **Icefoot:** A narrow fringe of ice attached to the coast, unmoved by tides and remaining after the *fast ice* has moved away.
- (c) **Anchor ice:** Submerged ice attached or anchored to the bottom, irrespective of the nature of its formation.

### (4) Floating ice

- (a) **Drift ice/Pack ice:** Term used in a wide sense to include any area of sea ice other than *fast ice* no matter what form it takes or how it is disposed. When *concentrations* are high, i.e. 7/10 or more, *drift ice* may be replaced by the term *pack ice*.
- (b) **Pancake ice:** Predominantly circular pieces of ice from 30 cm to 3 m in diameter, and up to about 10 cm thick, with raised rims due to the pieces striking against one another. It may be formed on a slight swell from *grease ice*, *shuga* or *slush* or as a result of the breaking of *ice rind*, *nilas* or, under severe conditions of swell or waves, of *grey ice*. It also sometimes forms at some depth at an interface between water bodies of different physical characteristics, from where it floats to the surface; it may rapidly cover wide areas of water.
- (c) **Floe:** Any contiguous piece of sea ice. *Floes* are subdivided according to horizontal extent as follows:
  - Floe giant:** Over 10 km across.
  - Floe vast:** 2-10 km across.
  - Floe big:** 500-2000 m across.
  - Floe medium:** 100-500 m across.

**Floe small:** 20-100 m across.

**Ice cake:** Less than 20 m across.

- (d) **Floeberg:** A massive piece of sea ice composed of a hummock, or a group of hummocks frozen together, and separated from any ice surroundings. It may typically protrude up to 5 m above sea-level.
- (e) **Ice breccia:** Ice of different stages of development frozen together.
- (f) **Brash ice:** Accumulations of *floating ice* made up of fragments not more than 2 m across, the wreckage of other forms of ice.

## (5) Ice of land origin

- (a) **Glacier ice:** Ice in, or originating from, a glacier, whether on land or floating on the sea as *icebergs*, *bergy bits* or *growlers*.
- (b) **Ice shelf:** A *floating ice* sheet of considerable thickness showing 2-50 m or more above sea-level, attached to the coast. Usually of great horizontal extent and with a level or gently undulating surface, nourished by annual snow accumulation and often also by the seaward extension of land glaciers. Limited areas may be aground. The seaward edge is termed an ice front.
- (c) **Calved ice of land origin:**

Iceberg: A massive piece of ice of greatly varying shape, protruding more than 5 m above sea-level, which has broken away from a glacier or ice shelf, and which may be afloat or aground. *Icebergs* may be described as tabular, dome-shaped, sloping, pinnacled, weathered or glacier bergs.

Bergy bit: A large piece of floating *glacier ice*, generally showing less than 5 m above sea-level but more than 1 m and normally about 100-300 m<sup>2</sup> in area.

Growler: Piece of ice smaller than a *bergy bit* and floating less than 1 m above the sea surface, a *growler* generally appears white but sometimes transparent or blue-green in color. Extending less than 1 m above sea surface and normally occupying an area of about 20 m<sup>2</sup>, *growlers* are difficult to distinguish when surrounded by sea ice or in high sea state.

### 10.2.2 Terms related to occurrence of sea ice

#### (1) Concentration

The ratio expressed in tenths describing the amount of the sea surface covered by ice as a fraction of the whole area being considered. Total *concentration* includes all stages of development that are present, partial *concentration* may refer to the amount of a particular stage or of a particular form of ice and represents only a part of the total.

- (a) **Compact ice:** *Floating ice* in which the *concentration* is 10/10 and no water is visible.
- (b) **Very close ice:** *Floating ice* in which the *concentration* is 9/10 to less than 10/10.
- (c) **Close ice:** *Floating ice* in which the *concentration* is 7/10 to 8/10, composed of *floes* mostly in contact.
- (d) **Open ice:** *Floating ice* in which the *concentration* is 4/10 to 6/10, with many *leads* and

*polynyas*, and the *floes* are generally not in contact with one another.

- (e) **Very open ice:** *Floating ice* in which the *concentration* is 1/10 to 3/10, and water preponderates over ice.
- (f) **Open water:** A large area of freely navigable water in which sea ice is present in *concentrations* less than 1/10. No *ice of land origin* is present.

## (2) Arrangement

- (a) **Ice field:** Area of *floating ice* consisting of any size of *floes*, which is greater than 10 km across.

Ice patch: An area of *floating ice* less than 10 km across.

- (b) **Strip:** Long narrow area of *floating ice*, about 1 km or less in width, usually composed of small fragments detached from the main mass of ice, and run together under the influence of wind, swell or current.
- (c) **Ice edge:** The demarcation at any given time between the open sea and sea ice of any kind, whether fast or drifting. It may be termed compacted or diffuse.
- (d) **Ice boundary:** The demarcation at any given time between *fast ice* and *drift ice* or between areas of *drift ice* of different *concentrations*.

## (3) Openings in the ice

- (a) **Fracture:** Any break or rupture through *very close ice*, *compact ice*, consolidated ice, *fast ice* or a single *floe* resulting from deformation processes. *Fractures* may contain *brash ice* and/or be covered with *nilas* and/or *young ice*. Length may vary from a few meters to many kilometers.
- (b) **Lead:** Any *fracture* or passage-way through sea ice navigable by surface vessels.
- (c) **Polynya:** Any non-linear shaped opening enclosed in ice. *Polynyas* may contain *brash ice* and/or be covered with *new ice*, *nilas* or *young ice*.

Shore polynya: A *polynya* between *drift ice* and the coast or *drift ice* and an ice front.

Flaw polynya: A *polynya* between *drift ice* and *fast ice*.

Recurring polynya: A *polynya*, which recurs in the same position every year.

## CHAPTER 11 Observation of Ice Accretion

Ice accretion (icing) is growth in size, particularly by accumulation, of ice formed on the ship's exposed surfaces. It can cause radio and radar failures due to the icing of aerials and sometimes cause a rollover due to the weight of ice. Observation of ice accretion is important for safe navigation.

### 11.1 Ice accretion on ship

Three main causes of ice accretion include

- 1) Spray and sea water thrown up by interaction between the ship and waves, and/or spray blown from the crests of waves
- 2) Freezing rain, drizzle or fog
- 3) Wet snow followed by a drop in temperature

Of the above, 1) poses the greatest concern for ships.

Studies on the meteorological effects on ice accretion in the western North Pacific have revealed conditions to consider as warning signs:

In sea surface temperature below 4°C, ice accretion starts with air temperature below - 3°C and wind speed over 8 m/s. Strong ice accretion (over 2 cm/hour) occurs with air temperature below - 6°C and wind speed over 10m/s. In sea surface temperature below 2°C, ice accretion starts with air temperature below - 2°C. The relation of air temperature and wind speed to ice accretion is shown in Fig. 11.1. With air temperature below - 17°C, spray freezes before it is thrown at the ship so the rate of ice accretion reduces. If navigating in sea surface temperature below 4°C and low air temperature and strong wind are expected, be careful of ice accretion on ship.

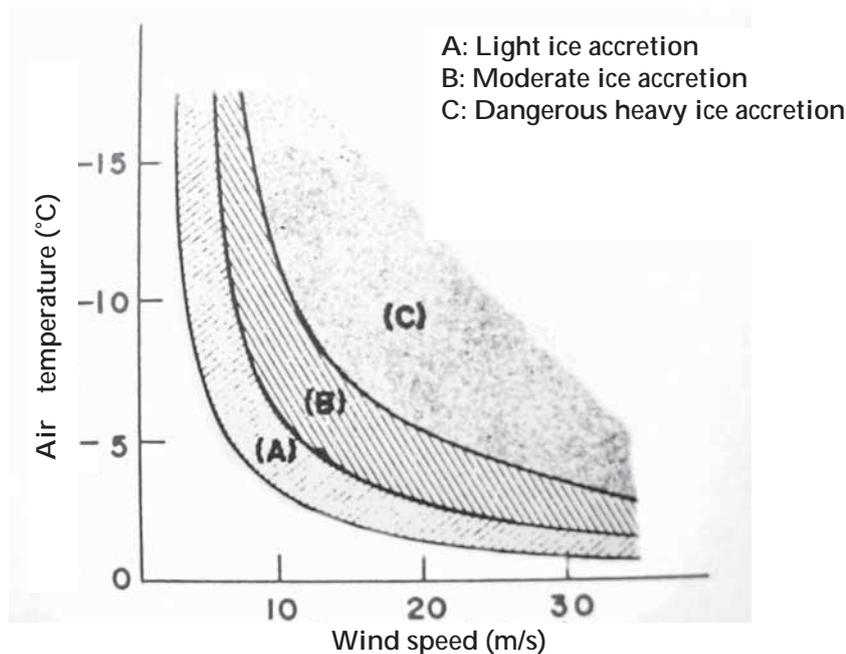


Fig. 11.1 General relation among air temperature, wind speed, and ice accretion  
Based on Figure 3 (b) of Sawada (1979)\*

## 11.2 Coding of observation

### 11.2.1 Causes of ice accretion on ships

Causes of ice accretion (referred to as "Is" in the Marine Meteorological Logbook) should be coded by one of five code figures (see Table 11.1 or "Ships' Weather Code Card" for details).

### 11.2.2 Thickness of ice accretion

Thickness of ice accretion (referred to as "EsEs") should be reported in centimeters. If there are different values of thickness on a ship, the maximum value should be reported.

### 11.2.3 Rate of ice accretion on ships

Find the description that best applies to the rate of ice accretion at observation time (referred to as "Rs") in Table 11.2 or "Ships' Weather Code Card".

Table 11.1 Ice accretion on ships code

Is	Ice accretion on ships
1	Icing from ocean spray
2	Icing from fog
3	Icing from ocean spray and fog
4	Icing from rain
5	Icing from ocean spray and rain

Table 11.2 Rate of ice accretion code

Rs	Rate of ice accretion
0	Ice not building up
1	Ice building up slowly
2	Ice building up rapidly
3	Ice melting or breaking up slowly
4	Ice melting or breaking up rapidly

\* T. Sawada (1975): Relation between the Rate of Ice Accretion on Ship and Meteorological Conditions. Journal of Meteorological Research, Vol.27, No.6, 229-236

## CHAPTER 12 Port Meteorological Officer (PMO)

The Port Meteorological Officer (PMO) is defined by WMO as the official of the Meteorological Service of a WMO Member who is stationed at a main seaport with the tasks of maintaining liaison with weather observers on board ships, checking instruments, providing advice, and contacting shipping authorities to enlist their cooperation in operating mobile ship stations (weather station aboard a moving ship). The PMO visits ships to encourage them to report weather and offers the following services free of charge:

- To check and calibrate instruments equipped on ships including barometers and give necessary advice.
- To provide instruction or assistance with regard to questions or inquiries on meteorology or oceanography, specifically on marine meteorological observation and its reporting.
- To explain such materials published by JMA as Marine Meteorological Logbook, Guide to Weather Observation for Ships, Ship's Weather Code Card, etc. If requested, the PMO will supply necessary copies.
- To inform the schedule of radio facsimile transmission from the meteorological services for ships and explain how to receive and utilize it.

There are three PMOs in Japan as shown below. They are in Yokohama, Nagoya and Kobe. Similar services are also available from Sapporo, Sendai, Osaka, Fukuoka and Okinawa Regional Headquarters of the Japan Meteorological Agency. The following E-mail address is common to all officers/offices.

### Port Meteorological Officers

Port Meteorological Officer, Yokohama Local Meteorological Office

99, Yamate-cho, Naka-ku, Yokohama 231 -0862, JAPAN

fax: +81-45-622-3520, E-mail: pmo@climar.kishou.go.jp

Port Meteorological Officer, Nagoya Local Meteorological Office

2-18, Hiyori-cho, Chikusa-ku, Nagoya 464 -0039, JAPAN

fax: +81-52-762-1242, E-mail: pmo@climar.kishou.go.jp

Port Meteorological Officer, Kobe Local Meteorological Office

1-4-3 Wakinohamakaigan-Dori, Chuo-ku, Kobe 651-0073, JAPAN

fax: +81-78-222-8946, E-mail: pmo@climar.kishou.go.jp

### Other Port Meteorological Service Offices

Climate and Marine Division, Sapporo Regional Headquarters, Japan Meteorological Agency

18-2 Kitanijo-nishi, Chuo-ku, Sapporo 060-0002, JAPAN

fax: +81-11-611-3206, E-mail: pmo@climar.kishou.go.jp

Climate and Marine Division, Sendai Regional Headquarters, Japan Meteorological Agency

1-3-15 Gorin, Miyagino-ku, Sendai 983-0842, JAPAN

fax: +81-22-291-8110, E-mail: pmo@climar.kishou.go.jp

Climate and Marine Division, Osaka Regional Headquarters, Japan Meteorological Agency

4-1-76 Otemae, Chuo-ku, Osaka 540-0008, JAPAN

fax: +81-6-6949-6160, E-mail: pmo@climar.kishou.go.jp

Climate and Marine Division, Fukuoka Regional Headquarters, Japan Meteorological Agency

1-2-36 Ohori, Chuo-ku, Fukuoka 810-0052 JAPAN

fax: +81-92-761-1726, E-mail: pmo@climar.kishou.go.jp

Climate and Marine Division, Okinawa Regional Headquarters, Japan Meteorological Agency

1-15-15 Higawa, Naha 900-8517, JAPAN

fax: +81-98-833-4292, E-mail: pmo@climar.kishou.go.jp

### Headquarters

Marine Division, Global Environment and Marine Department, Japan Meteorological Agency

1-3-4, Otemachi, Chiyoda-ku, Tokyo 100 -8122, JAPAN

fax: +81-3-3211-6908, E-mail: vos@climar.kishou.go.jp

To maintain the accuracy of barometers for meteorological observations, they should be checked every six months. If it is not possible for a ship to visit any of the above Japanese ports for a long time, barometers may be checked as follows:

- 1) Visit any foreign port in which a PMO is stationed to receive PMO service regardless of flag.
- 2) In Japanese ports other than above, barometers may be checked using email or facsimile. Please contact any Japanese PMO for details.

## Appendix 1

### List of supplies for ship weather observations and reports

JMA provides VOSs with the following materials free of charge:

### **Guide and tables for weather observation**

Guide to Weather Observations for Ships  
Guide to Ships' Weather Reports  
Ships' Weather Code Card  
Table for Finding the Dew-point  
JMA Cloud Plate  
Beaufort Scale of Wind Force

### **Materials for making weather observation reports**

OBSJMA (Software on CD-ROM) and writeable disks for data storage  
OBSJMA Operation Manual  
Ship's Weather Observation Field Note for OBSJMA  
Marine Meteorological Logbook  
Envelope to send logbooks, floppy disks or CD-Rs (postage free within Japan)

### **Brochures**

Marine Meteorological Observations and Port Meteorological Services  
Marine Meteorological Information Services for Shipping and Fishing

### **Bulletin**

The Ship and Maritime Meteorology

#### **Contact**

Marine Division  
Global Environment and Marine Department  
Japan Meteorological Agency (JMA)  
1-3-4, Otemachi, Chiyoda-ku, Tokyo 100-8122 Japan  
Facsimile No. +81 3 3211 6908  
E-mail vos@climar.kishou.go.jp

## Appendix 2

### Table for saturated vapor pressure

Table for saturated vapor pressure of water

(°C)	1/10 °C of temperature									
	0	1	2	3	4	5	6	7	8	9
	hPa	hPa	hPa	hPa	hPa	hPa	hPa	hPa	hPa	hPa
<b>0</b>	6.11	6.15	6.20	6.24	6.29	6.33	6.38	6.43	6.47	6.52
<b>1</b>	6.57	6.61	6.66	6.71	6.76	6.81	6.86	6.90	6.95	7.00
<b>2</b>	7.05	7.10	7.16	7.21	7.26	7.31	7.36	7.42	7.47	7.52
<b>3</b>	7.57	7.63	7.68	7.74	7.79	7.85	7.90	7.96	8.02	8.07
<b>4</b>	8.13	8.19	8.24	8.30	8.36	8.42	8.48	8.54	8.60	8.66
<b>5</b>	8.72	8.78	8.84	8.90	8.96	9.03	9.09	9.15	9.22	9.28
<b>6</b>	9.35	9.41	9.48	9.54	9.61	9.67	9.74	9.81	9.88	9.94
<b>7</b>	10.01	10.08	10.15	10.22	10.29	10.36	10.43	10.50	10.58	10.65
<b>8</b>	10.72	10.79	10.87	10.94	11.02	11.09	11.17	11.24	11.32	11.40
<b>9</b>	11.47	11.55	11.63	11.71	11.79	11.87	11.95	12.03	12.11	12.19
<b>10</b>	12.27	12.35	12.44	12.52	12.60	12.69	12.77	12.86	12.94	13.03
<b>11</b>	13.12	13.21	13.29	13.38	13.47	13.56	13.65	13.74	13.83	13.92
<b>12</b>	14.02	14.11	14.20	14.30	14.39	14.49	14.58	14.68	14.77	14.87
<b>13</b>	14.97	15.07	15.16	15.26	15.36	15.46	15.57	15.67	15.77	15.87
<b>14</b>	15.98	16.08	16.18	16.29	16.40	16.50	16.61	16.72	16.82	16.93
<b>15</b>	17.04	17.15	17.26	17.37	17.49	17.60	17.71	17.83	17.94	18.06
<b>16</b>	18.17	18.29	18.41	18.52	18.64	18.76	18.88	19.00	19.12	19.24
<b>17</b>	19.37	19.49	19.61	19.74	19.86	19.99	20.11	20.24	20.37	20.50
<b>18</b>	20.63	20.76	20.89	21.02	21.15	21.29	21.42	21.55	21.69	21.83
<b>19</b>	21.96	22.10	22.24	22.38	22.52	22.66	22.80	22.94	23.08	23.23
<b>20</b>	23.37	23.52	23.66	23.81	23.96	24.10	24.25	24.40	24.55	24.71
<b>21</b>	24.86	25.01	25.17	25.32	25.48	25.63	25.79	25.95	26.11	26.27
<b>22</b>	26.43	26.59	26.75	26.92	27.08	27.25	27.41	27.58	27.75	27.91
<b>23</b>	28.08	28.25	28.43	28.60	28.77	28.95	29.12	29.30	29.47	29.65
<b>24</b>	29.83	30.01	30.19	30.37	30.55	30.74	30.92	31.11	31.29	31.48
<b>25</b>	31.67	31.86	32.05	32.24	32.43	32.62	32.82	33.01	33.21	33.41
<b>26</b>	33.61	33.81	34.01	34.21	34.41	34.61	34.82	35.02	35.23	35.44
<b>27</b>	35.65	35.86	36.07	36.28	36.49	36.71	36.92	37.14	37.36	37.57
<b>28</b>	37.79	38.01	38.24	38.46	38.68	38.91	39.14	39.36	39.59	39.82
<b>29</b>	40.05	40.28	40.52	40.75	40.99	41.23	41.46	41.70	41.94	42.18
<b>30</b>	42.43	42.67	42.92	43.16	43.41	43.66	43.91	44.16	44.42	44.67
<b>31</b>	44.92	45.18	45.44	45.70	45.96	46.22	46.48	46.75	47.01	47.28
<b>32</b>	47.55	47.82	48.09	48.36	48.63	48.91	49.19	49.46	49.74	50.02
<b>33</b>	50.30	50.59	50.87	51.16	51.44	51.73	52.02	52.31	52.61	52.90
<b>34</b>	53.20	53.49	53.79	54.09	54.39	54.70	55.00	55.31	55.61	55.92
<b>35</b>	56.23	56.55	56.86	57.17	57.49	57.81	58.13	58.45	58.77	59.09
<b>36</b>	59.42	59.75	60.07	60.40	60.74	61.07	61.40	61.74	62.08	62.42
<b>37</b>	62.76	63.10	63.45	63.79	64.14	64.49	64.84	65.19	65.55	65.90
<b>38</b>	66.26	66.62	66.98	67.34	67.71	68.07	68.44	68.81	69.18	69.56
<b>39</b>	69.93	70.31	70.68	71.06	71.45	71.83	72.22	72.60	72.99	73.38
<b>40</b>	73.77	74.17	74.56	74.96	75.36	75.76	76.17	76.57	76.98	77.39
<b>41</b>	77.80	78.21	78.63	79.04	79.46	79.88	80.30	80.73	81.15	81.58
<b>42</b>	82.01	82.44	82.88	83.31	83.75	84.19	84.63	85.08	85.52	85.97
<b>43</b>	86.42	86.87	87.32	87.78	88.24	88.70	89.16	89.62	90.09	90.56
<b>44</b>	91.03	91.50	91.98	92.45	92.93	93.41	93.90	94.38	94.87	95.36
<b>45</b>	95.85	96.34	96.84	97.34	97.84	98.34	98.85	99.35	99.86	100.38
<b>46</b>	100.89	101.41	101.92	102.45	102.97	103.49	104.02	104.55	105.08	105.62
<b>47</b>	106.15	106.69	107.24	107.78	108.33	108.87	109.43	109.98	110.54	111.09
<b>48</b>	111.65	112.22	112.78	113.35	113.92	114.49	115.07	115.65	116.23	116.81
<b>49</b>	117.40	117.98	118.57	119.17	119.76	120.36	120.96	121.57	122.17	122.78
<b>50</b>	123.39	124.00	124.62	125.24	125.86	126.48	127.11	127.74	128.37	129.01

Table for saturated vapor pressure of supercooling water

(°C)	1/10 °C of temperature									
	0	1	2	3	4	5	6	7	8	9
	hPa	hPa	hPa	hPa	hPa	hPa	hPa	hPa	hPa	hPa
-50	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
-49	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.06
-48	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.07	0.07	0.07
-47	0.09	0.09	0.09	0.09	0.09	0.08	0.08	0.08	0.08	0.08
-46	0.10	0.10	0.10	0.10	0.10	0.09	0.09	0.09	0.09	0.09
-45	0.11	0.11	0.11	0.11	0.11	0.11	0.10	0.10	0.10	0.10
-44	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.11
-43	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.13	0.13	0.13
-42	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14
-41	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16	0.16
-40	0.19	0.19	0.19	0.18	0.18	0.18	0.18	0.18	0.17	0.17
-39	0.21	0.21	0.21	0.20	0.20	0.20	0.20	0.20	0.19	0.19
-38	0.23	0.23	0.23	0.23	0.22	0.22	0.22	0.22	0.21	0.21
-37	0.26	0.25	0.25	0.25	0.25	0.24	0.24	0.24	0.24	0.24
-36	0.28	0.28	0.28	0.28	0.27	0.27	0.27	0.27	0.26	0.26
-35	0.31	0.31	0.31	0.31	0.30	0.30	0.30	0.29	0.29	0.29
-34	0.35	0.34	0.34	0.34	0.33	0.33	0.33	0.32	0.32	0.32
-33	0.38	0.38	0.37	0.37	0.37	0.36	0.36	0.36	0.35	0.35
-32	0.42	0.42	0.41	0.41	0.41	0.40	0.40	0.39	0.39	0.39
-31	0.46	0.46	0.45	0.45	0.45	0.44	0.44	0.43	0.43	0.42
-30	0.51	0.50	0.50	0.49	0.49	0.49	0.48	0.48	0.47	0.47
-29	0.56	0.55	0.55	0.54	0.54	0.53	0.53	0.52	0.52	0.51
-28	0.61	0.61	0.60	0.60	0.59	0.59	0.58	0.58	0.57	0.56
-27	0.67	0.67	0.66	0.65	0.65	0.64	0.64	0.63	0.63	0.62
-26	0.74	0.73	0.72	0.72	0.71	0.70	0.70	0.69	0.69	0.68
-25	0.81	0.80	0.79	0.79	0.78	0.77	0.76	0.76	0.75	0.74
-24	0.88	0.88	0.87	0.86	0.85	0.84	0.84	0.83	0.82	0.81
-23	0.97	0.96	0.95	0.94	0.93	0.92	0.92	0.91	0.90	0.89
-22	1.05	1.04	1.04	1.03	1.02	1.01	1.00	0.99	0.98	0.97
-21	1.15	1.14	1.13	1.12	1.11	1.10	1.09	1.08	1.07	1.06
-20	1.25	1.24	1.23	1.22	1.21	1.20	1.19	1.18	1.17	1.16
-19	1.37	1.35	1.34	1.33	1.32	1.31	1.30	1.29	1.28	1.27
-18	1.49	1.48	1.46	1.45	1.44	1.43	1.41	1.40	1.39	1.38
-17	1.62	1.61	1.59	1.58	1.57	1.55	1.54	1.53	1.51	1.50
-16	1.76	1.75	1.73	1.72	1.70	1.69	1.67	1.66	1.65	1.63
-15	1.91	1.90	1.88	1.87	1.85	1.83	1.82	1.80	1.79	1.77
-14	2.08	2.06	2.04	2.03	2.01	1.99	1.98	1.96	1.94	1.93
-13	2.25	2.23	2.22	2.20	2.18	2.16	2.14	2.13	2.11	2.09
-12	2.44	2.42	2.40	2.38	2.36	2.34	2.33	2.31	2.29	2.27
-11	2.64	2.62	2.60	2.58	2.56	2.54	2.52	2.50	2.48	2.46
-10	2.86	2.84	2.82	2.80	2.77	2.75	2.73	2.71	2.69	2.67
-9	3.10	3.07	3.05	3.03	3.00	2.98	2.95	2.93	2.91	2.89
-8	3.35	3.32	3.30	3.27	3.25	3.22	3.20	3.17	3.15	3.12
-7	3.62	3.59	3.56	3.53	3.51	3.48	3.45	3.43	3.40	3.37
-6	3.91	3.88	3.85	3.82	3.79	3.76	3.73	3.70	3.67	3.65
-5	4.21	4.18	4.15	4.12	4.09	4.06	4.03	4.00	3.97	3.94
-4	4.54	4.51	4.48	4.44	4.41	4.38	4.34	4.31	4.28	4.25
-3	4.90	4.86	4.83	4.79	4.75	4.72	4.68	4.65	4.61	4.58
-2	5.28	5.24	5.20	5.16	5.12	5.08	5.05	5.01	4.97	4.93
-1	5.68	5.64	5.60	5.55	5.51	5.47	5.43	5.39	5.35	5.31
0	6.11	6.06	6.02	5.98	5.93	5.89	5.85	5.80	5.76	5.72

Table for saturated vapor pressure of ice

(°C)	1/10 °C of temperature									
	0	1	2	3	4	5	6	7	8	9
	hPa	hPa	hPa	hPa	hPa	hPa	hPa	hPa	hPa	hPa
- 0	6.11	6.06	6.01	5.96	5.91	5.86	5.81	5.76	5.72	5.67
- 1	5.62	5.58	5.53	5.48	5.44	5.39	5.35	5.30	5.26	5.22
- 2	5.17	5.13	5.09	5.04	5.00	4.96	4.92	4.88	4.84	4.80
- 3	4.76	4.72	4.68	4.64	4.60	4.56	4.52	4.48	4.45	4.41
- 4	4.37	4.33	4.30	4.26	4.23	4.19	4.15	4.12	4.08	4.05
- 5	4.01	3.98	3.95	3.91	3.88	3.85	3.81	3.78	3.75	3.72
- 6	3.68	3.65	3.62	3.59	3.56	3.53	3.50	3.47	3.44	3.41
- 7	3.38	3.35	3.32	3.29	3.26	3.24	3.21	3.18	3.15	3.12
- 8	3.10	3.07	3.04	3.02	2.99	2.96	2.94	2.91	2.89	2.86
- 9	2.84	2.81	2.79	2.76	2.74	2.71	2.69	2.67	2.64	2.62
-10	2.60	2.57	2.55	2.53	2.51	2.48	2.46	2.44	2.42	2.40
-11	2.38	2.35	2.33	2.31	2.29	2.27	2.25	2.23	2.21	2.19
-12	2.17	2.15	2.13	2.11	2.09	2.08	2.06	2.04	2.02	2.00
-13	1.98	1.97	1.95	1.93	1.91	1.90	1.88	1.86	1.84	1.83
-14	1.81	1.79	1.78	1.76	1.75	1.73	1.71	1.70	1.68	1.67
-15	1.65	1.64	1.62	1.61	1.59	1.58	1.56	1.55	1.53	1.52
-16	1.51	1.49	1.48	1.46	1.45	1.44	1.42	1.41	1.40	1.38
-17	1.37	1.36	1.35	1.33	1.32	1.31	1.30	1.28	1.27	1.26
-18	1.25	1.24	1.23	1.21	1.20	1.19	1.18	1.17	1.16	1.15
-19	1.14	1.12	1.11	1.10	1.09	1.08	1.07	1.06	1.05	1.04
-20	1.03	1.02	1.01	1.00	0.99	0.98	0.97	0.96	0.96	0.95
-21	0.94	0.93	0.92	0.91	0.90	0.89	0.88	0.88	0.87	0.86
-22	0.85	0.84	0.83	0.83	0.82	0.81	0.80	0.79	0.79	0.78
-23	0.77	0.76	0.76	0.75	0.74	0.73	0.73	0.72	0.71	0.71
-24	0.70	0.69	0.69	0.68	0.67	0.67	0.66	0.65	0.65	0.64
-25	0.63	0.63	0.62	0.61	0.61	0.60	0.60	0.59	0.58	0.58
-26	0.57	0.57	0.56	0.56	0.55	0.54	0.54	0.53	0.53	0.52
-27	0.52	0.51	0.51	0.50	0.50	0.49	0.49	0.48	0.48	0.47
-28	0.47	0.46	0.46	0.45	0.45	0.44	0.44	0.43	0.43	0.43
-29	0.42	0.42	0.41	0.41	0.40	0.40	0.40	0.39	0.39	0.38
-30	0.38	0.38	0.37	0.37	0.36	0.36	0.36	0.35	0.35	0.35
-31	0.34	0.34	0.34	0.33	0.33	0.33	0.32	0.32	0.31	0.31
-32	0.31	0.31	0.30	0.30	0.30	0.29	0.29	0.29	0.28	0.28
-33	0.28	0.27	0.27	0.27	0.27	0.26	0.26	0.26	0.25	0.25
-34	0.25	0.25	0.24	0.24	0.24	0.24	0.23	0.23	0.23	0.23
-35	0.22	0.22	0.22	0.22	0.21	0.21	0.21	0.21	0.20	0.20
-36	0.20	0.20	0.20	0.19	0.19	0.19	0.19	0.19	0.18	0.18
-37	0.18	0.18	0.18	0.17	0.17	0.17	0.17	0.17	0.16	0.16
-38	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15	0.15	0.15
-39	0.14	0.14	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.13
-40	0.13	0.13	0.13	0.12	0.12	0.12	0.12	0.12	0.12	0.12
-41	0.12	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.10	0.10
-42	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.09	0.09	0.09
-43	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.08	0.08	0.08
-44	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.07	0.07
-45	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
-46	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
-47	0.06	0.06	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.05
-48	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
-49	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
-50	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04

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## Sea Ice

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### 1. Introduction

Sea ice is any form of ice floating at sea which has originated from the freezing of seawater. Ice found at sea also contains ice of land origin, such as icebergs, lake ice which has formed on a lake, and river ice which has formed on a river. All these kinds of ice floating in water are called floating ice. Almost all the floating ice found in the vicinity of Japan is sea ice. The ice existing in the sea ice area is divided into fast ice (which is attached to the shore or an ice wall) and the remaining drift ice. Since sea ice reflects a higher fraction of solar radiation than the surrounding seawater and significantly reduces the exchange of heat and materials between the atmosphere and ocean, it plays an important role in shaping the polar climate. Therefore, the long-term standardized observation of sea ice provides important information on climate change in polar regions.

Sea ice observations can be conducted from the seashore on land, navigating ships, aircrafts, or satellite remote sensing. In this chapter we will describe the method and style of ship-based observations, focusing on the internationally standardized ship-based visual observation in section 2. The description includes an observation protocol designed for the Antarctic sea ice in sections 2-2 and 2-3, and one for Arctic sea ice in section 2-4. Nowadays, the data observed according to these protocols in various sea ice regions on the earth are being archived in Australia. Some results obtained from this observation method will be shown in section 2-5. In addition, visual observations from the seashore observatories on land have been continued for more than a century, and this dataset provides an important baseline for studies of climate change. Therefore, we also describe the present style of observation conducted by meteorological observatories by citing the observation guidelines of the Japan Meteorological Agency in section 3.

The classification of sea ice used in the standardized observation protocol is based on the sea ice nomenclature published by the World Meteorological Organization (WMO) in 1970. Therefore, all the nomenclature of WMO will be listed in section 4.

### 2. Ship based observation

#### *2-1 General description*

The ship-based sea ice observation is conducted from the ship by observers with a good knowledge of sea ice nomenclature, at fixed time intervals, to record the ice conditions around the ship while the ship is navigating in the sea ice area. Ice conditions such as ice concentration, floe size, and thicknesses of sea ice and snow are recorded according to the internationally standardized observation protocol. These data not only contribute to the safe navigation by providing an overview of the ice conditions along the ship, but also can be used as a validation tool for satellite remote sensing data. Besides, the statistics of the observational records for a long-term period provides useful information both for the intercomparison of the ice conditions between different sea ice regions and as a basic

dataset of climate change.

For Antarctic sea ice, the standardized ship-based observation protocol was defined by the ASPeCt (Antarctic Sea Ice Processes and Climate) program established by SCAR (Scientific Committee on Antarctic Research) in 1997 (<http://aspect.antarctica.gov.au/>). One of the important purposes of this program is to compile past records of the ice conditions observed in different formats in various Antarctic sea ice regions as well as to provide a guideline for ship-based ice observation. The observation protocol is based on the sea ice nomenclature published in 1970 by WMO (World Meteorological Organization) (see section 4), and requires the observers to record the ice thickness, floe size, surface topography, and the type and thickness of snow for the individual ice types classified. The observation manual is described by Worby and Allison (1999), and the relevant part of this manual which is essential to the observation is cited here in sections 2-2 and 2-3.

In this protocol, much attention is paid to the estimation of mean ice thickness including ridged ice. The observer is basically required to observe the thickness for level ice, and the thickness of ridged ice is estimated with the level ice thickness and the areal fraction and sail height of the ridge using a simple ridge model. The mean ice thickness is calculated by areally-weighted thicknesses of level ice and ridged ice (section 2-3-3). Another way to apply the results to climate research is to estimate the integrated albedo of the total sea ice region containing various types of sea ice by summing up the albedo prescribed for each ice type (section 2-3-4). Since first-year ice often takes common features in form and arrangement irrespective of the regions, it is expected that this observation protocol is applicable in other seasonal ice zones as well as in the Southern Ocean. The simplified version of the ASPeCt protocol for the Japanese Antarctic Research Expedition (JARE) is proposed by Ohshima et al. (2006).

On the other hand, for Arctic sea ice, as may be expected with a longer history of sea ice observation compared with Antarctic sea ice, ice conditions have been recorded in different formats by different organizations so far. Therefore, it was not easy to set the internationally standardized observation protocol (Worby and Eicken, 2009). However, recently with the rapid change of the ice conditions in the Arctic Ocean, the expectation for establishing a network of the in-situ observation and the interest in standardizing the observation method have been increasing. Taking this opportunity, the Climate and Cryosphere (CliC) Arctic Sea Ice Working Group was implemented in 2008 under the auspices of the World Climate Research Programme (WCRP), and has been making every effort to define an internationally standardized observation protocol for Arctic sea ice. While this protocol is based on the ASPeCt protocol designed for Antarctic sea ice, some observation parameters are added to apply to Arctic sea ice.

The major differences between Arctic and Antarctic sea ice are the surface conditions during the melting season and the concentration of inclusions such as sediments and ice algae within sea ice. In the Arctic, surface melting dominates (unlike the Antarctic sea ice), and a number of melt ponds develop on the surface in the melting season. Since melt ponds evolve with time and affect the surface heat budget significantly, additional codes are set for the observation of melt ponds. Reflecting the fact that sea ice often grows on widespread shallow continental shelves in the Arctic Ocean, it sometimes contains highly concentrated sediments. To record such conditions, observation codes relevant to

sediments are also added for Arctic sea ice. Another feature for the Arctic protocol is that it includes observation items relevant to marine creatures. Refer to the Website (<http://www.iarc.uaf.edu/icewatch>) for more details of the observation codes.

As supplemental information for ship-based sea ice observations, ship-borne electromagnetic induction sounding has been used by many icebreakers to monitor the ice thickness distribution along the ship track since the late 1990's. This device is composed of transmitter and receiver coils, which are set apart by a few meters, and is suspended at a distance of few meters from the ship during the navigation to avoid the effect of the ship (Fig.1). The transmitter generates a primary electromagnetic field which induces eddy currents just below the ice bottom, because the conductivity of sea ice ( $0\text{--}50\text{ mS m}^{-1}$ ) is negligible compared to that of seawater ( $2500\text{ mS m}^{-1}$ ). In turn the induced currents generate a secondary electromagnetic field. Then the distance to the ice bottom is calculated from the strength of the secondary electromagnetic field sensed by the receiver coil. If we mount a laser profilometer on the instrument and measure the height of the instrument above the combined snow and ice surface, ice thickness can be obtained by subtracting it from the EM results. The merit of this method is that ice thickness data can be obtained irrespective of level ice or ridged ice. Although the observational bias caused by taking the thinner route should sometimes be taken into account, this method has allowed us to obtain the ice thickness distribution quantitatively in a wide region while navigating. Refer to Haas (1998) and Uto et al. (2006) for details of this technique applied to the Southern Ocean and the Sea of Okhotsk, respectively. If the ice thickness monitoring by this method is continued along regular shipping routes, it is expected that these data will form a useful dataset for monitoring climate change.

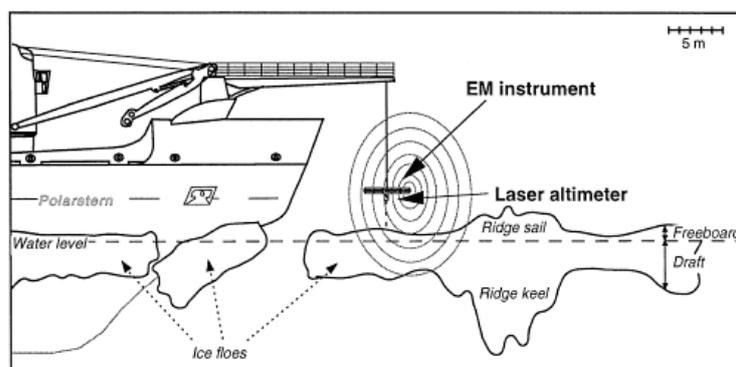


Fig. 1. Schematic picture showing the EM sensor suspended from the German ice breaker "Polarstern" (cited from Haas (1998))

## 2-2. Observational technique for Antarctic sea ice (cited from Worby and Allison (1999))

(Remarks by the author are shown in *Italics*.)

A standard set of observations are made hourly by an observer on the ship's bridge. These include the ship's position and total ice concentration, and an estimate of the areal coverage, thickness, floe size, topography and snow cover of the three dominant ice thickness categories within a radius of approximately 1 km of the ship. The three dominant ice categories are defined as those with the

greatest areal concentration, and the thickest of these is defined as the primary ice type. There may be times when only one or two different ice categories are present in which case only the primary, or primary and secondary, classifications are defined. The observations are entered on log sheets using a standard set of codes based on the WMO (1970) nomenclature and designed exclusively for Antarctic sea ice. A set of blank proformas is located in Table 1 and observation codes are shown in Table 2.

### *2-2-1 Ice Concentration (c)*

Total ice concentration is an estimate of the total area covered by all types of ice, expressed in tenths, and entered as an integer between 0 and 10. In regions of very high ice concentration (95-99%) where only very small cracks are present, the recorded value should be 10 and the open water classification should be 1 (small cracks). Regions of complete ice cover (100%) will be distinguished by recording an open water classification of 0 (no openings). An estimate of the concentration of each of the three dominant ice thickness categories is also made. These values are also expressed in tenths and should sum to the value of the total ice concentration. It is sometimes difficult to divide the pack into three distinct categories, and it may be necessary to group some categories together to ensure their representation.

### *2-2-2 Ice Type (ty)*

The different ice categories, together with the codes used to record the observations, are shown in Table 2.1. The ice categories are based on the [WMO, 1970] sea ice classifications. First year ice greater than approximately 0.1 m thick is classified by its thickness (e.g., young grey ice 0.1-0.15 m; first year ice 0.7-1.2 m), while thinner ice is generally classified by type (e.g., frazil, shuga, grease and nilas). A single category is defined for multi-year ice. There is also a category for brash, which is common between floes in areas affected by swell and where pressure ridging has collapsed.

### *2-2-3 Ice Thickness (z)*

Ice thickness is estimated for each of the three dominant ice types. It is helpful to the observer to suspend an inflatable buoy of known diameter (or other gauge) over the side of the ship, approximately 1 m above the ice, to provide a scaled reference against which floe thickness can be estimated. The ice thickness can then be determined quite accurately as floes turn sideways along the ship's hull (Fig.2). Only the thickness of level floes, or the level ice between ridges, is estimated. This is because ridges tend to break apart into their component blocks when hit by the ship, making it impossible to estimate their thickness. In order to determine the thickness of ridged floes, observations of the areal extent and mean sail height of the ridges are made (see Section 2-2-5) and combined with the level ice thickness data into a simple model (see Section



Fig. 2. A sample of floes turning sideways along the ship's hull.

2-3-3).

Thinner, snow-free ice categories, which are particularly important for ocean-atmosphere heat exchange, can be reliably classified by a trained observer from their apparent albedo, while the thickness of very thick floes may be estimated by their freeboard. The accuracy of careful observations will be within 10-20% of the actual thickness, and a large sample of observations can be expected to provide a good statistical description of the characteristics of the pack. This is particularly true at the thin end of the thickness distribution where changes are most important for both radiant and turbulent heat transfer (e.g., Worby and Allison, 1991).

On dedicated scientific voyages, it is usually possible to make regular *in situ* measurements of ice and snow thickness, both on level ice and across ridges, to “calibrate” the ship-based observations. Worby et al. (1996) demonstrated a technique for combining *in situ* and ship-based observations to estimate the ice thickness distribution in the Bellingshausen Sea. Dedicated scientific voyages also usually provide the opportunity to follow specific routes to optimise data quality, which may be compromised if the ship follows the most easily navigable routes. It is the observer’s responsibility to clearly indicate on the observation sheet when the ship is preferentially following leads so that this may be considered during data processing.

#### **2-2-4 Floe size (f)**

Floe size can be difficult to determine because it is not always clear where the boundary of a floe is located. Cracks and leads delineate floe boundaries whereas ridges do not. Where smaller floes have been cemented together to form larger floes, the larger dimension is recorded, but usually with a comment to indicate that smaller floes are visible. Where two floes have converged and ridged, the floe size is taken as the combined size of the two. A good rule of thumb is : if you could walk from point A to point B, then both points are on the same floe. This guide can be helpful when trying to determine floe size. The length of the ship (about 100 m for most ice breakers) can act as a good guide for estimating floe size. The ship’s radar can be useful for determining the size of very large floes.

Floe size is recorded using a code between 100 and 700. New sheet ice (code 200) is normally used for nilas. This code does not specify a floe size, but is a descriptor for refrozen leads and polynyas. It is often used in conjunction with topography codes 100 (level ice) and 400 (finger rafting).

#### **2-2-5 Topography (t)**

As discussed above, the ice thickness estimates are only made of the level ice in a floe. This is because the thickness of ridges can not reliably be estimated from a ship, since they tend to break up into their component blocks when hit by the ship, rather than turning sideways so that their thickness can be estimated. However, drilled transects across ridged ice floes indicate that the mass of ice in ridges is a major contributor to the total ice mass of the pack, hence it is important to quantify the extent of ridging within the pack. To do this, the areal extent and mean sail height of ridges is recorded for each ice type within the pack. The extent of surface ridging is estimated to the nearest 10%. It is important

that observers not look too far from the ship when estimating the areal extent of ridges, otherwise only the ridge peaks are seen and not the level ice between them. This gives a false impression of more heavily deformed ice than is actually present. The mean sail height is estimated to the nearest half metre below 2 m, and to the nearest metre above 2 m. It is important to remember that it is the *mean* sail height that is recorded. This can be difficult to estimate, particularly in flat light when the sky is overcast. Our experience has shown that ridge height is generally underestimated due to the vertical perspective from the bridge.

Ridges are classified using a three digit code between 500 and 897. The first digit (5-8) is a description of the type of ridge, which may be unconsolidated, consolidated or weathered. This is determined from the appearance of the ridge and is useful for estimating ridge sail density. The second digit (0-9) describes the areal coverage of ridges, and the third digit (0-7) records the mean sail height to the nearest 0.5 m. These observations are probably the most subjective of those made from the ship, and it is particularly important to standardise them between observers.

The observations of surface ridging are input to a model formulation as described in section 2-3-3, to estimate the mass of ice in ridges.

#### **2-2-6 Snow type (s)**

This is a descriptor for the state of the snow cover on sea ice floes. It is important for estimating the area-averaged albedo of the pack as discussed in Section 2-3-4. The snow classification is an integer between 0 and 10. For accurate surface albedo calculations, the snow cover classification describes the surface snow. Hence, in a case where fresh snow has fallen over older wind-packed snow, the classification code should describe the freshly fallen snow cover. However, it is very important that the total snow cover thickness is still recorded.

#### **2-2-7 Snow thickness (sz)**

An estimate of the snow cover thickness is made for each of the three dominant ice thickness categories. Snow thickness is relatively straight forward to estimate for floes turned sideways along the ship's hull, although at times the ice/snow interface is difficult to distinguish, particularly when the base of the snow layer has been flooded and snow-ice has formed.

#### **2-2-8 Open water (o/w)**

The codes for open water are descriptors for the size of the cracks or leads between floes, not a concentration value (in tenths). As discussed above, the length and breadth of the ship can act as a useful guide when estimating lead dimensions. The ship's radar can also be useful, particularly at night.

#### **2-2-9 Meteorological Observations**

Instantaneous conditions are usually recorded hourly, but this may be reduced to three hourly. The standard set of observations include water temperature, air temperature, true wind speed and direction, total cloud cover, visibility, and current weather. On most research vessels, water temperature, air temperature and wind speed and direction will be displayed on the bridge, and may even be logged for

the duration of the voyage. Cloud cover can be estimated by the observer in eighths, and visibility is estimated in kilometres from the ship. Wind speed is recorded in  $\text{ms}^{-1}$  and wind direction relative to north ( $^{\circ}\text{T}$ ). The current weather is recorded using the Australian meteorological observer's two digit codes that are provided in Table 4.

#### 2-2-10 Photographic Records

During daylight hours a photographic record of ice conditions can be kept. Slides are usually taken from the bridge at the time of each observation, and the log book has a column for recording film and frame numbers. There is also scope for recording the frame number for a time lapse video recorder which the authors have mounted on the ship's rail. This captures a single video frame every 8 seconds, providing a comprehensive visual record of ice conditions on a single video tape for each 30 day period. This photographic archive is not generally used for quantitative analyses, but provides an excellent reference that can be used in conjunction with the ship-based observations. At night the camera is angled closer to the ship to view an area that can be adequately lit by flood lights mounted on the ship's rail.

*\*Note: Taking three photos at the fixed observation time from the bridge or the upper bridge of the ship is recommended to record ice conditions, as shown in Fig.3.*



Fig. 3 Sample photos taken from the upper bridge of P/V “Soya”  
in the southern Sea of Okhotsk (From left to right, portside, front, and starboard)

#### 2-2-11 Comments

In addition to the hourly observations entered by code, there is scope for additional comments to be recorded. These usually include a brief description of the characteristics of the pack, in particular features which are not covered by the observation codes, such as frost flowers on dark nilas or swell penetrating the pack. Brief details of sampling sites, buoy deployments or other ‘on ice’ activities may also be recorded and, if necessary, a comment on how typical the ice along the ship's route is of the surrounding region.

Table 1. An observation sheet for ASPeCt (1)

Day/Date (Z):		SEA ICE OBSERVATIONS																		
POSITION		SEA ICE OBSERVATIONS																		
hr (Z)	Lat (°S) dd mm	Long (°E/W) ddd mm	Conc. (tenths)	PRIMARY			SECONDARY			TERTIARY			OW	hr (Z)						
				c	ty	z	f	t	s	sz	c	ty	z	f	t	s	sz			
0																			0	
1																			1	
2																			2	
3																			3	
4																			4	
5																			5	
6																			6	
7																			7	
8																			8	
9																			9	
10																			10	
11																			11	
12																			12	
13																			13	
14																			14	
15																			15	
16																			16	
17																			17	
18																			18	
19																			19	
20																			20	
21																			21	
22																			22	
23																			23	

NOTES:  
 \* PRIMARY SEA ICE IS OF GREATEST THICKNESS. HENCE  $t_1 > t_2 > t_3$

Table 1. An observation sheet for ASPeCt (2)

Day/Date (Z):													
METEOROLOGICAL OBSERVATIONS							PHOTO	VIDEO	COMMENTS			OBSERVER	
hr	T <sub>water</sub> (Z) (°C)	T <sub>air</sub> (°C)	Wind (sp/d)	Cloud (oktas)	Visib (v)	Weath (ww)	Film/ Frame	Tape No./ Reading	Text	Ref. no.	Name	hr (Z)	
0												0	
1												1	
2												2	
3												3	
4												4	
5												5	
6												6	
7												7	
8												8	
9												9	
10												10	
11												11	
12												12	
13												13	
14												14	
15												15	
16												16	
17												17	
18												18	
19												19	
20												20	
21												21	
22												22	
23												23	

NOTES:  
\* ADDITIONAL COMMENTS MAY BE MADE ON THE FOLLOWING PAGE. PROVIDING DUE REFERENCE IS GIVEN

Table 2. Observation codes for ASPeCt

<b>ICE TYPE (ty)</b>	<b>FLOE SIZE (D)</b>	<b>TOPOGRAPHY (t)</b>	<b>SNOW TYPE (s)</b>
10 Frazil	100 Pancakes	100 Level ice	0 No snow observation
11 Shuga	200 New sheet ice	200 Rafterd pancakes	1 No snow, no ice or brash
12 Grease	300 Brash/broken ice	300 Cemented pancakes	2 Cold new snow, <1 day old
20 Nilas	400 Cake ice, <20 m	400 Finger rafting	3 Cold old snow
30 Pancakes	500 Small floes, 20-100 m	5xy New, unconsolidated ridges (no snow)	4 Cold wind-packed snow
40 Young grey ice, 0.1-0.15 m	600 Medium floes, 100-500 m	6xy New ridges filled with snow or a snow cover	5 New melting snow (wet new snow)
50 Young grey-white ice, 0.15-0.3 m	700 Large floes, 500-2000 m	7xy Consolidated ridges (no weathering)	6 Old melting snow
60 First year, 0.3-0.7 m	800 Vast floes, >2000 m	8xy Older, weathered ridges	7 Glaze
70 First year, 0.7-1.2 m		x values: 0 0-10% areal coverage	8 Melt slush
80 First year, >1.2 m		1 10-20%	9 Melt puddles
85 Multiyear floes		2 20-30%	10 Saturated snow (waves)
90 Brash		3 30-40%	11 Sastrugi
95 Fast ice		4 40-50%	
		5 50-60%	
		6 60-70%	
		7 70-80%	
		8 80-90%	
		9 90-100%	
		y values: 1 0.5 m av. sail height	
		2 1.0 m	
		3 1.5 m	
		4 2.0 m	
		5 3.0 m	
		6 4.0 m	
		7 5.0 m	
<b>ICE CONC<sup>n</sup> (c)</b> to be expressed in tenths		<b>OPEN WATER</b>	0 No openings
		1 Small cracks	1 Very narrow breaks, <50m
		2 Very narrow breaks, <50m	2 Narrow breaks, 50-200 m
		3 Narrow breaks, 50-200 m	3 Wide breaks, 200-500 m
		4 Wide breaks, 200-500 m	4 Very wide breaks, >500 m
		5 Very wide breaks, >500 m	5 Lead/coastal lead
		6 Lead/coastal lead	6 Polyalya/coastal polylynya
		7 Polyalya/coastal polylynya	7 Water broken only by small scattered floes
		8 Water broken only by small scattered floes	8 Open sea
		9 Open sea	
<b>SEA ICE (z) AND SNOW THICKNESS (sz)</b> to be expressed in centimetres			

## **2-3 Data entry and processing for Antarctic sea ice**

(cited from Worby and Allison (1999))

### **2-3-1 Quality control**

Checks are made to identify errors and inconsistencies in the data. These include, but are not limited to:

- snow thicker than ice
- thin ice types greater than 0.1 m thick
- total concentration greater than 10/10, or not adding up to the sum of the concentrations of the three dominant categories
- ice thickness categories not matching assigned thickness values
- topography or floe size codes incompatible with ice type (e.g., consolidated ridges on nilas)
- primary ice category thinner than secondary or tertiary categories
- distance between consecutive hourly observations greater than 20 km.

### **2-3-2 Editing data**

The data set may be edited to exclude observations within a prescribed distance of the previous observation. This is to prevent biasing in areas of heavy ice where the ship's speed is reduced. The distance is usually set to 6 nautical miles, corresponding to a straight line speed of 6 knots which most ice breakers are capable of maintaining in moderate pack ice. The processing software enables the user to specify this distance, or to use all observations regardless of spacing.

Observations are also removed when there is obvious biasing caused by the ship following easily navigable routes. The most common example of this is near the ice edge, when the ship may constantly pick its way through leads. This is usually avoidable on voyages dedicated to sea ice research, but may otherwise prove to be a problem. It is at the discretion of the observer to either note that the data may be biased, or not record data under such circumstances.

### **2-3-3 Estimating the area-averaged ice and snow thicknesses**

Estimates of the area-averaged ice and snow thicknesses may be made over the ice covered region of the pack only, or for the total pack ice zone including the open water fraction. Each observation is equally weighted unless eliminated by the minimum distance rule described above. For each hourly observation, the estimated ice thickness values for each of the three dominant ice thickness categories are weighted by the ice concentration. This provides a mean thickness of the level ice within the pack.

To account for the mass of ice in ridges, the observations described in section 2-2-5 are used in conjunction with a simple model to calculate a corrected mean floe thickness ( $Z_r$ ). The model takes the undeformed floe thickness ( $Z_u$ ), average sail height ( $S$ ) and an estimate of the areal extent of surface ridging ( $R$ ) as input parameters, and calculates the mean thickness of the floe ( $Z_r$ ), assuming a triangular sail, isostasy and a ratio of ice and snow above sea level to ice below sea level as 5:1. The assumption of a triangular sail cross section is consistent with the formulation of Hibler et al. (1974) for calculating the effective thickness of ridged ice. Their formulation used a fixed slope angle of 26°;

however the present study uses an implied variable slope angle which is dependent upon the areal coverage of ridges and the average sail height. In this way broader ridges are flatter which is consistent with the theory that ridges should build laterally once the limiting height is reached (Tucker and Govoni, 1981). The assumption of a triangular ridge is therefore not likely to induce large errors. Published literature on sea ice density is sparse; however Buynitskiy (1967) presented mean densities from East Antarctic sea ice for summer and winter ice of  $875 \text{ kg m}^{-3}$  and  $920 \text{ kg m}^{-3}$  respectively, and these are consistent with the value of  $900 \text{ kg m}^{-3}$  used in the model formulation. The assumption of hydrostatic equilibrium must also hold on the large scale; however the effect of snow drifts around ridges may induce errors in both the observations and the model. In particular, observers may not be able to differentiate ridge sails from adjoining snow drifts, hence the observations of the areal coverage (and to a lesser extent, height) or ridging will include the fraction covered by snow. This will affect the value  $r$  defined as the ratio of ice thickness below sea level to the combined thickness of ice and snow above sea level. Hence, the assumption that ridge sails are solid ice with a density of  $900 \text{ kg m}^{-3}$  is incorrect, and this is accounted for in the model.

To determine  $r$  in the vicinity of ridges, data from drilled thickness transects that intersected ridges were examined. Only transects, or parts thereof, with peaks in freeboard (the height of the snow/ice interface above the sea level)  $>0.5 \text{ m}$  were considered, and the mean ice and snow thicknesses were calculated. A total of 339 drill holes from 9 thickness transects had mean ice and snow thicknesses of  $1.18 \text{ m}$  and  $0.16 \text{ m}$  respectively. By assuming densities of  $900 \text{ kg m}^{-3}$  and  $360 \text{ kg m}^{-3}$  for ice and snow respectively the mean draft was calculated to be  $1.12 \text{ m}$ . Hence,  $r = 5$  in areas of ridged ice. The snow density value was derived from data collected on two voyages to the East Antarctic pack (V9 92/93 and V1 95/96), with a mean value of  $360 \pm 110 \text{ kg m}^{-3}$  over the range  $120\text{-}760 \text{ kg m}^{-3}$ .

In order to calculate only the thickness of ice in ridges it is necessary to remove the snow from the calculation. The ratio of ice below sea level : ice above,  $r'$ , is defined as :

$$r' = [1 - (0.16/1.18)] r = 4.3$$

based on the mean ice and snow thickness given above. The value  $r' = 4.3$  compares well with the value of 4 used by Dierking (1995), which was based on drilled transect measurements by Lange and Eicken (1991) and Wadhams et al. (1987).

The model formulation to calculate the average thickness of ridged floes ( $Z_r$ ) can now be written as :

$$Z_r = (r' + 1)(0.5RS) + Z_u$$

where  $R$  is the areal extent of surface ridging,  $S$  is the average sail height of ridges, and  $Z_u$  is the thickness of level (undeformed) ice in the floe (Fig.4).

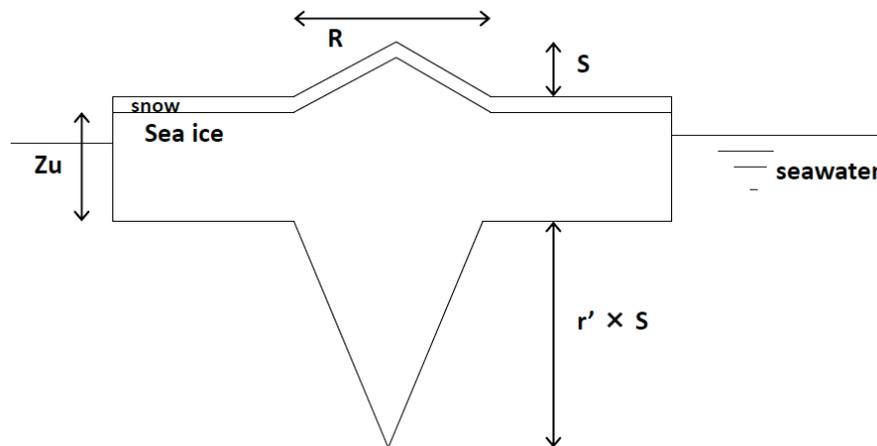


Fig. 4 A simple ridge model for estimating the thickness of ridged ice

#### 2-3-4 Calculating area-averaged albedo

The area-averaged albedo is computed from the ice concentration and allwave albedo for each ice type. The allwave albedo values for different ice and snow thickness categories were originally taken from Allison et al. (1993); however these have recently been updated (Brandt et al., 2005; Table 3). The average albedo is calculated over the entire pack, including the open water fraction. A value is calculated at each hourly observation site, from which zonal averages may also be calculated.

Table 3. Representative all-wave solar albedos of surface types in the East Antarctic sea ice zone in spring and summer. Values in bold are derived from measurements; all others were estimates by interpolation or extrapolation. SON: September to November, DJF: December to February.

(cited from Brandt et al. (2005))

Ice type	Ice thickness (cm)	No snow		Thin snow (<3 cm)				Thick snow (>3 cm)			
				Clear		Cloudy		Clear		Cloudy	
		Clear	Cloudy	SON	DJF	SON	DJF	SON	DJF	SON	DJF
Open water	0	<b>0.07</b>	<b>0.07</b>	—	—	—	—	—	—	—	—
Grease	<1	<b>0.09</b>	<b>0.09</b>	—	—	—	—	—	—	—	—
Nilas	<10	<b>0.14</b>	<b>0.16</b>	<b>0.42</b>	<b>0.39</b>	<b>0.45</b>	<b>0.42</b>	—	—	—	—
Young grey ice	10–15	<b>0.25</b>	<b>0.27</b>	0.55	0.51	0.59	0.56	0.72	0.67	0.76	0.72
Young grey-white ice	15–30	0.32	0.34	0.64	0.59	0.68	0.64	0.76	0.70	0.81	0.76
First-year ice <0.7 m	30–70	0.41	0.45	<b>0.74</b>	<b>0.69</b>	<b>0.79</b>	<b>0.74</b>	0.81	0.75	0.87	0.82
First-year ice >0.7 m	>70	<b>0.49</b>	<b>0.54</b>	<b>0.81</b>	<b>0.75</b>	<b>0.87</b>	<b>0.82</b>	<b>0.81</b>	<b>0.75</b>	<b>0.87</b>	<b>0.82</b>

## 2-4 Observational technique for Arctic sea ice

Arctic sea ice observations are normally conducted at hourly intervals from the ship's bridge, as with Antarctic sea ice. The standardization of the ship-based sea ice observation protocol for Arctic sea ice is now being made by the Arctic Ice Watch program, with the aim to establish a PC software called ASSIST (Arctic Shipborne Sea Ice Standardization Tool) for operational management. Since the observation protocol is based on ASPeCt, the observations added for Arctic sea ice are listed here. (For details, refer to <http://www.iarc.uaf.edu/icewatch/assist>)

### \*Melt pond (Puddle)

Melt pond concentration	tenths
Melt pond pattern	1. linked 2. discrete
Melt pond surface type	1. Frozen (dry or wet snow) 2. Open 3. Bottom up
Melt pond freeboard	centimeters
Melt pond depth	centimeters
Melt pond bottom type	1. Solid 2. Some have thaw holes 3. All have thaw holes
Melt dried ice	logical
Melt rotten ice	logical

### \*Ice algae and Sediments

Algae concentration	0. 0 1. <30 2. <60 3. >60%
Algae density	-1. Not visible 0. Trace 1. Light 2. Medium 3. Strong
Algae location	10. Top 20. Middle 30. bottom
Sediment concentration	0. 0 1. <30 2. <60 3. >60%

# As for algae concentration, select from the color menu below:

 0 <3 mg chl a m <sup>-2</sup>	 2 ~10 mg chl a m <sup>-2</sup> [Pantone 110c]
 1 ~4.5 mg chl a m <sup>-2</sup> [Pantone 100C]	 3 ~30 mg chl a m <sup>-2</sup> [Trumatch 49b]

Additional observation items include the type and amount of cloud for upper, medium, and low cloud. The observation codes are listed in Table 4.

Table 4. Observation codes for ASSIST

ASSIST Menu						
Data fields and coding tables used in observations.						
* * P, S, or T if Primary, Secondary, or Tertiary Ice, respectively. All Concentrations are in tenths, see coding values in SO. Ex. 3/10 concentration code is 3.						
<b>OPEN WATER (OW)</b>	<b>ICE TYPE (IT)</b>	<b>ICE SIZE (IS)</b>	<b>SNOW TYPE (ST)</b>	<b>TOPOGRAPHY TYPE (TO)</b>	<b>MP PATTERN (MP)</b>	<b>MP SURFACE TYPE (MS)</b>
0 :: No openings	10 :: Frost	200 :: Pancakes	00 :: No snow observation	00 :: No snow observation	100 :: Level ice	1 :: Frozen
1 :: Small cracks	11 :: Single	200 :: Pancakes	01 :: No snow, ice or brash	01 :: No snow, ice or brash	200 :: Mixed Pancakes	2 :: Open
2 :: Very narrow breaks, <50m	12 :: Grease	300 :: Beak/Broken Ice	02 :: Cold new snow, <1 dry old	02 :: Cold new snow, <1 dry old	300 :: Cemented Pancakes	3 :: Bottom up
3 :: Narrow breaks, 50-200m	20 :: Nilas	400 :: Cake Ice, <20m	03 :: Cold old snow	03 :: Cold old snow	400 :: Railing	
4 :: Wide breaks, 200-500m	30 :: Pancakes	500 :: Small Floes, 20-500m	04 :: Cold wind-packed snow	04 :: Cold wind-packed snow	500 :: Risks	
5 :: Very wide breaks >500m	40 :: Young Grey Ice, 10-15cm	600 :: Medium Floes, 100-500m	05 :: New melting snow (wet new)	05 :: New melting snow (wet new)		
	50 :: Young Grey Ice, 15-30cm	700 :: Large Floes, 500-2000m	06 :: Old melting snow	06 :: Old melting snow		
	60 :: First Year, <70cm	800 :: Vast Floes, >2000m	07 :: Glare	07 :: Glare	<b>POND DEPTH (MPD)</b>	<b>POND BOTTOM (MBT)</b>
	70 :: First Year, 70-120cm	900 :: Berry floes	08 :: Melt slush	08 :: Melt slush	1 :: 0-30cm	1 :: Solid
	80 :: First Year, >120cm		09 :: Melt puddles	09 :: Melt puddles	2 :: 30-50cm	2 :: Some have thin holes
	90 :: Second Year		10 :: Saturated snow	10 :: Saturated snow	3 :: 50-50cm	3 :: All have thin holes
	95 :: Multiyear		11 :: Saturated	11 :: Saturated	4 :: >50cm	4 :: Unknown
	99 :: Badly					
	95 :: First Ice					
<b>ALGAL CONC. (AC)</b>	<b>ALGAL LOCATION (AL)</b>	<b>OTHER ICE TYPE THICK (OT)</b>	<b>OTHER ICE TYPE THIN (OT)</b>	<b>HIGH CLOUD TYPE (HT)</b>	<b>MED. CLOUD TYPE (MT)</b>	<b>LOW CLOUD TYPE (LT)</b>
0 :: 0%	1 :: Top	30 :: Pancakes	40 :: Young Grey Ice, 10-15cm	C :: Cirrus	A :: Alcotratus	ST :: Stratus
1 :: <5%	2 :: Middle	40 :: Young Grey Ice, 15-30cm	50 :: Young Grey Ice, 15-30cm	CS :: Cirrostratus	AC :: Alcotratus	SC :: Strato-cumulus
2 :: <5%	3 :: Bottom			CC :: Cirrocumulus		NS :: Nimbostratus
3 :: >5%				20 :: Nilas		CU :: Cumulus
				30 :: Pancakes		CN :: Cumulonimbus
<b>SEMI-OPAC CONC. (SO)</b>	<b>ALGAL DENSITY (AD)</b>					
0 :: 0%	0 :: Not Visible					
1 :: <5%	1 :: Trace					
2 :: <5%	2 :: Light					
3 :: >5%	3 :: Medium					
	4 :: Strong					
	5 :: Badly					
	95 :: First Ice					
	See Color Chart					
<b>WEATHER (WX)</b>						
00-03 Change of Sky During Past Hour						
000 :: Clouds not observable/observed						
001 :: Clouds dissolving or becoming less developed						
002 :: State of sky as a whole unchanged						
003 :: Clouds forming or developing						
20-29 Phenomena in Past Hour but NOT at OB						
020 :: Drizzle not freezing or snow grains						
021 :: Rain not freezing or snow grains						
022 :: Snow not freezing or snow grains						
023 :: Rain and snow or ice pellets						
024 :: Drizzle or rain, freezing						
025 :: Showers of rain						
026 :: Showers of snow, or of rain and snow						
027 :: Showers of hail, or rain and hail						
028 :: Fog in past hour, not at present						
029 :: Thunderstorm, with or without precip						
36-39 Unlapse Snow Conditions						
036 :: Drifting snow below eye level, slight/moderate						
037 :: Drifting snow below eye level, heavy						
038 :: Blowing snow, above eye level, slight/moderate						
039 :: Blowing snow, above eye level, heavy						
40-49 Fog at the Time of OB						
040 :: Fog at a distance but not at ship in past hour**Vis may be >1/2sm						
041 :: Fog in patches						
042 :: Fog drifting in last hour, sky discernible						
043 :: Fog drifting in last hour, sky not discernible						
044 :: Fog unchanged in last hour, sky not discernible						
045 :: Fog unchanged in last hour, sky not discernible						
046 :: Fog beginning/thickening in last hour, sky not discernible						
047 :: Fog beginning/thickening in last hour, sky not discernible						
048 :: Fog dissipating, thin, sky not discernible						
049 :: Fog dissipating, thin, sky not discernible						
50-59 Drizzle						
050 :: Slight drizzle, intermittent						
051 :: Slight drizzle, continuous						
052 :: Moderate drizzle, intermittent						
053 :: Moderate drizzle, continuous						
054 :: Dense drizzle, intermittent						
055 :: Dense drizzle, continuous						
056 :: Freezing drizzle, slight						
057 :: Freezing drizzle, moderate or dense						
058 :: Drizzle and rain, slight						
059 :: Drizzle and rain, moderate or dense						
60-69 Rain NOT falling as Showers						
060 :: Slight rain, intermittent						
061 :: Slight rain, continuous						
062 :: Moderate rain, intermittent						
063 :: Moderate rain, continuous						
064 :: Heavy rain, intermittent						
065 :: Heavy rain, continuous						
066 :: Freezing rain, slight						
067 :: Freezing rain, moderate or heavy						
068 :: Rain or drizzle and snow, slight						
069 :: Rain or drizzle and snow, moderate/heavy						
70-79 Solid Precip. Not falling as Showers						
070 :: Slight fall of snow flakes, intermittent						
071 :: Slight fall of snow flakes, continuous						
072 :: Moderate fall of snow flakes, intermittent						
073 :: Moderate fall of snow flakes, continuous						
074 :: Heavy fall of snow flakes, intermittent						
075 :: Heavy fall of snow flakes, continuous						
076 :: Ice crystals, with/without fog						
077 :: Snow grains, with/without fog						
078 :: Isolated star like crystals						
079 :: Ice pellets						
80-84 Rain Showers						
080 :: Slight rain showers						
081 :: Moderate or heavy rain showers						
082 :: Violent rain showers						
083 :: Slight showers of rain and snow						
084 :: Moderate/heavy showers of rain and snow						
85-90 Solid Precipitation in Showers						
085 :: Slight snow showers						
086 :: Moderate or heavy snow showers						
087 :: Slight showers of soft or small hail						
088 :: Moderate/heavy showers of soft/small hail						
089 :: Slight showers of hail						
090 :: Moderate or heavy showers of hail						

## 2-5 Some achievements

In this section, some results obtained from the ASPeCt protocol will be shown. One of the most important achievements is that this method was the first to reveal a circumpolar map of ice thickness distribution around the Antarctica. Worby et al. (2008) showed the mean annual ice thickness distribution (including ridged ice) for the whole Antarctic region, from a compilation of 21,710 individual ship-based observations collected from 81 voyages to Antarctica for the period of 1981 to 2005, following the ASPeCt protocol (Fig.5). This figure clearly shows that the sea ice near the Antarctic Peninsula in the western Weddell Sea and around the coastal area of the eastern Ross Sea is comparatively thicker, and the maximum thickness exceeds 2 m in these regions. In other regions, ice thickness is about 0.3-0.5 m in the marginal ice zone and about 1 m near the coast. These characteristics almost coincide with the results predicted with numerical sea ice models (e.g. Timmermann et al., 2002), providing a valuable ground truth data. However, the observational data and period still does not appear to be sufficient to analyze the interannual variability of ice conditions in the Southern Ocean. For discussion of climate change, further continuous observations are strongly encouraged.

On the other hand, in the southern Sea of Okhotsk, cooperative sea ice observations have been conducted by Hokkaido University and the Japan Coast Guard onboard P/V “Soya” in February every winter since 1996. In particular, after 2001, the ship-based visual observation has been conducted according to the ASPeCt protocol (Toyota et al., 2007). Since the Sea of Okhotsk is a seasonal sea ice zone like most of the Southern Ocean, and since the sea ice there has many common properties with the Antarctic sea ice, the ASPeCt protocol is considered to be useful to obtain the properties of the ice conditions there. Fig. 6 shows the histograms of floe size and thickness obtained by compiling 546 ship-based observations collected in the southern Sea of Okhotsk for the period of 2001 to 2015. It is found from this figure that the dominant floe size category is ice cake (2 m to 20 m) and nilas even though vast floes which comprise a few percents of the floe size distribution. The dominant thickness category is thin first-year ice (30 cm to 70 cm) and nilas, which contrasts to the result obtained along the ship track of JARE off East Antarctica (Ohshima et al., 2006) where a small floe (20 m to 100 m) and medium first-year ice (70 cm to 120 cm) are dominant. The mean ice thicknesses of level ice, ridged ice, and total ice floes are estimated to be 0.29 m, 1.58 m, and 0.63 m, which is somewhat thinner than those of winter Antarctic sea ice (level ice: 0.38 – 0.49 m, total thickness: 0.54 – 0.72 m; Worby et al., 2008).

Thus standardized ship-based observations are not only useful to find the properties of the ice conditions in the individual sea ice area, but they also enable us to compare properties between different ice areas. One of the advantages seems to be the feasibility at only a small cost. It is hoped that the continuity of ship-based observations will contribute to the understanding of climate change.

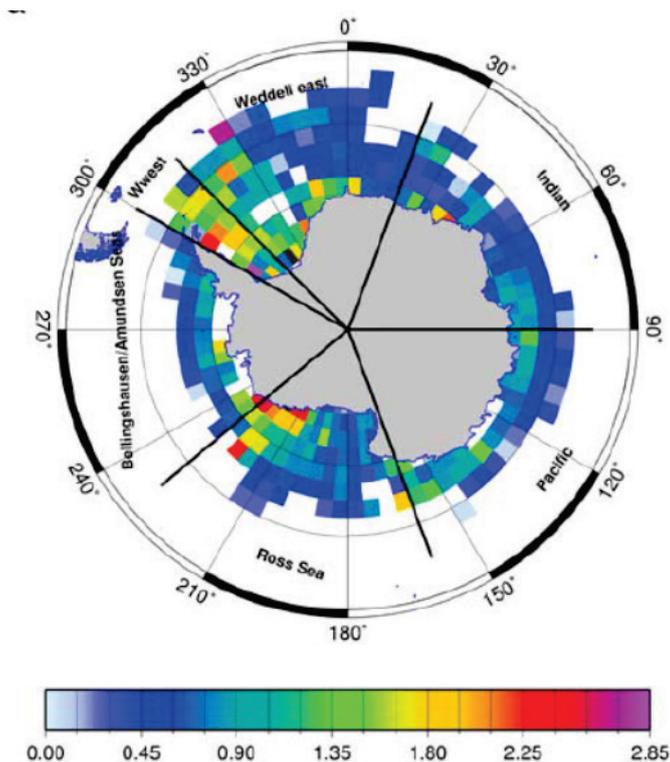


Fig. 5 Circumpolar map of mean annual ice thickness distribution, including ridged ice, obtained by compiling ASPeCt data on 2.5o lat × 5.0o lon grid (cited from Worby et al., 2008).

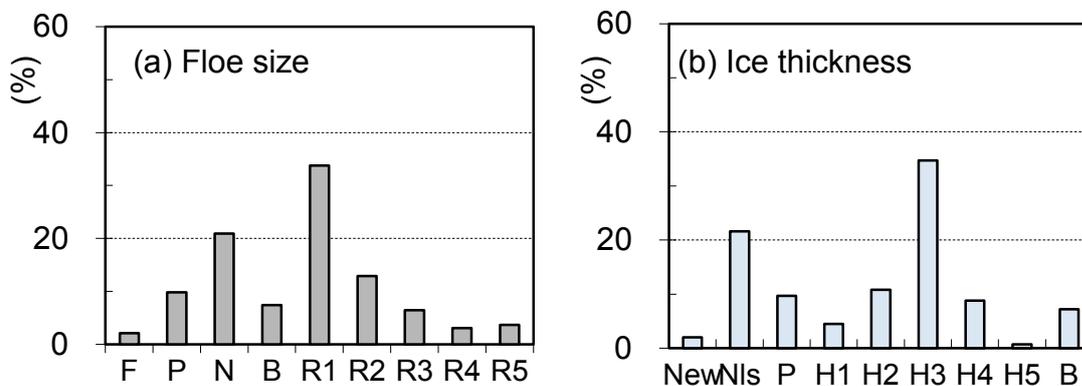


Fig. 6 Mean histograms obtained from ASPeCt observations conducted in the southern Sea of Okhotsk for the period of 2001 to 2015 for

(a) floe size (F: Frazil ice, P: Pancake ice, N: Nilas, B: Brash ice, R1: 2-20m, R2: 20-100m, R3: 100-500m, R4: 500m-2km, R5: >2km)

(b) thickness category (New: New ice, NIs: Nilas, P: Pancake ice, B: Brash ice, H1: 10-15cm, H2: 15-30cm, H3: 30-70cm, H4: 70-120cm, H5: >120cm)

### 3. Coastal observation#

(#Translation of “Guideline for oceanographical observations” published by the Japan Meteorological Agency in 2004)

Coastal sea ice observations are conducted by observers with a good knowledge of sea ice nomenclature at a fixed time of the day at a fixed coastal site. By transmitting the observed ice conditions immediately via a telecommunications network, the data can be used for disaster prevention (e.g., safe ship navigation) as well as ground truth data for sea ice observations conducted by satellite remote sensing. Furthermore, the long-term collection of sea ice observations serves as important baseline dataset for the monitoring of climate.

#### 3-1 Observation site

The observation site should be a relatively high place such as the roof of a building so that observers can view a wide area including the coastline and large sea of the sea surface.

#### 3-2 Observation parameters

Observation parameters include total ice amount, ice conditions around the coastal region, and the first and last dates of sea ice coverage.

#### 3-3 Observation time

Coastal observations are conducted at 09:00 am (LST). If the first or last sea ice phenomena are observed at another time, the observation time should be recorded in the column for remarks on the observation sheet.

#### 3-4 Observation period

In principle, the observation period is from the date when sea ice first appears in sight until the date when sea ice disappears completely out of sight.

#### 3-5 Observation of total ice amount

Ice amount is defined as the areal fraction covered with all types of sea ice within the observation area. Ice amount is classified into *true ice amount*, defined as the fraction of real sea ice area to the real total observation area, and *apparent ice amount* defined as the fraction of apparent sea ice area seen from the slant view to the apparent total observation area. Observers observe the *apparent ice amount*, and record the total ice amount, which is defined as the *apparent ice amount* for the whole sea surface in sight (including the port area), at the fixed observation time.

Ice amount is recorded according to the grade shown in Table 5. This expression is analogous to the total cloud amount (in tenths) in surface meteorological observations. Ice amount is determined solely by the area covered with sea ice, independent of ice types. Some matters which require more attention are listed below:

1. As for the sea ice near the horizon, observers should avoid unreasonable judgments and determine ice amount simply from the appearance because the precise conditions such as a space between floes are often hard to see.
2. Observers should take care to notice relatively dark new sheet ice when present.
3. When only part of the sea surface can be seen due to bad visibility caused by blowing snow or fog, ice amount should be observed within the visible range. In this case, observers should write down the data in parenthesis and put remarks about the reason for the bad visibility in the observation sheet.

Table 5 Grade of total ice amount

Code	Ice amount	Fraction of sea ice to the total sea surface in sight
0	0	No sea ice
1	0+	Sea ice is present in sight but the fraction is < 10%.
1	1	Sea ice occupies 10% of the total sea surface in sight.
2	2	Sea ice occupies 20% of the total sea surface in sight.
2	3	Sea ice occupies 30% of the total sea surface in sight.
3	4	Sea ice occupies 40% of the total sea surface in sight.
4	5	Sea ice occupies 50% of the total sea surface in sight.
5	6	Sea ice occupies 60% of the total sea surface in sight.
6	7	Sea ice occupies 70% of the total sea surface in sight.
6	8	Sea ice occupies 80% of the total sea surface in sight.
7	9	Sea ice occupies 90% of the total sea surface in sight.
7	10-	Total sea surface in sight is mostly covered with sea ice except for small open area.
8	10	The sea surface is covered completely with sea ice.
/	/	Unknown

### 3-6 Observation of ice conditions around the coastline

Ice conditions around the coastline are observed, to report the presence of fast ice or drift ice, and the state of open water such as fractures, leads, and whatever in the sea ice area. The observed data are recorded with the 11 grades listed in Table 6, according to the format of coastal sea ice observation report: JM305.

Although in general sea ice is classified into *fast ice* and *drift ice*, it is hard to judge the classification in some cases. Since this matter is relevant to the definition of the first and last dates of sea ice phenomena, however, some standardization is required.

*Fast ice* is defined by the sea ice which forms and remains fast along the coast, where it is attached to the shore. *Fast ice* may be formed *in situ* from sea water or by freezing of pack ice of any age to the

shore.

On the other hand, *drift ice* is the term used in a wide sense to include any area of sea ice, other than *fast ice*, no matter what form it takes. Therefore, *drift ice* includes not only sea ice which has been advected from the northern or central Sea of Okhotsk, far away from the observation site along the Hokkaido coast, but also *new ice* which has formed off shore, and also sea ice which was once originated from *fast ice* but is now drifting after becoming detached from the shore. Although the judgment between *fast ice* and *drift ice* is often difficult from coastal observation, sea ice extending from the shore should be recorded as *fast ice* even if coastal leads covered with new sheet ice may intervene between the shore and the off-shore ice. In the case where the sea ice is not contiguous with the coast, it should be recorded as *drift ice*.

The most important thing in the coastal sea ice observation is the judgment of the presence of coastal leads, or whether *drift ice* is attached to the shore. While it is relatively easy to discriminate *fast ice* from *drift ice* at the beginning of the ice season, the judgment becomes difficult when *drift ice* gets stuck after becoming attached to the shore or when leads form offshore after becoming attached to the shore. In such cases, the offshore ice should be recorded as *drift ice* though the origin is unknown. However, sea ice which is grounded on the shore or remaining in the port after the ice pack retreats should be recorded as *fast ice*, not *drift ice*.

Table 6 Ice conditions along the coast (Report format: JM305)

Report code	Ice condition along the coast
0	No sea ice
1	Drift ice on the horizon.
2	Fast ice around the shore, while drift ice offshore separated by leads.
3	Leads along the shore and drift ice offshore.
4	Drift ice along the shore and no ice offshore.
5	Drift ice along the shore and another drift ice offshore separated by leads.
6	Drift ice attached to fast ice along the shore.
7	Drift ice mostly covering all the sea surface in sight.
8	Drift ice mostly approaching the port.
9	Drift ice entering the inside of the port.
/	Unknown
Remarks	(1) If more than two codes are fitting, the larger number should be selected. (2) Ice conditions are independent of ice amount and concentration. However, note that code 7 should be used for ice amount greater than 9.

### 3-7. Observation of the first and last dates of sea ice coverage

The observation of the first and last dates indicates the duration of ice coverage for the year. The definitions of the first and last dates of sea ice phenomena are as follows:

#### 1. The first date of drift ice in sight

This is defined by the date when *drift ice* advected from offshore beyond the observation area first appears in sight in the ice season. This observation is not for when sea ice first forms in the observation area, or the first formation of *fast ice*.

#### 2. The last date of drift ice in sight

This is defined by the last date when *drift ice* is found in sight within the observation area. *Drift ice* which remains offshore after the retreat of the ice pack should be taken into account, irrespective of ice amount. A small amount of ice floes remaining around the port or near the coast should be excluded.

#### 3. The first date of drift ice attached to the shore

This is defined by the date when *drift ice* first becomes attached to the shore in the ice season, resulting in the closure of coastal lead for the ship navigation.

#### 4. The first date of reopening of the coastal lead (“*Umiake*” in Japanese)

This is defined by the date when reopening of the sea along the coast allows a ship navigation along the shore, with total ice amount less than 5. In the case when *drift ice* gets attached to the shore again after the first formation of coastal lead and this state continues for more than 3 days, the next reopening date should be recorded as “*Umiake*”. In the case when the short-term (< 2 days) attachments of *drift ice* to the shore are repeated and the interval between attachments is less than 15 days, the reopening date after the final attachment period should be recorded as “*Umiake*”. On the other hand, in the case where there is no opening along the shore even if total ice amount is less than 5, the date should not be recorded as “*Umiake*”.

Since the sight range and the distance to the horizon are determined by the observation height, the observational results about the first and last dates of sea ice phenomena should not be recorded except when the observation is conducted at a fixed site. Furthermore, to determine the last date, the observation should be continued for several days even after sea ice coverage appears to end because sea ice coverage may occur again later in the ice season. Particularly when the last date is earlier than normal, monitoring should be continued until around the normal date.

### ***3-8 Monthly observation sheet***

In the monthly observation sheet, the following matters should be filled in, compiled and archived as the original data sources. A sample of the records is shown in Table 7. In the case where there is no sea ice, total ice amount should be recorded as “0” and the ice conditions as “-”.

1. Total ice amount is filled in according to the grades in Table 5. In the case of bad visibility, apparent ice amount should be filled in with parenthesis. When observation is difficult, “×” should be filled in.

2. The coastal ice condition section is filled in according to the transmitting codes listed in Table 6 (JM305). When observation is difficult due to bad visibility or other reasons, “×” should be filled in.

3. Remarks should be written when the first date of drift ice in sight or drift ice attached to the shore is observed or in the case where total ice amount is recorded as “×” or in parentheses.

4. The first and last dates of sea ice phenomena and the difference from the dates of the normal year and the previous year should be filled in.

5. General conditions are described briefly about the sea ice conditions during the month. In addition, when disasters related to sea ice have occurred, these should be described in the observation sheet.

Table 7. Monthly observation sheet for coastal sea ice observation

2004年2月

## 沿岸海水観測月表

定時観測 (09時)

○×地方气象台

日	全氷量	沿岸海水の分布状況	記 事
1	×	×	雪のため不明
2	5	3	
3	3	2	
4	10-	6	流水接岸初日
5	10-	7	
6	10-	7	
7	10-	7	
8	9	6	
9	10-	7	
10	6	3	
11	4	3	
12	5	3	
13	3	3	
14	10-	6	
15	×	×	雪のため不明
16	7	2	
17	10-	6	
18	10-	6	
19	10-	6	
20	10-	6	
21	10-	6	
22	7	2	
23	8	2	
24	10-	7	
25	10-	7	
26	(10)	(9)	雪のため視界内海面での観測
27	3	3	
28	0	—	
29	0	—	
30			
31			

海水現象初終日	平年差	昨年差
流水初日	1月23日	3日遅い
流水接岸初日	2月4日	12日早い
海明け	月 日	
流水終日	月 日	

## 概 況

月を通して流水に覆われた日が続いたが、月末には沖合いへ後退して流水は視界外へ去った。

## 4. Sea ice nomenclature (cited from WMO (1970))

### 4-1 FLOATING ICE

Any form of ice found floating in water. The principal kinds of floating ice are *lake ice*, *river ice*, and *sea ice* which form by the freezing of water at the surface, and *glacier ice* (*ice of land origin*) formed on land or in an *ice shelf*. The concept includes ice that is stranded or grounded.

#### 4-1-1 Sea ice:

Any form of ice found at sea which has originated from the freezing of sea water.

#### 4-1-2 Ice of land origin:

Ice formed on land or in an *ice shelf*, found floating in water. The concept includes ice that is stranded or grounded.

#### 4-1-3 Lake ice:

Ice formed on a lake, regardless of observed location.

#### 4-1-4 River ice:

Ice formed on a river, regardless of observed location.

### 4-2 DEVELOPMENT

#### 4-2-1 New ice:

A general term for recently formed ice which includes *frazil ice*, *grease ice*, *slush* and *shuga*. These types of ice are composed of ice crystals which are only weakly frozen together (if at all) and have a definite form only while they are afloat.

**FRAZIL ICE:** Fine spicules or plates of ice, suspended in water.

**GREASE ICE:** A later stage of freezing than frazil ice when the crystals have coagulated to form a soupy layer on the surface. Grease ice reflects little light, giving the sea a matt appearance.

**SLUSH:** Snow which is saturated and mixed with water on land or ice surfaces, or as a viscous floating mass in water after a heavy snowfall.

**SHUGA:** An accumulation of spongy white ice lumps, a few centimetres across; they are formed from grease ice or slush and sometimes from anchor ice rising to the surface.

#### 4-2-2 Nilas:

A thin elastic crust of ice, easily bending on waves and swell and under pressure, thrusting in a pattern of interlocking “fingers” (*finger rafting*). Has a matt surface and is up to 10 cm in thickness. May be subdivided into *dark nilas* and *light nilas*.

**DARK NILAS:** *Nilas* which is under 5 cm in thickness and is very dark in colour.

**LIGHT NILAS:** *Nilas* which is more than 5 cm in thickness and rather lighter in colour than

*dark nilas.*

ICE RIND: A brittle shiny crust of ice formed on a quiet surface by direct freezing or from *grease ice*, usually in water of low salinity. Thickness to about 5 cm. Easily broken by wind or swell, commonly breaking in rectangular pieces.

**4-2-3 Pancake ice:**

cf. 4-4-3

**4-2-4 Young ice:**

Ice in the transition stage between *nilas* and *first-year ice*, 10-30 cm in thickness. May be subdivided into *grey ice* and *grey-white ice*.

GREY ICE: *Young ice* 10-15 cm thick. Less elastic than *nilas* and breaks on swell. Usually rafts under pressure.

GREY-WHITE ICE: *Young ice* 15-30 cm thick. Under pressure more likely to ridge than to raft.

**4-2-5 First-year ice:**

*Sea ice* of not more than one winter's growth, developing from *young ice*; thickness 30 cm – 2 m. May be subdivided into *thin first-year ice* / white ice, *medium first-year ice* and *thick first-year ice*.

THIN FIRST-YEAR ICE / WHITE ICE: *First-year ice* 30-70 cm thick.

MEDIUM FIRST-YEAR ICE: *First-year ice* 70-120 cm thick.

THICK FIRST-YEAR ICE: *First-year ice* over 120 cm thick.

**4-2-6 Old ice:**

*Sea ice* which has survived at least one summer's melt. Most topographic features are smoother than on *first-year ice*. May be subdivided into *second-year ice* and *multi-year ice*.

SECOND-YEAR ICE: *Old ice* which has survived only one summer's melt. Because it is thicker and less dense than *first-year ice*, it stands higher out of the water. In contrast to *multi-year ice*, summer melting produces a regular pattern of numerous small *puddles*. Bare patches and puddles are usually greenish-blue.

MULTI-YEAR ICE: *Old ice* up to 3 m or more thick which has survived at least two summers' melt. *Hummocks* even smoother than in *second-year ice*, and the ice is almost salt-free. Colour, where bare, is usually blue. Melt pattern consists of large interconnecting irregular *puddles* and a well-developed drainage system.

**4-3 FORMS OF FAST ICE**

**4-3-1 Fast ice:**

*Sea ice* which forms and remains fast along the coast, where it is attached to the shore, to an *ice wall*, to an ice front, between shoals or grounded *icebergs*. Vertical fluctuations may be observed

during changes of sea-level. Fast ice may be formed *in situ* from sea water or by freezing of *pack ice* of any age to the shore, and it may extend a few metres or several hundred kilometres from the coast. Fast ice may be more than one year old and may then be prefixed with the appropriate age category (*old*, *second-year*, or *multi-year*). If it is thicker than about 2 m above sea-level it is called an *ice shelf*.

**YOUNG COASTAL ICE:** The initial stage of *fast ice* formation consisting of *nilas* or *young ice*, its width varying from a few metres up to 100-200 m from the shoreline.

#### **4-3-2 Icefoot:**

A narrow fringe of ice attached to the coast, unmoved by tides and remaining after the *fast ice* has moved away.

#### **4-3-3 Anchor ice:**

Submerged ice attached or anchored to the bottom, irrespective of the nature of its formation.

#### **4-3-4 Grounded ice:**

*Floating ice* which is aground in shoal water (cf. *stranded ice*).

**STRANDED ICE:** Ice which has been floating and has been deposited on the shore by retreating high water.

**GROUNDING HUMMOCK:** Hummocked *grounded ice* formation. There are single grounded *hummocks* and lines (or chains) of grounded *hummocks*.

### **4-4 PACK ICE**

Term used in a wide sense to include any area of *sea ice*, other than *fast ice*, no matter what form it takes or how it is disposed.

#### **4-4-1 Ice cover:**

The ratio of an area of ice of any concentration to the total area of sea surface within some large geographic local; this local may be global, hemispheric, or prescribed by a specific oceanographic entity such as Baffin Bay or the Barents Sea.

#### **4-4-2 Concentration:**

The ratio in tenths of the sea surface actually covered by ice to the total area of sea surface, both ice-covered and *ice-free*, at a specific location or over a defined area.

**COMPACT PACK ICE:** *Pack ice* in which the *concentration* is 10/10 (8/8) and no water is visible.

**Consolidated pack ice:** *pack ice* in which the *concentration* is 10/10 (8/8) and the *floes* are frozen together.

**VERY CLOSE PACK ICE:** *Pack ice* in which the *concentration* is 9/10 to less than 10/10 (7/8 to less than 8/8).

**CLOSE PACK ICE:** *Pack ice* in which the *concentration* is 7/10 to 8/10 (6/8 to less than 7/8), composed of *floes* mostly in contact.

- OPEN PACK ICE: *Pack ice* in which the *ice concentration* is 4/10 to 6/10 (3/8 to less than 6/8), with many *leads* and *polynyas*, and the *floes* are generally not in contact with one another.
- VERY OPEN PACK ICE: *Pack ice* in which the *concentration* is 1/10 to 3/10 (1/8 to less than 3/8) and water preponderates over ice.
- OPEN WATER: A large area of freely navigable water in which *sea ice* is present in *concentrations* less than 1/10 (1/8). When there is no sea ice present, the area should be termed *ice-free*, even though icebergs are present.
- ICE-FREE: No *sea ice* present. There may be some *ice of land origin* (cf. *open water*).

#### 4-4-3 Forms of floating ice

- PANCAKE ICE: Predominantly circular pieces of ice from 30 cm – 3 m in diameter, and up to about 10 cm in thickness, with raised rims due to the pieces striking against one another. It may be formed on a slight swell from *grease ice*, *shuga* or *slush* or as a result of the breaking of *ice rind*, *nilas* or, under severe conditions of swell or waves, of *grey ice*. It also sometimes forms at some depth, at an interface between water bodies of different physical characteristics, from where it floats to the surface; its appearance may rapidly cover wide areas of water.
- FLOE: Any relatively flat piece of *sea ice* 20 m or more across. Floes are subdivided according to horizontal extent as follows:  
*Giant*: Over 10 km across.  
*Vast*: 2-10 km across.  
*Big*: 500-2,000 m across.  
*Medium*: 100-500 m across.  
*Small*: 20-100 m across.
- ICE CAKE: Any relatively flat piece of *sea ice* less than 20 m across.  
*Small ice cake*: An ice cake less than 2 m across.
- FLOEBERG: A massive piece of *sea ice* composed of a *hummock*, or a group of *hummocks*, frozen together and separated from any ice surroundings. It may float up to 5 m above sea-level.
- ICE BRECCIA: Ice pieces of different age frozen together.
- BRASH ICE: Accumulations of *floating ice* made up of fragments not more than 2 m across, the wreckage of other forms of ice.
- ICEBERG: cf. 4-10-4
- GLACIER BERG: cf. 4-10-4
- TABULAR BERG: cf. 4-10-4
- ICE ISLAND: cf. 4-10-4
- BERGY BIT: cf. 4-10-4
- GROWLER: cf. 4-10-4

## 4-4-4 Arrangement

- ICE FIELD:** Area of *pack ice* consisting of any size of *floes*, which is greater than 10 km across (cf. *patch*).  
*Large ice field:* An *ice field* over 20 km across.  
*Medium ice field:* An *ice field* 15-20 km across.  
*Small ice field:* An *ice field* 10-15 km across.  
*Ice patch:* An area of *pack ice* less than 10 km across.
- ICE MASSIF:** A concentration of *sea ice* covering hundreds of square kilometres, which is found in the same region every summer.
- BELT:** A large feature of *pack ice* arrangement; longer than it is wide; from 1 km to more than 100 km in width.
- TONGUE:** A projection of the ice edge up to several kilometres in length, caused by wind or current.
- STRIP:** Long narrow area of *pack ice*, about 1 km or less in width, usually composed of small fragments detached from the main mass of ice, and run together under the influence of wind, swell or current.
- BIGHT:** An extensive crescent-shaped indentation in the *ice edge*, formed by either wind or current.
- ICE JAM:** An accumulation of broken *river ice* or *sea ice* caught in a narrow channel.
- ICE EDGE:** The demarcation at any given time between the open sea and *sea ice* of any kind, whether fast or drifting. It may be termed *compacted* or *diffuse* (cf. *ice boundary*).  
*Compacted ice edge:* Close, clear-cut *ice edge* compacted by wind or current; usually on the windward side of an area of *pack ice*.  
*Diffuse ice edge:* Poorly defined *ice edge* limiting an area of dispersed ice; usually on the leeward side of an area of *pack ice*.  
*Ice limit:* Climatological term referring to the extreme minimum or extreme maximum extent of the *ice edge* in any given month or period based on observations over a number of years. Term should be preceded by minimum or maximum (cf. *mean ice edge*).  
*Mean ice edge:* Average position of the *ice edge* in any given month or period based on observations over a number of years. Other terms which may be used are mean maximum ice edge and mean minimum ice edge (cf. *ice limit*).  
*Fast-ice edge:* The demarcation at any given time between *fast ice* and *open water*.
- ICE BOUNDARY:** The demarcation at any given time between fast ice and pack ice or between areas of *pack ice* of different *concentrations* (cf. *ice edge*).  
*Fast-ice boundary:* The *ice boundary* at any given time between *fast ice* and

*pack ice.*

*Concentration boundary:* A line approximating the transition between two areas of *pack ice* with distinctly different *concentrations*.

ICEBERG TONGUE: cf. 5-4-10-4

#### **4-5 PACK-ICE MOTION PROCESSES**

##### **4-5-1 Diverging:**

*Ice fields* or *floes* in an area are subjected to diverging or dispersive motion, thus reducing ice concentration and / or relieving stresses in the ice.

##### **4-5-2 Compacting:**

Pieces of *floating ice* are said to be compacting when they are subjected to a converging motion, which increases ice concentration and / or produces stresses which may result in ice deformation.

##### **4-5-3 Shearing:**

An area of *pack ice* is subject to shear when the ice motion varies significantly in the direction normal to the motion, subjecting the ice to rotational forces. These forces may result in phenomena similar to a *flaw* (q.v.).

#### **4-6 DEFORMATION PROCESSES**

##### **4-6-1 Fracturing:**

Pressure process whereby ice is permanently deformed, and rupture occurs. Most commonly used to describe breaking across *very close pack ice*, *compact pack ice* and *consolidated pack ice*.

##### **4-6-2 Hummocking:**

The pressure process by which *sea ice* is forced into *hummocks*. When the floes rotate in the process it is termed screwing.

##### **4-6-3 Ridging:**

The pressure process by which *sea ice* is forced into *ridges*.

##### **4-6-4 Rafting:**

Pressure processes whereby one piece of ice overrides another. Most common in *new* and *young ice* (cf. *finger rafting*).

**FINGER RAFTING:** Type of rafting whereby interlocking thrusts are formed, each floe thrusting “fingers” alternately over and under the other. Common in *nilas* and *grey ice*.

##### **4-6-5 Weathering:**

Processes of ablation and accumulation which gradually eliminate irregularities in an ice surface.

#### 4-7 OPENINGS IN THE ICE

##### 4-7-1 Fracture:

Any break or rupture through *very close pack ice*, *compact pack ice*, *consolidated pack ice*, *fast ice*, or a single *floe* resulting from deformation processes. Fractures may contain *brash ice* and / or be covered with *nilas* and / or *young ice*. Length may vary from a few metres to many kilometres.

CRACK: Any *fracture* which has not parted.  
*Tide crack*: Crack at the line of junction between an immovable *ice foot* or *ice wall* and *fast ice*, the latter subject to rise and fall of the tide.  
*Flaw*: A narrow separation zone between *pack ice* and *fast ice*, where the pieces of ice are in chaotic state; it forms when pack ice shears under the effect of a strong wind or current along the *fast ice boundary* (cf. *shearing*).

VERY SMALL FRACTURE: 0 to 50 m wide.  
 SMALL FRACTURE: 50 to 200 m wide.  
 MEDIUM FRACTURE: 200 to 500 m wide.  
 LARGE FRACTURE: More than 500 m wide.

##### 4-7-2 Fracture zone:

An area which has a great number of fractures.

##### 4-7-3 Lead:

Any *fracture* or passage-way through *sea ice* which is navigable by surface vessels.

SHORE LEAD: A *lead* between *pack ice* and the shore or between *pack ice* and an *ice front*.

FLAW LEAD: A passage-way between *pack-ice* and *fast ice* which is navigable by surface vessels.

##### 4-7-4 Polynya:

Any non-linear shaped opening enclosed in ice. Polynyas may contain *brash ice* and / or be covered with *new ice*, *nilas* or *young ice*; submariners refer to these as *skylights*. Sometimes the polynya is limited on one side by the coast and is called a *shore polynya* or by *fast ice* and is called a *few polynya*. If it recurs in the same position every year, it is called a *recurring polynya*.

SHORE POLYNIA: A *polynya* between *pack ice* and the coast or between *pack ice* and an *ice front*.

FLAW POLYNIA: A *polynya* between *pack ice* and *fast ice*.

RECURRING POLYNIA: A *polynya* which recurs in the same position every year.

#### 4-8 ICE-SURFACE FEATURES

##### 4-8-1 Level ice:

*Sea ice* which is unaffected by deformation.

**4-8-2 Deformed ice:**

A general term for ice which has been squeezed together and in places forced upwards (and downwards). Subdivisions are *rafted ice*, *ridged ice* and *hummocked ice*.

**RAFTED ICE:** Type of *deformed ice* formed by one piece of ice overriding another (cf. *finger rafting*).

*Finger rafted ice:* Type of *rafted ice* in which *floes* thrust “fingers” alternately over and under the other.

**RIDGE:** A line or wall of broken ice forced up by pressure. May be fresh or weathered. The submerged volume of broken ice under a ridge, forced downwards by pressure, is termed an *ice keel*.

*New ridge:* Ridge newly formed with sharp peaks and slope of sides usually 40°. Fragments are visible from the air at low altitude.

*Weathered ridge:* Ridge with peaks slightly rounded and slope of sides usually 30° to 40°. Individual fragments are not discernible.

*Very weathered ridge:* Ridge with tops very rounded, slope of sides usually 20°-30°.

*Aged ridge:* Ridge which has undergone considerable weathering. These ridges are best described as undulations.

*Consolidated ridge:* A ridge in which the base has frozen together.

*Ridged ice:* Ice piled haphazardly one piece over another in the form of ridges or walls.

Usually found in first-year ice (cf. *ridging*).

*Ridged ice zone:* An area in which much *ridged ice* with similar characteristics has formed.

**HUMMOCK:** A hillock of broken ice which has been forced upwards by pressure. May be fresh or weathered. The submerged volume of broken ice under the hummock, forced downwards by pressure, is termed a *bummock*.

*Hummocked ice:* Sea ice piled haphazardly one piece over another to form an uneven surface. When weathered, has the appearance of smooth hillocks.

**4-8-3 Standing floe:**

A separate *floe* standing vertically or inclined and enclosed by rather smooth ice.

**4-8-4 Ram:**

An underwater ice projection from an *ice wall*, *ice front*, *iceberg* or *floe*. Its formation is usually due to a more intensive melting and erosion of the unsubmerged part.

**4-8-5 Bare ice:**

Ice without snow cover.

**4-8-6 Snow-covered ice:**

Ice covered with snow.

**SASTRUGI:** Sharp, irregular ridges formed on a snow surface by wind erosion and deposition. On mobile *floating ice* the ridges are parallel to the direction of the prevailing wind at the time they were formed.

**SNOWDRIFT:** An accumulation of wind-blown snow deposited in the lee of obstructions or heaped by wind eddies. A crescent-shaped snowdrift, with ends pointing down-wind, is known as a snow barchans.

**4-9 STAGES OF MELTING****4-9-1 Puddle:**

An accumulation on ice of melt-water, mainly due to melting snow, but in the more advanced stages also to the melting of ice. Initial stage consists of patches of melted snow.

**4-9-2 Thaw holes:**

Vertical holes in *sea ice* formed when surface *puddles* melt through to the underlying water.

**4-9-3 Dried ice:**

*Sea ice* from the surface of which meltwater has disappeared after the formation of *cracks* and *thaw holes*. During the period of drying, the surface whitens.

**4-9-4 Rotten ice:**

*Sea ice* which has become honeycombed and which is in an advanced state of disintegration.

**4-9-5 Flooded ice:**

*Sea ice* which has been flooded by melt-water or river water and is heavily loaded by water and wet snow.

**4-10 ICE OF LAND ORIGIN****4-10-1 Firn:**

Old snow which has recrystallized into a dense material. Unlike snow, the particles are to some extent joined together; but, unlike ice, the air spaces in it still connect with each other.

**4-10-2 Glacier ice:**

Ice in, or originating from, a *glacier*, whether on land or floating on the sea as *icebergs*, *berg bits* or *growlers*.

**GLACIER:** A mass of snow and ice continuously moving from higher to lower ground or, if afloat, continuously spreading. The principal forms of glacier are: inland ice

sheets, *ice shelves*, *ice streams*, ice caps, ice piedmonts, cirque glaciers and various types of mountain (valley) glaciers.

ICE WALL: An ice cliff forming the seaward margin of a *glacier* which is not afloat. An ice wall is aground, the rock basement being at or below sea-level (cf. *ice front*).

ICE STREAM: Part of an inland ice sheet in which the ice flows more rapidly and not necessarily in the same direction as the surrounding ice. The margins are sometimes clearly marked by a change in direction of the surface slope but may be indistinct.

GLACIER TONGUE: Projecting seaward extension of a *glacier*, usually afloat. In the Antarctic, glacier tongues may extend over many tens of kilometres.

#### 4-10-3 *Ice shelf:*

A floating ice sheet of considerable thickness showing 2-50 m or more above sea-level, attached to the coast. Usually of great horizontal extent and with a level or gently undulating surface. Nourished by annual snow accumulation and often also by the seaward extension of land *glaciers*. Limited areas may be aground. The seaward edge is termed an *ice front* (q.v.)

ICE FRONT: The vertical cliff forming the seaward face of an *ice shelf* or other floating *glacier* varying in height from 2-50 m or more above sea-level (cf. *ice wall*).

#### 4-10-4 *Calved ice of land origin*

CALVING: The breaking away of a mass of ice from an *ice wall*, *ice front* or *iceberg*.

ICEBERG: A massive piece of ice of greatly varying shape, more than 5 m above sea-level, which has broken away from a *glacier*, and which may be afloat or aground. Icebergs may be described as *tabular*, dome-shaped, sloping, pinnacled, weathered or *glacier bergs*.

*Glacier berg:* An irregularly shaped *iceberg*.

*Tabular berg:* A flat-topped *iceberg*. Most tabular bergs form by *calving* from an *ice shelf*

and show horizontal banding (cf. *ice island*).

*Iceberg tongue:* A major accumulation of *icebergs* projecting from the coast, held in place

by grounding and joined together by *fast ice*.

ICE ISLAND: A large piece of floating ice about 5 m above sea-level, which has broken away from an Arctic ice shelf, having a thickness of 30-50 m and an area of from a few thousand square metres to 500 sq. km or more, and usually characterized by a regularly undulating surface which gives it a ribbed appearance from the air.

BERGY BIT: A large piece of floating *glacier ice*, generally showing less than 5 m above sea-level but more than 1 m and normally about 100-300 sq. m in area.

GROWLER: Smaller piece of ice than a *berg bit* or *floeberg*, often transparent but appearing green or almost black in colour, extending less than 1 m above the sea surface and normally occupying an area of about 20 sq. m.

#### **4-11 SKY AND AIR INDICATIONS**

##### **4-11-1 Water sky:**

Dark streaks on the underside of low clouds, indicating the presence of water features in the vicinity of *sea ice*.

##### **4-11-2 Ice blink:**

A whitish glare on low clouds above an accumulation of distant ice.

##### **4-11-3 Frost smoke:**

Fog-like clouds due to contact of cold air with relatively warm water, which can appear over openings in the ice, or leeward of the *ice edge*, and which may persist while ice is forming.

#### **4-12 TERMS RELATING TO SURFACE SHIPPING**

##### **4-12-1 Beset:**

Situation of a vessel surrounded by ice and unable to move.

##### **4-12-2 Ice-bound:**

A harbour, inlet, etc. is said to be ice-bound when navigation by ships is prevented on account of ice, except possibly with the assistance of an icebreaker.

##### **4-12-3 Nip:**

Ice is said to nip when it forcibly presses against a ship. A vessel so caught, though undamaged, is said to have been nipped.

##### **4-12-4 Ice under pressure:**

Ice in which deformation processes are actively occurring and hence a potential impediment or danger to shipping.

##### **4-12-5 Difficult area:**

A general qualitative expression to indicate, in a relative manner, that the severity of ice conditions prevailing in an area is such that navigation in it is difficult.

##### **4-12-6 Easy area:**

A general qualitative expression to indicate, in a relative manner, that ice conditions prevailing in an area are such that navigation in it is not difficult.

##### **4-12-7 Iceport:**

An embayment in an *ice front*, often of a temporary nature, where ships can moor alongside and unload directly onto the *ice shelf*.

#### **4-13 TERMS RELATING TO SUBMARINE NAVIGATION**

##### **4-13-1 Ice canopy:**

*Pack ice* from the point of view of the submariner.

##### **4-13-2 Friendly ice:**

From the point of view of the submariner, an *ice canopy* containing many large *skylights* or other features which permit a submarine to surface. There must be more than ten such features per 30 nautical miles (56 km) along the submarine's track.

##### **4-13-3 Hostile ice:**

From the point of view of the submariner, an *ice canopy* containing no large *skylights* or other features which permit a submarine to surface.

##### **4-13-4 Bummock:**

From the point of view of the submariner, a downward projection from the underside of the *ice canopy*; the counterpart of a *hummock*.

##### **4-13-5 Ice keel:**

From the point of view of the submariner, a downward-projecting ridge on the underside of the *ice canopy*; the counterpart of a ridge. Ice keels may extend as much as 50 m below sea-level.

##### **4-13-6 Skylight:**

From the point of view of the submariner, thin places in the *ice canopy*, usually less than 1 m thick and appearing from below as relatively light, translucent patches in dark surroundings. The under-surface of a skylight is normally flat. Skylights are called large if big enough for a submarine to attempt to surface through them (120 m), or small if not.

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**Appendix: sea ice photo samples**

Frazil ice



(Sea of Okhotsk)

Nilas



(Sea of Okhotsk)

Grease ice



(Sea of Okhotsk)

Shuga



(Sea of Okhotsk)

Pancake ice



(Sea of Okhotsk)

Pancake ice



(off East Antarctica)

Snow covered first-year ice



(Sea of Okhotsk)

Nilas & Young ice



(Sea of Okhotsk)

Small floe



(Sea of Okhotsk)

Vast floe



(Weddell Sea)

Ridge



(Weddell Sea)



(Sea of Okhotsk)

Puddle



(Arctic Ocean)

Thaw holes



(Arctic Ocean)

Iceberg



(Weddell Sea)



(Off East Antarctica)

Ice algae



(Sea of Okhotsk)

Frost flower



(Off East Antarctica)

# The photos in the Sea of Okhotsk were taken with the support of P/V “Soya”.

## Conductivity-Temperature-Depth profiler (CTD) (Blue-water measurements)

○ Hiroshi UCHIDA (Japan Agency for Marine-Earth Science and Technology)

In this guideline, a manual entitled “Notes on CTD/O<sub>2</sub> Data Acquisition and Processing Using Seabird Hardware and Software (as Available)” by McTaggart et al. (2010) [Available online at [http://www.go-ship.org/Manual/McTaggart\\_et\\_al\\_CTD.pdf](http://www.go-ship.org/Manual/McTaggart_et_al_CTD.pdf)] of GO-SHIP Repeat Hydrography Manual is adopted as the manual of conductivity-temperature-depth profiler (CTD) for blue-water measurements, although the manual should be updated with the passage of time. Findings about the uncertainty of deep ocean temperature measurement evaluated after publication of the manual is mentioned below.

Although the Sea-Bird Electronics *9plus* thermometers (SBE 3 or SBE *3plus*) can be corrected relative to a deep ocean standards thermometer (SBE 35), the pressure sensitivity of the in situ reference thermometer had not yet been examined. Therefore, it was difficult to estimate the measurement uncertainty. Uchida et al. (2015) reported that the deep ocean standards thermometers have no pressure sensitivity and the temperature readings of the deep ocean standards thermometers agreed with the realized temperature of the national standard fixed-point temperature cells of Japan. The overall expanded uncertainty of the deep ocean (depths deeper than 20 MPa [2000 m]) temperature measurement by the CTD thermometer calibrated in reference to the deep ocean standards thermometer is estimated to be 0.7 mK.

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## **CTD Oxygen Sensor Calibration Procedures**

○Hiroshi UCHIDA (Japan Agency for Marine-earth Science and Technology)

A manual of “CTD Oxygen Sensor Calibration Procedures” can be obtained from a GO-SHIP web site as below.

[http://www.go-ship.org/Manual/Uchida\\_CTDO2proc.pdf](http://www.go-ship.org/Manual/Uchida_CTDO2proc.pdf)

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## Lowered Acoustic Doppler Current Profiler (LADCP)

○ Shinya KOUKETSU (JAMSTEC)

An acoustic Doppler current profiler (ADCP) attached to the frame of a Rosette water sampling system (CTD frame hereafter) is known as a lowered ADCP (LADCP) and can be used to measure velocity profiles in deep layers during water sampling and measurement of water conductivity, temperature, and depth. Because the current velocities are measured relative to the instrument, it is important to know the velocity of the CTD frame at all times. Two methods that are commonly used to obtain current velocities from LADCP measurements are by integration of vertical velocity shears (Fischer and Visbeck, 1993) and by using an inverse method to infer the velocities of both the CTD frame and the currents (Visbeck, 2002). Data processing programs for both of these methods are publicly available (Thurnherr, 2004). The Global Ocean Ship-Based Hydrographic Investigations Program (GO-SHIP) manual (Thurnherr et al., 2010) provides detailed technical information about the LADCP but only summarizes data processing principles.

Despite recent improvements in instrument design and data processing methods, it is often difficult to extract accurate velocity profiles from the surface to the ocean floor from LADCP observations (ex. due to weak echo intensities). For this reason, the data processing methods that are summarized in the GO-SHIP manual are described here in more detail, but excluding instrument- and region-specific technical information.

The two available methods of velocity estimation are presented in section 1 and the data requirements and processing procedures are described in section 2. Section 3 provides an example of an instrument system and its configuration, and section 4 describes onboard observation procedures.

### 1 Methods for calculation of current velocity profiles

#### *1-a Vertical shear of water velocity and current estimation by integration*

Although LADCP data can be obtained for up to ten layers for each ping, the instrument (CTD frame) velocity is unknown, so (unlike the data from shipboard ADCPs; see Kouketsu, 2015) current velocities cannot be measured directly. However, the vertical differences between the data measured for each layer for a particular ping represent vertical shears of water velocity. Absolute ocean currents velocities can then be estimated by integrating the vertical shear profiles from the available reference velocities (Fischer and Visbeck, 1993), as described below.

A downward-looking LADCP measures current velocity relative to the CTD frame ( $u_{i,n}^m, v_{i,n}^m, w_{i,n}^m$ ) for the  $i$ -th ping in the  $n$ -th layer (Fig. 1). These measurements are strongly influenced by any movement of the CTD frame.

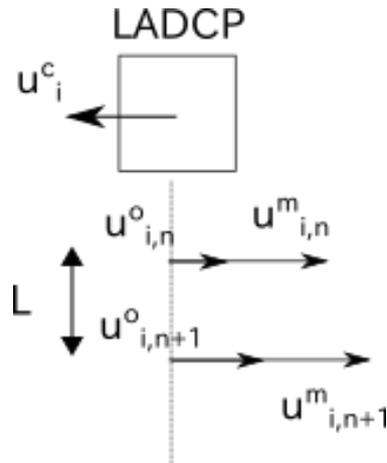


Figure 1 Measured velocity ( $u^m$ ) for each LADCP ping.  $u_{i,n}^m$  is the velocity measured by the  $i$ -th ping in the  $n$ -th layer.  $u_{i,n}^o$  is the ocean current corresponding to  $u_{i,n}^m$ . Note that  $u_{i,n}^m$  includes the CTD frame velocity  $u_i^c$ .

Ocean currents ( $u_{i,n}^o, v_{i,n}^o, w_{i,n}^o$ ) are the sum of the measured velocities and the CTD frame velocities ( $u_i^c, v_i^c, w_i^c$ ):

$$\begin{aligned} u_{i,n}^o &= u_i^c + u_{i,n}^m \\ v_{i,n}^o &= v_i^c + v_{i,n}^m \\ w_{i,n}^o &= w_i^c + w_{i,n}^m. \end{aligned}$$

The vertical velocity shear ( $u_{i,n}^L, v_{i,n}^L, w_{i,n}^L$ ) between the  $n$ -th and  $(n+1)$ -th layers is calculated for a layer of thickness  $L$ , which is initially set equal to the vertical dimension of the observation cell (Fig. 1). These vertical velocity shears are not strongly influenced by movements of the CTD frame and are defined as follows:

$$\begin{aligned} u_{i,n}^L &= \frac{u_{i,n}^o - u_{i,n+1}^o}{L} = \frac{u_{i,n}^m - u_{i,n+1}^m}{L} \\ v_{i,n}^L &= \frac{v_{i,n}^o - v_{i,n+1}^o}{L} = \frac{v_{i,n}^m - v_{i,n+1}^m}{L} \\ w_{i,n}^L &= \frac{w_{i,n}^o - w_{i,n+1}^o}{L} = \frac{w_{i,n}^m - w_{i,n+1}^m}{L}. \end{aligned}$$

By integration of the continuously measured vertical velocity in a layer, the vertical location of the shear measurement ( $z_{i,n}$ ) is determined as a function of time. For this calculation, data from the first or second layer ( $w_{i,1}^m$  or  $w_{i,2}^m$ ) or their average are generally used.

$$z_{i,n} = \int_{t=0}^{t=t_i} w dt + L(n-1) + L_0$$

Here, the distance between LADCP and the first layer is  $L_0$ , and the  $i$ -th ping is emitted at time  $t = t_i$ . Note that the vertical position of the LADCP that corresponds to the first term of the right-hand side of

the above equation can also be determined from CTD pressure data. To calculate the vertical shear ( $u_z^L$ ) of the velocities at depth  $z$ , the ensemble average of the shear measurements in a layer of thickness  $h$  is used.

$$u_z^L = \sum_{i,n \in |z_{i,n} - z| < \frac{h}{2}} u_{i,n}^L / N$$

Here,  $N$  is the number of shear measurements in the layer centered at depth  $z$ . The vertical velocity profile is obtained by vertical integration of the averaged shears with the reference velocities ( $u_{ref}(z_{ref})$ ) for each level:

$$u(z) = \int_{z'=z_{ref}}^{z'=z} u_{z'}^L dz' + u_{ref}(z_{ref}).$$

If the LADCP is used to obtain bottom-track data, the velocity of the deepest layer is used as the reference velocity. Without bottom-track data, the reference velocity is assumed to be the average ship velocity calculated from GPS locations at the start ( $x_s, t = t_s$ ) and end ( $x_e, t = t_e$ ) of the cast. The measured velocity ( $u^m(t)$ ) is the difference between the velocities of the LADCP and the ocean current, and the temporal integration of the LADCP velocity during the cast is equal to the distance travelled by the ship. The current velocity is the sum of the temporal integration of the velocities ( $u(z(t))$ ) obtained from the first term of the right-hand side of the above equation and the reference velocity. The reference velocity ( $u_{ref}$ ) can be calculated as follows.

$$\int_{t=t_s}^{t=t_e} u^m(t) dt = u_{ref} (t_e - t_s) + \int_{t=t_s}^{t=t_e} u(z(t)) dt - (x_e - x_s).$$

Note that  $u(z(t))$  is obtained from the right-hand side of above equation with  $z$  as the temporal function.

### ***1-b Inverse estimation of current and CTD frame velocities***

The measured velocities from a single ping for each layer include the same CTD frame velocity (Fig. 1). Assuming that high-frequency disturbances of ocean currents during the cast were negligible, the current profiles are a function of depth and individual ocean currents are measured by successive LADCP pings, thus providing linear equations that represent the unknown ocean current and CTD frame velocities during the cast. The unknown ocean current velocities can be obtained by solving those linear equations (Visbeck, 2002).

In this method, it is assumed that the CTD frame velocity and the ocean current velocity are functions of time and depth, respectively. If the vertical and temporal resolutions of the estimated ocean current velocity profile and CTD frame velocity time series are  $h$  and  $\tau$ , the measured horizontal velocity ( $u_{i,n}^m, v_{i,n}^m$ ) can be determined as the current ( $u_k, v_k$ ) averaged over the depths from  $z_k = kh$  to  $(k + 1)h$  and CTD frame velocity ( $u^c, v^c$ ) averaged over the period from  $t_1 = l\tau$  to  $(l + 1)\tau$  as follows:

$$u_{i,n}^m = -u_l^c + u_k + \varepsilon_{u_{i,n}}^m$$

$$v_{i,n}^m = -v_l^c + v_k + \varepsilon_{v_{i,n}}^m,$$

where  $\varepsilon_{u_{i,n}}^m$  and  $\varepsilon_{v_{i,n}}^m$  are residuals.

If for a particular ping the measured velocity in another vertical bin ( $n'$ ) is observed at a different level  $z_k$ ,  $u_{i,n'}^m$  can be obtained by the equation

$$u_{i,n'}^m = -u_l^c + u_{k'} + \varepsilon_{u_{i,n'}}^m.$$

In this case the frame velocity is the same as in the other bin because  $u_{i,n'}^m$  is obtained from the same ping as  $u_{i,n}^m$ .

If the measured velocity  $u_{i',n''}^m$  for a different ( $i'$ -th) ping is the sum of the CTD frame velocity at  $t_{i'}$  and the ocean current at  $z_k$ ,  $u_{i',n''}^m$  is defined as

$$u_{i',n''}^m = -u_{l'}^c + u_k + \varepsilon.$$

In this case, the CTD frame velocity is different from  $u_l^c$ , but the ocean current is the same as  $u_k$ .

As shown above, equations can be obtained for all observed layers. For sufficiently large  $h$  (generally 5–20 m) and  $\tau$  (e.g., during three pings), the unknown CTD frame and ocean current velocities can be estimated by solving the equations so as to minimize residuals. In this method, additional variable conditions can easily be included. For example, if known velocity data  $u^K$  are available, the following equation can be added.

$$u^K = u_k + \varepsilon.$$

Furthermore, the equation indicating that the integration of the CTD frame velocity for the cast period is equal to the distance the ship has moved during the cast can also be used.

## 2 Data processing

Before doing the calculations described in the previous section, corrections are necessary for both the difference between magnetic and true north (Thébault et al., 2015) and the velocity of sound in water (see Kouketsu, 2015). Furthermore, it is preferable to calculate observation depths from CTD pressure observations rather than by the integration of vertical velocities. This preprocessing can be done with publicly available software (Thurnherr, 2004).

The following data are needed.

- LADCP raw data
- CTD time series data (the average time bin of 1 second)
- Start and end locations of casts (from GPS data)

- Shipboard ADCP profiles
- Local deviation of magnetic north from true north

### **3 Instrument configurations for LADCP observations**

In addition to the ADCP, the essential equipment for an LADCP survey is a battery and PCs for operational data recording and subsequent processing. The battery provides power to the onboard PC (Windows operating system) and to the submerged LADCP. For a single LADCP operation, the LADCP is attached to the CTD frame in a downward-looking orientation. For a dual LADCP operation, one LADCP usually looks down and the other up. RS232C or USB cables are used to connect the onboard PC to the ADCP and battery.

At present, a 300 kHz ADCP provided by Teledyne RD Instruments is available to members of the Oceanographic Society of Japan; previously, a 150 kHz ADCP was provided. Higher frequency instruments are conveniently small, but larger instruments with lower operating frequencies can measure currents more distant from the instrument, albeit with lower vertical resolution. Because reflection amplitudes for deep layers in the subtropics tend to be weak, lower frequency instruments may provide better results there.

Maintenance by the manufacturer are required every few years, during which the magnetometer should be re-calibrated.

The configuration for the LADCP is set by commands sent from the onboard PC to the instrument. For 300 kHz instruments, an 8 m cell size (and 16 observation layers per ping) is recommended. Although smaller cell sizes increase noise levels, a smaller cell size (e.g., 4 m with 25 observation layers) is worth considering for observations of deep layers, as reflections from greater depths tend to be too weak to obtain multi-bin observations per ping. Table 1 lists the commands that control the configuration of an LADCP and the recommended settings. More detailed information about settings is provided in the manufacturer's manuals.

### **4 Onboard observation processes**

To optimize data processing and obtain accurate current velocities, the onboard ADCP observations should be recorded during lowering of the apparatus to within about 100 m of the seafloor and for about 2 minutes at maximum depth to ensure capture of bottom-track data. To ensure that bottom-track data can be obtained, the LADCP should remain about 30 m above the seafloor. Concurrent collection of CTD data is recommended. Both ship travel distance and CTD frame rotations should be kept to a minimum during each cast. Frame rotations can be minimized by a fin attached to the frame and by ensuring that instruments mounted on the frame are evenly balanced. Short-term movements of the CTD frame and ship can be inferred from CTD pressure data and GPS data, respectively. If the variance of these short-term movements is large, the uncertainty of LADCP data may also be large. To

avoid inaccuracies due to deviations of the orientation of the LADCP from the vertical, directional data from the inner gyro sensor should be used. Under normal operational conditions, standard deviations of current velocity data are typically about 10 cm/s.

Onboard observation processes can be summarized as follows.

1. Connect LADCP and PC to battery case.
2. Transfer initial settings from PC to LADCP and start LADCP observations.
3. Detach cable between battery and PC and begin CTD cast.
4. After completion of cast, with CTD frame on deck, re-connect PC to battery case.
5. Transfer data to PC by using the recovery command. Transfer generally takes over 20-30 minutes for a 6000 m cast.
6. After completion of data recovery, activate sleep command.

Table 1 LADCP configuration

<b>Command</b>	<b>Configuration</b>
CR1	Reset to default values
WM15	Use ADCP as LADCP
CF11101	Output settings
EA0	Instrument attachment angle (0 for LADCP)
EB0	Alignment adjustment (0 for LADCP)
ED0	Instrument depth (0 for LADCP)
ES35	Salinity for calculation of sound velocity
EX11111	Coordinate system for output. This setting means geographic coordinates.
EZ0111101	Available sensors to output. This setting means that sound velocity is constant, and that pressure, gyro, inclinometer, and temperature sensors are available.
LW1	Set Narrow band mode (noisy but longer range)
LD111000000	Velocity, correlation, and echo intensity data are stored
LF176	Distance from instrument to first bin (1.76 m)
LN16	Number of observation layers (16)
LS800	Observation layer thickness (8 m)
LV175	Maximum velocity (measurements above this value are discarded)
TE00:00:01.00	Ensemble period
TP00:01.00	Time between pings
CK	Save above configurations
CS	Start pinging

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# Radiometric determination of anthropogenic radionuclides in seawater samples

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## Abstract

Anthropogenic radionuclides in seawater have been concerned with their ecological effects and oceanographically used as a tracer. Current concentrations of anthropogenic radionuclides in the oceanic waters are generally extremely low except areas affected by Fukushima Dai-ichi Nuclear Power plants accident in 11 March 2011. Determination of anthropogenic radionuclides in seawater has been traditionally performed with radiometric method such as  $\gamma$ -spectrometry,  $\beta$ -counting and  $\alpha$ -spectrometry. The radiometric method is still a useful tool to determine concentrations of anthropogenic radionuclides, although recently mass spectrometric methods have been developed extensively. In this paper, the radiometric methods to determine typical anthropogenic radionuclides,  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$  in seawater, which includes recent development of the radiometric methods such as extremely low background  $\gamma$ -spectrometry are presented.

*Keywords:* Anthropogenic radionuclides;  $^{137}\text{Cs}$ ;  $^{90}\text{Sr}$ , Radioanalytical method

## 1. Introduction

Huge amounts of anthropogenic radionuclides have been introduced in marine environments as global fallout of the large-scale atmospheric nuclear-weapon testing, discharge from nuclear facilities and ocean dumping of nuclear wastes (UNSCEAR, 2000) and Fukushima Dai-ichi Nuclear Power plants accident. The radiological and ecological effects of anthropogenic radionuclides are still world concern. To assess the marine environmental effects of anthropogenic radionuclides, it is significant to clarify their behavior and fate in the marine environments. Therefore, concentrations of anthropogenic radionuclides in seawater are an important tool to evaluate the ecological effect of anthropogenic radionuclides.

$^{137}\text{Cs}$  is one of the most important anthropogenic radionuclides in the field of environmental radioactivity because of a long physical half-life of 30.2 years. It is a major fission product (fission yield: 6-7 %) from both plutonium and uranium (UNSCEAR, 2000).  $^{137}\text{Cs}$  in the ocean has been mainly derived from global fallout (Reiter, 1978; Bowen et al., 1980; UNSCEAR, 2000; Livingston, 2001, Aoyama et al., 2006), together with close-in fallout from the Pacific Proving Ground nuclear explosions (Bowen et al., 1980; Livingston, 2001), discharge of radioactive wastes from nuclear facilities and others (Sigiura et al., 1959; Pentreath, 1989; Hirose et al., 1999).  $^{137}\text{Cs}$  in seawater has been determined since 1957 to elucidate radioecological effects of anthropogenic radioactivity in the marine environment (Miyake & Sugiura, 1955; Rocco & Broecker, 1963; Shirasawa & Schuert, 1968; Saruhashi et al., 1975, Bowen et al., 1980; Folsom, 1980; Nagaya & Nakamura, 1987a; Nagaya & Nakamura, 1987b; Miyake et al., 1988; Miyake et al., 1988; Hirose et al., 1992; Aoyama & Hirose, 1995; Hirose et al., 1999; Aoyama et al., 2000; Aoyama et al., 2001; Aoyama & Hirose, 2003; Hirose & Aoyama, 2003a; Hirose & Aoyama, 2003b; Ito et al., 2003; Povinec et al., 2003; 2004; 2011;

Hirose et al., 2005, Aoyama et al., 2008; 2009; 2012a; 2012b; 2013, 2015). Additionally,  $^{137}\text{Cs}$  in seawater is a powerful chemical tracer of water mass motion at the time scale of several decades (Bowen et al., 1980; Folsom, 1980; Miyake et al., 1988; Miyao et al., 2000; Tsumune et al., 2001; Tsumune et al., 2003a; Tsumune et al., 2003b; Aoyama et al., 2008, Tsumune et al., 2011) because most of the  $^{137}\text{Cs}$  in water columns is present as a dissolved form. Another advantage of the use of  $^{137}\text{Cs}$  as an oceanographic tracer is the quantity and accessibility of marine radioactivity during the past four decades in contrast with other chemical tracers such as CFCs (Warner et al., 1996).

Another important fission product is  $^{90}\text{Sr}$ , which is  $\beta$ -emitter with a half-life of 28.8 years. It has been believed that the oceanic behavior of  $^{90}\text{Sr}$  is very similar to that of  $^{137}\text{Cs}$  because both  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$ , a typical non particle-reactive element, exist as an ionic form in seawater. In contrast of  $^{137}\text{Cs}$ , measurements of  $^{90}\text{Sr}$  in seawater are generally inconvenient because of complicated radioanalytical processes. However, the behavior of  $^{90}\text{Sr}$  in the ocean differs from that of  $^{137}\text{Cs}$ ; for example, a significant amount of  $^{90}\text{Sr}$  has been introduced to the ocean via river discharge in contrast of  $^{137}\text{Cs}$  (Livingston, 1988), which is tightly retained in soil mineral surface. In fact, the  $^{90}\text{Sr}/^{137}\text{Cs}$  ratios in oceanic waters varied spatially and temporally.  $^{90}\text{Sr}$  may be considered to be a tracer of effect of river discharge to the ocean. These findings suggest that there is a need independently to determine  $^{90}\text{Sr}$  in seawater.

In this paper, improved radiometric methods of  $^{137}\text{Cs}$  and traditional radiometric method  $^{90}\text{Sr}$  in seawater are presented.

## 2. Analytical method of $^{137}\text{Cs}$ in seawater

### 2-1 Background

$^{137}\text{Cs}$  decays to stable  $^{137}\text{Ba}$  to emit  $\beta$ -ray (188 keV) and  $\gamma$ -ray (661.7 keV). Cs, which exists ionic form in the natural water, is one of the alkali metals and chemically shows less affinity with other chemicals. The concentration of stable Cs in the ocean is only 3 nM. Known adsorbents to collect Cs in seawater are limited; ex., ammonium phosphomolybdate (AMP) and hexacyanoferrate compounds (Folsom & Sreekumaran, 1966; La Rosa, et al., 2001). The AMP has been an effective ion exchanger of alkali metals (Van R. Smit, et al., 1989). It has been known that AMP forms insoluble compound with Cs. AMP, therefore, has been used to separate other ions and concentrate Cs in environmental samples. In the late 1950s, determination of  $^{137}\text{Cs}$  in seawater was carried out with  $\beta$ -counting because of underdevelopment of  $\gamma$ -spectrometry. Two to several ten mg of Cs carrier is usually added when the radiocaesium is determined by  $\beta$ -counting because of formation of precipitate of  $\text{Cs}_2\text{PtCl}_4$  and calculation of chemical yields of caesium throughout the procedure (Yamagata & Yamagata, 1958; Rocco & Broecker, 1963). After the development of  $\gamma$ -spectrometry using Ge detectors, the AMP procedure with  $\gamma$ -spectrometry became a convenient concentration procedure for the determination in the environmental samples.

Traditionally (AMP) method was believed as adsorption method (Wong et al., 1994), therefore a receipt of AMP method was very simple that pH should be adjusted between 1 -4 by nitric acid and AMP of 0.2 g per 1 liter was added without stable caesium carrier. In a

modified method (Baskaran et al., 2009), they recommended to add 20 mg of Cs carrier to the sample, however, they did not care about stoichiometry between AMP and Cs.

In Japan, the AMP method is recommended for radiocaesium measurements in seawaters, in which Cs carrier is stated to be unnecessary (Science and Technology Agency, 1982). It, however, must be noted that large volumes of seawater samples (more than 100 liters) were required to determine  $^{137}\text{Cs}$  because of relatively low efficiency of Ge-detectors (around 10 %).

In the previous literatures, the weight yield of AMP has not been used because the chemical yield of Cs could be obtained and the loss of small amount of AMP during the treatment did not cause serious problems. Actually, the use of AMP reagent produced in the 1960s and in the mid 1980s gave the range from 70% to 90 % as weight yields of AMP without Cs carrier in the laboratory experiment in 1996. These weight yields are in good agreement with the records of weight yields of AMP in our laboratory during the 1970s and 1980s. However, the weight yield of AMP without Cs carrier had been decreasing from the end of the 1980s and it sometimes became very low, less than 10%, in the mid 1990s. To improve  $^{137}\text{Cs}$  determination in seawater, Aoyama et al. (2000) re-examined the ammonium phosphomolybdate (AMP) procedure. Their experiments revealed that the stable Cs carrier of the same equivalent amount as AMP is required to form insoluble the Cs-AMP compound in an acidic solution (pH = 1.2 to 2.2). The improved method has been achieved to have high chemical yields of more than 95% for sample volumes of less than 100 liters. Another improvement is to succeed to reduce the amount of AMP from several ten grams to 4 grams to adsorb  $^{137}\text{Cs}$  from seawater samples. As a result, it has been reduced the sample volume from around 100 liters to less than 20 liters to be able to use high-efficiency well-type Ge-detectors. This improvement of  $^{137}\text{Cs}$  is favorable to use the chemical tracer of  $^{137}\text{Cs}$  in the oceanographic field.

However, there was a serious problem regarding the  $^{137}\text{Cs}$  measurement; i.e., large-volume sampling of more than 100 liters has been required to determine  $^{137}\text{Cs}$  concentrations in deep waters because of very low concentrations of  $^{137}\text{Cs}$  (less than  $0.1 \text{ Bq m}^{-3}$ ). A major problem not to improve sensitivity of high-efficiency well-type Ge-detectors results in higher background accompanied with  $\gamma$ -spectrometry in ground-level laboratories. Especially, it is difficult to determine accurate  $^{137}\text{Cs}$  concentrations in deep waters (>1000 m) because of the difficulty acquiring large, non-contaminated samples. However, recently, Komura (Komura, 2004; Komura & Hamajima, 2004) has established an underground facility (Ogoya Underground Laboratory: OUL) to achieve extremely low background  $\gamma$ -spectrometry using Ge detectors with high efficiency and low background materials.

The OUL has been constructed in the tunnel of former Ogoya copper mine (235m height from sea level, Ishikawa prefecture) in 1995 by Low Level Radioactivity Laboratory, Kanazawa University. Depth of the OUL is 270 meters water equivalent and contributions of muon and neutron are more than two orders of magnitude lower than those at ground level. In order to achieve extremely low background  $\gamma$ -spectrometry, high efficiency well type Ge detectors specially designed for low level counting were shielded with extremely low background lead prepared from the very old roof tile of the

Kanazawa Castle. As a result, background of  $\gamma$ -ray corresponding to an energy range of  $^{137}\text{Cs}$  is two orders of magnitude lower than that in ground-level facilities as shown in Table 1. A detection limit of  $^{137}\text{Cs}$  at the OUL is 0.18 mBq for a counting time of 10000 minutes (Hirose et al., 2005).

There is a residual problem of underground  $\gamma$ -spectrometry for  $^{137}\text{Cs}$  measurements. AMP adsorbs trace amounts of potassium when Cs is extracted from seawater because K is a major component in seawater and radioactive potassium ( $^{40}\text{K}$ ) contains 0.0118 % of total in the natural materials. Trace amounts of  $^{40}\text{K}$  cause elevation of background corresponding to energy range of  $^{137}\text{Cs}$  due to Compton scattering of  $^{40}\text{K}$ . If  $^{40}\text{K}$  can be removed in AMP/Cs compound samples, the full performance of underground  $\gamma$ -spectrometry for  $^{137}\text{Cs}$  measurements is established. To remove  $^{40}\text{K}$  from the AMP/Cs compound, a precipitation method including insoluble platinate salt of Cs was applied for purification of Cs. This method performed to be able to trace amounts of  $^{40}\text{K}$  from the AMP/Cs compound with a chemical yield of around 90 % for  $^{137}\text{Cs}$  (Hirose et al., 2006b).

## ***2-2 Sampling and materials***

Seawater samples should be collected without contamination. When using a CTD-rosette sampler, which collected seawater of each 12 liters at 24 -36 different depth layers, before sample seawater collection outside of the sampler bottles should be washed by clean water. When use a bucket, the bucket should be rinsed a few time before sample seawater collection.

In general, sample seawater should be filtered using a filter with pore size of 0.45 micrometer.

All reagents used for  $^{137}\text{Cs}$ ,  $^{90}\text{Sr}$  and Pu assay are special (G.R.) grade for analytical use. All experiments and sample treatments are carried out at ambient temperatures. It is very important to know background  $\gamma$  activity of reagents. The  $^{137}\text{Cs}$  activity in CsCl was less than 0.03 mBq  $\text{g}^{-1}$  by using extremely low background  $\gamma$ -spectrometry. The  $^{137}\text{Cs}$  activity in AMP was less than 0.00<sub>8</sub> mBq  $\text{g}^{-1}$ . There is no serious contamination of  $^{137}\text{Cs}$  from other reagents.

## ***2-3 Recommended AMP procedure***

Proposed improved AMP procedure with the ground-level  $\gamma$ -spectrometer is as follows:

- 1) Measure the seawater volume (5-100 liters) and put into a tank with appropriate size.
- 2) pH should be adjusted to be 1.6-2.0 by adding concentrated  $\text{HNO}_3$  (addition of 40 ml conc. $\text{HNO}_3$  for 20 litre seawater sample makes pH of sample seawater about 1.6).
- 3) Add CsCl of 0.26g and stir at a rate of 25 litre per minute or alternative method for one hour.
- 4) Weigh AMP of 4g and pour it into a tank to disperse the AMP with seawater.
- 5) 1 hour stirring at the rate of 25 litre air per minute or alternative method.
- 6) Settle until the supernate becomes clear. A settling time is usually 6 hours to overnight, but no longer than 24 hours.
- 7) Take an aliquot of 50 ml supernate to calculate the amount of the residual caesium in the supernate.
- 8) Loosen the AMP/Cs compound from the bottom of the tank and transfer into a 1-2

litre of beaker, if it is necessary do additional step of decantation.

- 9) Collect the AMP/Cs compound onto 5B filter by filtration and wash the compound with 1M HNO<sub>3</sub>
- 10) Dry up the AMP/Cs compound for several days in room temperature
- 11) Weigh the AMP/Cs compound and determine weight yield
- 12) Transfer the AMP/Cs compound into a Teflon tube of 4ml volume and subject to  $\gamma$ -ray spectrometry

#### **2-4 Underground $\gamma$ -spectrometry**

- 1) the same procedure from step 1) to step 12)
- 2) Dissolve AMP/Cs compound by adding alkali solution
- 3) pH should be adjusted to be ca. 8.1 by adding 2M HCl and adjust the volume of solution ca. 70-100ml.
- 4) Perform precipitation of Cs<sub>2</sub>Pt(Cl)<sub>4</sub> to add chloroplatinic acid (1g/5ml D.W) at pH = 8.1 and keep in refrigerator during a half-day.
- 5) Collect the Cs<sub>2</sub>Pt(Cl)<sub>4</sub> precipitate onto filter by filtration and wash the compound with solution (pH = 8.1)
- 10) Dry up the Cs<sub>2</sub>Pt(Cl)<sub>4</sub> precipitate for several days in room temperature
- 11) Weigh the Cs<sub>2</sub>Pt(Cl)<sub>4</sub> precipitate and determine weight yield
- 12) Transfer the Cs<sub>2</sub>Pt(Cl)<sub>4</sub> precipitate into a Teflon tube of 4ml volume and subject to underground  $\gamma$ -spectrometry

### **3. Analytical method of <sup>90</sup>Sr**

#### **3-1 Background**

<sup>90</sup>Sr decays to stable <sup>90</sup>Zr via <sup>90</sup>Y (half life: 2.67 day) with  $\beta$  emitter (934 keV) to emit  $\beta$ -ray (196 keV). Strontium comprises about 0.025 percent of the Earth's crust and the concentration of stable Sr in the ocean is  $8.7 \times 10^{-5}$  M. It is widely distributed with calcium. The chemistry of strontium is quite similar to that of calcium, of which concentration in the ocean is  $10^{-2}$ M. The biological behaviors of strontium in the ocean are also very close to those of calcium, and then the behavior of Sr in the ocean can be considered to be different from that of Cs.

The radiometric method of <sup>90</sup>Sr is only  $\beta$ -counting. Therefore, radiochemical separation is required for determination of <sup>90</sup>Sr. An essential step in <sup>90</sup>Sr analytical methodologies is the separation and purification of the strontium, both to remove radionuclides which may interfere with subsequent  $\beta$ -counting and to free it from the large quantities of inactive substances typically present, i.e., calcium in seawater. In the 1950s, oxalate technique was used to separate Sr and Ca (Miyake & Sugiura, 1955). They applied the fuming HNO<sub>3</sub> method for purification of Sr. On the other hand, carbonate techniques (Sugihara et al., 1959) was used for <sup>90</sup>Sr determination for the Atlantic sample in the 1950s. Shirasawa et al.

(Shirasawa et al., 1968) used similar procedures to those developed by Rocco and Broecker (1966) in which the oxalate technique was adapted. During the GEOSECS period, the oxalate technique was applied for  $^{90}\text{Sr}$  determination for the world ocean (Bowen et al., 1980). Classical methods for the separation of strontium from calcium rely upon the greater solubility of calcium nitrate in fuming nitric acid. Those procedures require numerous steps including repeated precipitation in strong nitric acid. Therefore, various alternative methods for separation have been proposed; precipitation methods of strontium sulfate and strontium rhodizonate (Weiss and Shipman, 1957), sorption of strontium on an ion-exchange resin from a solution of chelating agent such as CyDTA and EDTA (Noshkin & Mott, 1967). These methods, however, had not improved to shorter analytical steps because the precipitation and extraction methods yield strontium fractions containing significant amounts of calcium. In the late 1970s, Kimura et al. (1978) proposed the use of macrocyclic polyethers for the separation of strontium and calcium. In the 1990s, extraction chromatography, using a solution of 4,4'(5')-bis (tert-butylcyclohexano)-18-crown-6 in 1-octanol sorbed on an inert substrate, has been developed for the separation of strontium and calcium (Horwitz et al., 1991). Recently membrane filter coating crown ether was developed for separation of strontium from others (Lee et al., 2000; Miró et al., 2002). These modern techniques have contributed for downsizing small volumes of samples. On the other hand, large volumes of seawater have been used for determination of  $^{90}\text{Sr}$  in seawater due to low concentrations of  $^{90}\text{Sr}$ . Therefore, the current practical method for separation and purification of  $^{90}\text{Sr}$  in seawater still contains preparing precipitation at the first step.

There are some problems in the current practical methods using the carbonate technique; one is a lower recovery of Sr with a range from 30-60% and another is along radiochemical separation. Since the radioactivity of  $^{90}\text{Sr}$  in seawater was lower even in the surface water in present days, improvement of the Sr recovery is one of the key issues for determination of  $^{90}\text{Sr}$  activity in seawater. Another key point is that Ca/Sr ratio in the carbonate precipitates is remarkably reduced from that in seawater. To improve  $^{90}\text{Sr}$  determination in seawater, we re-examined the Sr separation technique for seawater samples (Aoyama & Hirose, 2006).

### **3-2 Method**

#### **3-2-1 Sampling and materials**

Seawater samples should be collected without contamination. When using a CTD-rosette sampler, which collected seawater of each 12 liters at 24 -36 different depth layers, before sample seawater collection outside of the sampler bottles should be washed by clean water. When use a bucket, the bucket should be rinsed a few time before sample seawater collection.

In general, sample seawater should be filtered using a filter with pore size of 0.45 micrometer.

$^{90}\text{Sr}$  were assayed as  $^{90}\text{Y}$  using  $\beta$ -counting following radiochemical separation described in detail as follows:

### 3-2-2 Preconcentration of $^{90}\text{Sr}$

Coprecipitation method has been practically used for extracting Sr from large volumes of seawater. Both oxalate technique and carbonate technique has been carried out for preconcentration of Sr from large volume water samples. The preconcentration of  $^{90}\text{Sr}$  due to carbonate was performed 500g  $\text{NH}_4\text{Cl}$  and 500g  $\text{Na}_2\text{CO}_3$  in 100 liter seawater. To improve the lower recovery of  $^{90}\text{Sr}$  using carbonate technique, it is essential to remove Mg as hydroxide at pH=12 from the sample seawater before performing the carbonate precipitation.

### 3-2-3 Radiochemical separation

At the first step of radiochemical separation of Sr from Ca, Sr is separated from calcium as oxalate precipitation at pH=4. After dissolution of oxalate precipitation, Ra and Ba are removed with Ba chromate. After Ra and Ba are removed as precipitates, Sr is recovered as carbonate precipitation. Further purification of Sr is carried out by using fuming nitric acid to remove Ca. After the  $^{90}\text{Sr}$ - $^{90}\text{Y}$  equilibrium has been attained, coprecipitation of  $^{90}\text{Y}$  with ferric hydroxide was formed and mounted on a disk for counting.

### 3-2-4 $\beta$ -counting

$\beta$ -counting of  $^{90}\text{Y}$  were carried out by gas proportional counters for external solid samples. A typical efficiency of  $^{90}\text{Y}$  counting by gas proportional counters with window is ca. 40 percent. The detection limit of  $^{90}\text{Y}$  is several mBq when counting time is 360 minutes.

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## Microplastics (Surface Water Trawl Surveys for Small Debris Items)

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### 1. Introduction

Plastic debris in the marine environment has significant impacts on marine ecosystem, and is one of the major global issues. Although small plastic debris is generally undetectable with shipboard sighting survey, there is no doubt that they have been steadily increasing within marine environment in spite of efforts to control the problem (UNEP, 2014). Moreover, it has been revealed that marine plastics carry toxic chemicals of smaller molecular size ( $MW < 1000$ ), which can penetrate through the cell membrane (Takada et al., 2009; Teuten et al., 2009). There is evidence that plastics are fragmenting in the marine environment (Barnes et al., 2009), as a consequence, small fragments of plastics are distributed throughout the ocean, occurring on shorelines, in surface waters, seabed sediments from Arctic to Antarctic and in a wide variety of biota such as invertebrates, fish, seabirds, mammals etc. (Ryan et al., 2009; Reisser et al., 2014).

The term “microplastics” was introduced in mid-2000s, and are used pragmatically to describe plastic particles “smaller than 5mm” (Arthur et al., 2009; GESAMP, 2015). Microplastics are often classified into two main categories: (i) “primary” microplastics are originally manufactured to be of a microscopic size for particular industrial or domestic applications, and (ii) “secondary” microplastics are tiny plastic fragments derived from the breakdown of larger plastic debris, both at sea and on land (Cole et al., 2011).

In this chapter, the methodology of the surface water trawl surveys for small size debris, mainly microplastics, is described. Trawl surveys are less subjective than direct sighting survey (Ryan et al, 2009), but currently no universally accepted standard method is available for observations of microplastics. Therefore, the following is only suggested procedures derived from many previous reports.

Incidentally, examinations of stomach contents of seabirds and sea turtles can also bring information about microplastics; however, they need quite another technique and equipment.

### 2. Objectives and Setting of Surveys

#### 2-1 Objectives

Shipboard surface water trawl surveys obtain samples of floating microplastics and collect information on their distribution, types and amounts. Typical objectives for open-water trawling surveys are, for example, as follows:

- to obtain samples of microplastics;
- to identify types of microplastics;
- to estimate concentration of microplastics;
- to detect temporal and spatial variation in the occurrence of microplastics.

It is desirable that trawl surveys are accompanied with direct sighting survey for floating pollutants

(see Chapter 4) in order to interpret the connection with the distribution of larger debris. While field sampling and sample processing, avoid wearing clothes made of polymer or synthetic fibers, because they may contaminate plastic samples. Woodall et al. (2015) gave more strict information on the contamination of the samples.

## ***2-2 Site Selection***

There has been little information about abundance and types of floating microplastics, as well as about larger pollutants, in the open-ocean. The survey sites should be selected in consideration of the following matters:

- to deliberate on sources and transportation process affected by the wind system and the major currents;
- to focus on areas that are known to accumulate pollutants;
- not to impact on marine ecosystems by the navigation of the observation ship.

For example, the area of concentrated small debris, known as “Great Pacific Garbage Patch”, lies off the Japan Archipelago and between Hawaii and California, almost coincident with the Subtropical Convergence Zone, affected by Ekman dynamics (Dautel, 2009; EPA, 2011). Isobe et al. (2014) described that they sought oceanic fronts before trawl survey, because floating plastic debris is likely to be trapped there. Needless to say, sampling site should not be located close to shore and considered to be shallow, taking the dimension of observation ship into account.

## ***2-3 Frequency and Timing of Surveys***

For long-standing monitoring in fixed region, the minimum sampling frequency should be annually. Ideally quarterly sampling is recommended, allowing an interpretation on seasonal changes.

It is well known that depth profiles of microplastics are mainly affected by turbulence in the surface layer; especially smaller particles are more susceptible to vertical transport (Kukulka et al., 2012; Reisser et al., 2015). Therefore, it is desirable that trawl surveys for microplastics floating within thin surface layer are conducted under calm sea state as far as possible.

# **3. Shipboard Trawl Survey**

## ***3-1 Outline of Sampling Method***

Surface water trawl surveys are conducted in the daytime, so that the state of sampling net can be seen clearly. Sampling net attached to the end of the towrope is deployed while the ship is moving at constant speed, and trawled for preset time duration (details will be described later). During the trawl, towrope length may be adjusted to ensure approximately half of net mouth is under water, so that sampling net skims the sea surface. In order to stabilize sampling net posture, weights can be attached at the bottom of net mouth frame, or floats can be put on both sides of the net mouth. Sampling net should be placed on a boom, if possible, in order to avoid the wake behind the ship.

After sampling net is recovered, filtered surface water sample is collected in small net bag in the end bucket (traditionally called “cod-end”), washing gently with natural seawater from the outside of the

sampling net. Next, the end bucket is detached and entire contents of small net bag are transferred into another container, rinsing with natural seawater or fresh water. Extra care must be taken because small items are easily lost under rainy or windy condition.

The sample may be consequently processed on shipboard or stored in labeled container for laboratory processing (see 3-4). In the latter case, sample should be frozen or chemically preserved (add formalin etc.) as soon as possible not to turn putrid. Note that the chemically preserved samples generally cannot be submitted for persistent organic pollutants (POPs: such as PCBs, DDTs etc.) analysis.

### 3-2 Equipment

The observation ship should equip GPS unit for determination of ship position and speed continuously. Appropriate trawling system (crane, winch, capstan etc.) and sampling net with supplies (cable/rope, shackles, swivels etc.) are required for net-based surveys.

Although many kinds of sampling nets are available for trawl surveys, among them neuston net and manta net, shown in Figure 3-1, seem to have been most frequently used (Ribic et al., 1992; Lattin et al., 2004; Ryan et al., 2009; Lippiatt et al., 2013; Reisser et al., 2013; Isobe et al., 2014). Both of them are implements for sea surface sampling, the latter is supposed to resemble a manta ray, with metal wings and broad rectangular mouth.

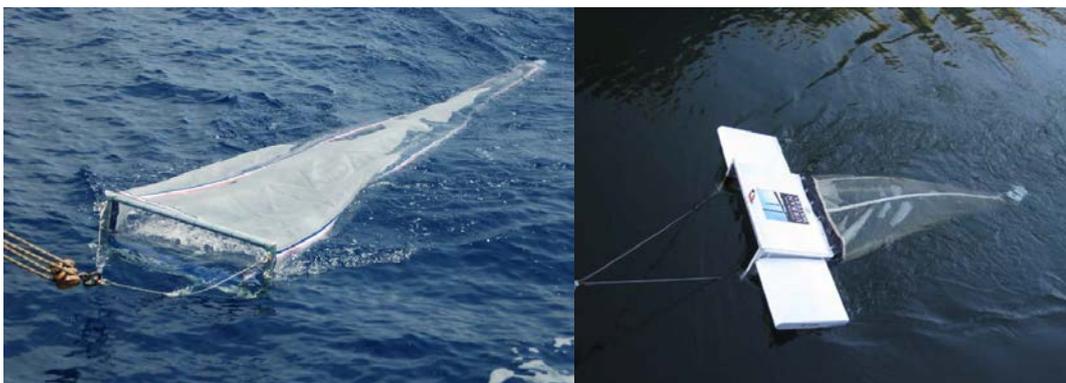


Figure 3-1 Neuston net (left; [[http://www.aoml.noaa.gov/ocd/ocdweb/waltonsmith\\_photos.html](http://www.aoml.noaa.gov/ocd/ocdweb/waltonsmith_photos.html)]) and manta net (right; Masura et al., 2015).

Desforges et al. (2014) collected subsurface water sample (4.5m below the water line) using the saltwater intake system of the vessel. Although their sampling method is independent on sea state and needs no trawling system on the vessel, the plastic concentration in subsurface water is generally lower than the surface water. Consequently, it is likely that total amount of microplastics in the water column may be underestimated.

## 4. Sample Processing

Microplastics in surface water samples are often classified by their size, external characteristics (function of the original product) and polymer types. Efforts of classification are important as obtained information can be used to pinpoint sources of plastic debris and target reduction measures.

### *4-1 Sieving and Visual Separation*

First, any obvious large debris items, larger than centimeter-sizes, are manually removed from surface water samples. Consequently, samples are filtered through double-stacked sieves, which have equivalent mesh size of upper and lower limit size of target microplastics (Masura et al. (2015) used 5.6-mm (No. 3.5) and 0.3-mm (No. 50)), to separate debris into two size fractions, and rinsed with distilled water. Material retained on the upper limit size sieve can be discarded or archived for another studies. The sample collected on the lower limit size sieve is transferred into some other container.

Next, plastic fragments are visually (by naked eye or with the aid of magnifier/microscope) identified and picked up and placed in a graduated petri dish with forceps or some adequate instrument from the sample. This is an almost obligatory step to separate plastics from other materials, such as organic debris, small pieces of hull-originated paint, metal, glass, etc., and, if possible, should be confirmed by more than one people for quality assurance (Lippiatt, et al., 2013). Plastic fragments on the petri dish can be counted for every different size categories, and can be weighed after drying process. Pre-weighed vials or dishes are useful for the procedures described here.

### *4-2 Classification by External Characteristics*

Microplastics can be classified by external characteristics into several types. For example, Erikson (2005) introduced seven types: i) Pellet, ii) Fragment, iii) Film – plastic debris of bags or wrappers, iv) Foam, v) Filament, vi) Cigarette parts and vii) Other debris (including glass, rubber, metal, etc.). Lattin et al. (2004) sorted plastics into six categories – i) fragments, ii) styrofoam, iii) pellets, iv) polypropylene/monofilament line, v) thin plastic films and vi) resins. UNEP/IOC (2009) also showed the detail “standard categories” for remote benthic and floating observation, including seven classes; i) Containers, ii) Fishing & Boating, iii) Food and Beverage, iv) Packing, v) Sanitary, vi) Smoking and vii) Other.

### *4-3 Density Separation*

Although many different types of plastics are produced globally, 6 classes are dominant in the market (GESAMP, 2015) and given “SPI Resin Identification Code” (see American Chemistry Council, 2007) as follows: i) polyethylene terephthalate (PET), ii) high-density polyethylene (HDPE), iii) polyvinyl chloride (PVC), iv) low-density polyethylene (LDPE), v) polypropylene (PP), vi) polystyrene (PS, including expanded polystyrene EPS) and vii) others. Their code and density data for typical virgin resins are summarized in Table 3-1.

Table 3-1. SPI Resin Identification Code and Plastic Classes (American Chemistry Council, 2007) with density data (Eriksen, 2005).

Code	Plastic Class		Density <sup>#</sup> [g · cm <sup>-3</sup> ]
1	PET	Polyethylene terephthalate	1.38 – 1.39
2	HDPE	High-density Polyethylene	0.95 – 0.97
3	PVC	Polyvinyl Chloride	1.16 – 1.35
4	LDPE	Low-density Polyethylene	0.92 – 0.94
5	PP	Polypropylene	0.90 – 0.91
6	PS	Polystyrene	1.05 – 1.07
7	(Others)	Cellulose Acetate etc.	-

# Densities given here are approximate value for typical virgin resins. Note that plastic products are often mixed with fillers and additives that may alter their density.

The plastic fragments can be separated based on the difference of densities among plastic classes (Corcoran, 2009). Plastics that float in seawater and fresh water are EPS, high and low density PE, and PP. Solid form PS also floats in a saturated NaCl solution ( $\rho \cong 1.2$  [g·cm<sup>-3</sup>]). Plastics that finally float in sodium polytungstate solution include flexible/rigid PVCs and PETs. As the density of 30% CaCl<sub>2</sub> solution is approximately equal to 1.3 [g·cm<sup>-3</sup>], it can be used to separate PET and PVC. Ethanol-water mixtures of various densities, including  $\rho = 1.0, 0.9408$  and  $0.911$  [g·cm<sup>-3</sup>], can be used to separate PS, high and low density PE and PP. Cooking oils, their densities are approximately equal to ethanol-water mixtures, may also be used for plastic separation (Eriksen, 2005).

#### 4-4 Spectroscopic Identification

There are some concern that density separation method may make misidentification, because the density of plastic fragments may vary considerably depending not only on the polymer type, but also on the manufacturing process, fillers and additives. For that reason, use of spectroscopic method (FT-IR spectroscopy, near-infrared spectroscopy, Raman spectroscopy etc.) is recommended, because it may bring certain determination of the polymer types of plastic samples (Hidalgo-Ruz et al., 2012).

If plastic samples are not submitted for chemical analysis of PCBs, DDTs etc., they should be disposed appropriately.

## 5. Variables to Consider

### 5-1 Weather Condition and Sea State

As mentioned above, depth profiles of microplastics are mainly affected by turbulence in the surface layer. If the sea surface is choppy, net mouth might be submerged and/or be out of the water, and sampling net will not collect “surface water sample”. Trawl surveys should not be conducted in strong wind and rough sea surface condition. It is also recommended to avoid the area where tide and/or current may be strong.

### ***5-2 Ship Speed and Time Duration***

Trawl surveys should be conducted at a ship speed of 1-3 knots, if there is interest to size distribution of microplastics. In recent studies, trawling speed are usually 2-4 knots (Lattin et al., 2004; UNEP/IOC, 2009; Reisser et al., 2013; Isobe et al., 2014), if the ship speed is too fast, objects collected in sampling net may be increasingly susceptible to fragmentation resulting from abrasion, wave-action and turbulence (Cole et al., 2011).

Time duration of the trawl shot depends on the ship speed, and should be set supposing the amount of floating objects; however, as often conducted in neuston study, every trawl shot should be made for at least 10 minutes.

### ***5-3 Mesh Size of Sampling Net***

It is a natural consequence that the minimum size of debris collected by sampling net depends on its mesh size. The majority of sampling nets used in recent studies have a mesh size of around 333  $\mu\text{m}$ . For example, Ribic et al. (1992) recommended 333  $\mu\text{m}$ -mesh net. In “NOAA Marine Debris Program”, Lipiatt et al. (2013) and Masura et al. (2015) introduced the 330  $\mu\text{m}$ -mesh and 335  $\mu\text{m}$ -mesh net, respectively. Isobe et al. (2014) used neuston net with mesh size of 0.35mm. Therefore, recommended mesh size of sampling nets is around 333  $\mu\text{m}$ , in this guidance. All the particles smaller than the mesh size go through sampling net.

### ***5-4 Personnel***

Required personnel include technical experts, who are experienced in or trained in the use of sampling net and visual separation.

## **6. Data and Metadata**

### ***6-1 Classification of Microplastics***

As mentioned in 3-4-2, microplastics can be classified by external characteristics. Their color, shape, dimension (length/width), wear etc. may be close observed and recorded. Microplastics can be counted and weighed for every different size categories or polymer types. Although there is no universally authorized classification of microplastics has, it should be decided based upon previous works, depending on observation purpose.

### ***6-2 Concentration of Microplastics***

For quantitative comparisons of different surveys, it is strongly recommended that concentration of microplastics should be reported in either unit among next ones: [ $\text{items}\cdot\text{km}^{-2}$ ], [ $\text{items}\cdot\text{m}^{-3}$ ] and [ $\text{mg}\cdot\text{m}^{-3}$ ].

Sea surface area skimmed by sampling net can be calculated multiplying net mouth width by trawl shot length. Consequently, water volume passed through the sampling net can be estimated from the area found above and net mouth height under water. To measure water volume directly and more exactly, sampling net should be equipped with a flowmeter, calibrated under the calm sea surface condition, at

the net mouth (e.g. Lippiatt et al., 2013; Isobe et al., 2014).

### 6-3 Metadata

Metadata should include following information:

- Survey name, date, time, location
- Ship characteristics: Name, type (e.g. research, fishing, regular ferry etc.), tonnage, length, width etc.
- Detail of trawl shot: latitude/longitude of start/end point, trawl shot length, ship speed, time duration
- Detail of sampling net: Type, dimension (net mouth width and height), mesh size
- Environmental conditions: wind speed/direction, wave and swell (direction/height/period), surface current etc.

## 7. Concluding Remarks

Surface water trawling survey can provide valuable information on the concentration and types of microplastics. From results of previous trawl surveys, however, variability in amounts of microplastics may be considerably large. Therefore, increase of sample water volume or number of trawl surveys will be expected for a given level of confidence. Careful consideration to survey design and standardization should be paid in order to develop robust estimates of concentration of microplastics.

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# Floating Marine Pollutants (Shipboard Sighting Surveys for Macro-Debris Items)

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## 1. Introduction

Floating marine pollutants (debris) are widely distributed in the world's oceans. They can comprise anything from cigarette butts and plastic bags to discarded or lost fishing nets, endangering marine and coastal wildlife by their ingestion and entanglement; interfere with navigation; cause economic losses; and threaten human health and safety. Above all, plastics are the most prevalent of marine pollutants, and remain in the environment for a long time and have a negative impact upon marine ecosystems. Moreover, with time they break down into small fragments becoming difficult to remove. The source of these pollutants source is human activities, especially improper waste disposal and mismanagement of trash and industrial products.

In this chapter, the dedicated shipboard sighting surveys for visible size debris from an elevated platform on a moving ship are mainly described. Other possible platforms are airplanes, with the disadvantage that only large debris items could be determined. In fact, shipboard sighting surveys do not necessarily need to occur as a stand-alone activity, and are relatively easy method for crowd-sourcing marine debris sighting (i.e. from ships of opportunity). In spite of their differences in environmental factors, they can provide useful information on the spatial and temporal variability of floating debris. Nonetheless, it should be noted that results of sighting survey will be skewed toward larger debris items.

## 2. Objectives and Setting of Surveys

### 2-1 Objectives

Sighting surveys collect information on the distribution and amounts of visible floating debris during specific time periods. The most common variables of interest for open-ocean sighting surveys are density and types of pollutants. Therefore, typical objectives for open-water sighting surveys are, for example, as follows:

- to identify types of floating marine pollutants;
- to estimate densities of floating marine pollutants;
- to detect temporal and spatial variation in the occurrence of floating marine pollutants.

### 2-2 Site Selection

There have been many studies on macro-debris in beaches and coastal waters, but little information about abundance and types of floating marine debris in the open-ocean. The survey sites should be chosen in consideration of the following matters:

- to deliberate on sources and transportation process affected by the wind system and the major currents;
- to focus on areas that are known to accumulate pollutants;
- not to impact on marine ecosystems by the navigation of the observation ship.

### ***2-3 Frequency and Timing of Surveys***

For long-standing monitoring in fixed region, the minimum sampling frequency should be annually. Ideally quarterly sampling is recommended, allowing an interpretation on seasonal changes.

Bearing in mind that the sighting survey for floating debris is affected by environmental factors, in particular, sea state and wind speed. Thus the sighting survey should be conducted after a minimum duration of calm sea, so that there is no bias by floating debris which has been mixed into the water column by recent storms. In addition, it is desirable that the wind speed should be less than 2 in Beaufort scale (i.e.  $3.3 \text{ [m s}^{-1}\text{]})$  (DeFishGear, 2013).

## **3. Field Measurement**

### ***3-1 General Information***

Sighting surveys must be conducted in the daytime, from the sunrise to the sunset. Observer(s) on a moving ship stand on the bridge or other elevated section, and continuously focused on the port or starboard side of the ship. Observer heights above the water line vary according to the type of ship. The cruising track does not need to be straight, although it is easier to handle the data. Observer(s) will record type, size, number and other information on the provided data sheet, as well as ancillary data (date, time, latitude, longitude etc.), whenever floating pollutant is found (it may be convenient to use tablet computer instead of the data sheet). If the line transect sampling (see Figure 4-2) is adopted, perpendicular distance from the cruising track (or sighting distance and angle from the bow) must be also recorded.

It is desirable that observer(s) use only glare-free side of the ship, especially in dedicated surveys. Debris object should be detected with the naked eye, and binoculars can be used only for the purpose to confirm the identity or to estimate sizes of objects. The number of observers on a survey varies, but it is recommended that a minimum of two observers be employed in any survey (Ribic, 1990).

### ***3-2 Equipment***

The observation ship must equip GPS unit for determination of ship position and speed. It is desirable that some system for visually marking the observation area also can be used.

As mentioned above, debris object should be detected with the naked eye, polarizing glasses may be useful for protecting eyes from strong sunrays and glare of sea-surface. Binoculars (8 to 10-power might be adequate) are indispensable to sighting survey. Range finder and/or inclinometer can be used to measure the distance to detected debris. Digital cameras are also useful to re-confirm the identity of debris, to re-estimate sizes of objects, and to record detailed images of floating debris with the complicated appearance.

### 3-3 Variables to Consider

#### (1) Weather Condition

It is desirable to avoid making sighting surveys under poor visibility. For example, Yoshida and Baba (1985) made no surveys when visibility fell below 200m. Unfavorable sea state, that is high wave and swell, also affect detectability.

#### (2) Characteristics of Debris

It is well known that color, size, shape, and buoyancy of floating objects affect their detectability. Large and fine-colored debris are found with ease. Especially, buoyancy has a large effect; light plastic bottles appeared from the sea surface can be much easily detected than fishing nets floating just beneath the sea surface. If the line transect approach is taken, the detection function should be configured for each category of debris.

#### (3) Vessel Variability

Ship speed and observer heights above the water line affect detectability of floating debris. On large vessels, observers are typically stand higher above the water line and farther from the bow, which causes objects close to the bow to become undetected. It is considerable fact that ship body shape might also make undetectable area.

#### (4) Personnel

Sighting survey of floating marine pollutants should be made by dedicated observer(s) who do not have other duties at the same time (Ribic et al., 1992; DeFishGear, 2013). Required personnel include at least two observers experienced or trained in sighting objects floating at sea, who can measure distances and angle or who can use measuring equipment (range finder, inclinometer, binoculars with reticles etc.). Experienced observers often detect more debris items and estimate distance more accurate than inexperienced observers. Novice observers should be trained pairing with skilled observers.

## 4. Data and Metadata

### 4-1 Pollutant Density

It is strongly recommended that density of debris should be reported in unit of [*items km<sup>-2</sup>*]; not in [*items km<sup>-1</sup>*] etc., for quantitative comparisons of different survey. Scanned area can be calculated based on the transect length and width. The transect length will determined from the latitude and longitude of transect start and end points. Therefore, the ship position should be recorded whenever a course-change occurred.

Calculating formulas of the pollutant density are given, dividing the number of detected objects by scanned area. The method of scanning area estimation should be selected from following two approaches, depending on the actual situation of the survey.

### (1) Strip Transect Approach

When a strip transect approach is adopted, only debris within a specific distance from the side of the ship are detected (Figure 1). It is assumed that all objects within the strip transect are counted and any objects seen outside the strip transect are not recorded.

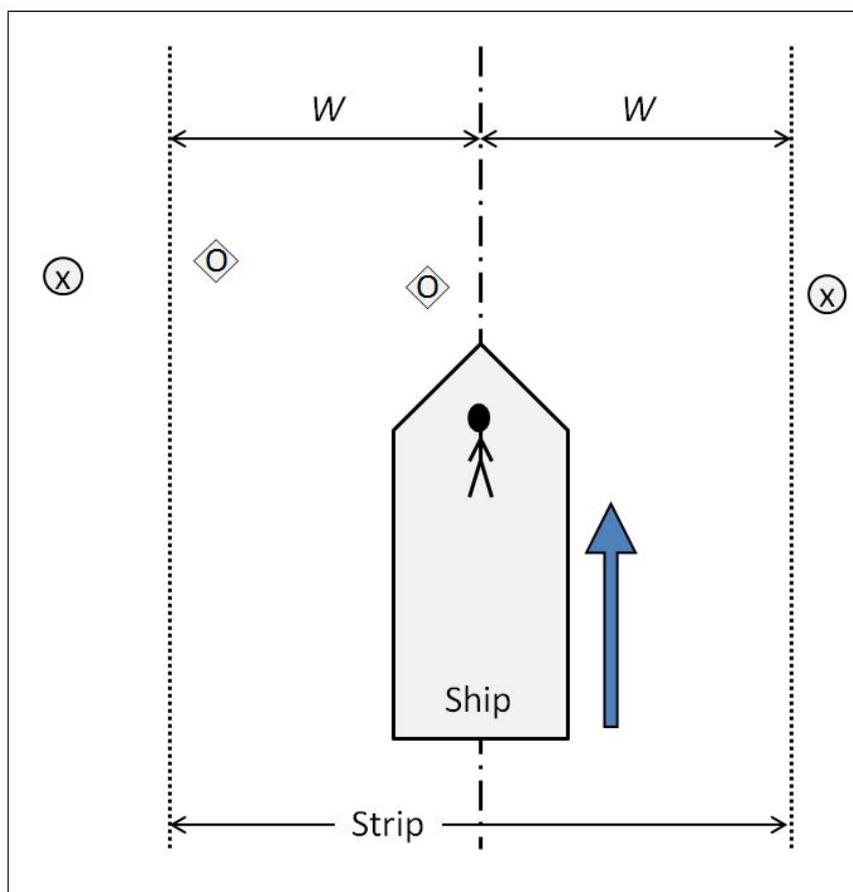


Figure 1 Schematic diagram of a strip transect [after Ribic et al., 1992].

$W$  is strip width. Objects labelled "O" are inside of the strip and can be recorded, while object labelled "x" are outside of the strip and not recorded (even if detected).

The practical strip transect width will depend on the debris size and ship speed. In case that surveys ensure the detection of debris at 2.5cm size, the preliminary strip transect widths based on observation height and ship speed are given in Table 4-1. The strip widths most commonly used are 50m and 100m (Ribic et al., 1992), or maximum perpendicular distance from the transect center to detected debris (Thiel et al. 2003; Shiimoto and Kameda, 2005), for larger debris sighting. Generally, however, these suggested strip widths need to be tested (Lippiatt et al., 2013) and should be determined taking into account survey objectives.

Table 1. Preliminary strip transect widths from different observer height above the water line and the ship speed (MSFD Technical Subgroup on Marine Litter, 2013).

Observer height above the water line	Ship speed		
	2 knots	6 knots	10 knots
1 m	6 m	4 m	3 m
3 m	8 m	6 m	4 m
6 m	10 m	8 m	6 m
10 m	15 m	10 m	5 m

Finally, the density of floating debris is given by

$$D = \frac{N}{A} = \frac{N}{2 \cdot W \cdot L} \quad [\text{items } km^{-2}]$$

where

$N$ : number of object counted,

$A$ : scanned area [ $km^2$ ],

$W$ : width of strip transect [ $km$ ],

$L$ : length of transect [ $km$ ].

## (2) Line Transect Approach

When a line transect approach is adopted, all objects are assumed to be counted regardless the distance from the observer, and perpendicular distance from the ship to the object is measured (Figure 2). However, probability of detection equal 1 on the transect center line, and will decreases according to “detection function” with perpendicular distance. For example, commonly employed detection functions are:

i) The half-normal:  $g(x) = \exp\left(-\frac{x^2}{2\sigma^2}\right)$ ,

ii) The exponential:  $g(x) = \exp\left(-\frac{x}{\sigma}\right)$ ,

iii) The hazard-rate:  $g(x) = 1 - \exp\left[-\left(\frac{x}{\sigma}\right)^{-b}\right]$ ,

where,  $x$  is perpendicular distance,  $\sigma(> 0)$  and  $b(> 1)$  are, respectively, the scale and shape parameters to be estimated based on survey data.

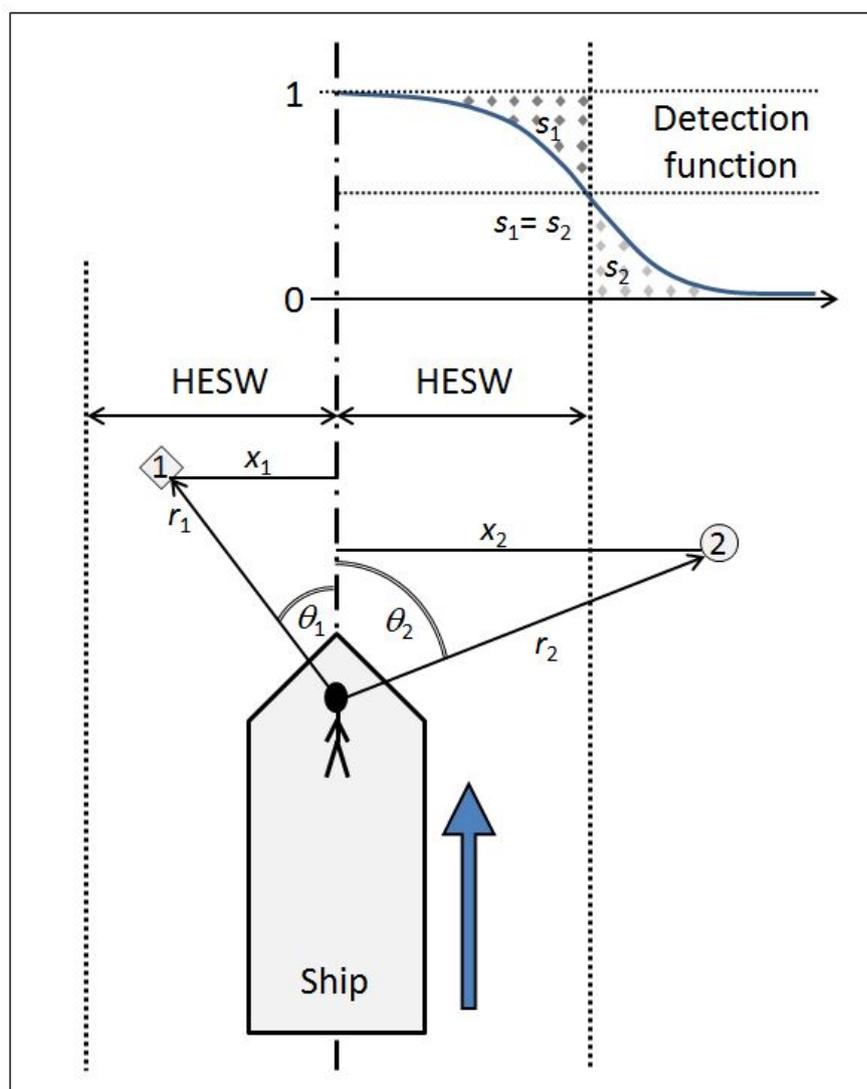


Figure 2 Schematic diagram of a line transect [after Ribic et al., 1992].

All objects are assumed to be recorded regardless the distance from the observer. Perpendicular distances to the object  $i$  ( $X_i$ ) can be estimated directly when they pass just beside observer, or can be calculated from sighting distance ( $r_i$ ) and angle from the bow ( $\theta_i$ ). Using “detection function”, HESW, within which theoretically all floating debris are detected (meaning in upper part of the figure,  $s_1 = s_2$ ), can be estimated.

Using an adequate detection function, “Half the effective strip width: HESW”, within which theoretically all floating debris are detected, is estimated. Finally, the density of floating debris is given by

$$D = \frac{N}{A} = \frac{N}{2 \cdot HESW \cdot L} \quad [items \ km^{-2}]$$

where

$N$ : number of object counted,

$A$ : scanned area [ $km^2$ ],

$W$ : Half the effective strip width( $HESW$ ) [ $km$ ],

$L$ : length of transect [ $km$ ].

More precisely, detection function should be configured for each category of debris, as mentioned above.

For further detail of line transect sampling method, see Buckland et al. (2001).

#### **4-2 Classification of Floating Marine Pollutants**

##### (1) Size Classes

Floating marine pollutants in the size of a 2.5cm to 1m (in the longest dimension), so-called “macro-debris” (GESAMP, 2015), should be monitored. However, sighting survey will not permit the correct measuring of object sizes, the following size classification, introduced by DeFishGear (2013) and MSFD Technical Subgroup on Marine Litter (2013), are suggested as an example ( $L$  denotes the longest dimension of the pollutants):

- i)  $2.5cm \leq L < 5cm$
- ii)  $5cm \leq L < 10cm$
- iii)  $10cm \leq L < 20cm$
- iv)  $20cm \leq L < 30cm$
- v)  $30cm \leq L < 50cm$
- vi)  $50cm \leq L$

These classes can be merged appropriately at statistical processing. When small debris cannot be detected owing to observer heights, the lower limit size should be changed.

##### (2) Identification of Debris

The categories of items for floating debris are desirable to be consistent with the categories selected for beach litter, seafloor litter and others. For example, UNEP/IOC (2009) introduced seven large classes based on their appearances as follows: i) Containers, ii) Fishing & Boating, iii) Food & Beverage, iv) Packaging, v) Sanitary, vi) Smoking and vii) Other. The Master List of items (MSFD Technical Subgroup on Marine Litter, 2013) include eight large groups; i) Artificial polymer materials, ii) Rubber, iii) Cloth/textile, iv) Paper/Cardboard, v) Processed/worked wood, vi) Metal, vii) Glass/ceramics, viii) Others (chemicals, food waste, unidentified).

However, the world common classification does not yet exist. For the practical use during the monitoring, the categories of items can be arranged by object occurrence frequency so that the data acquisition can be easily done in short time.

### 4-3 Metadata

Metadata should include following information:

- Survey name, date, time, location
- Ship characteristics: Name, type (e.g. research, fishing, regular ferry etc.), tonnage, length, width etc.
- Detail of transect: latitude/longitude of start/end point, ship speed, observer height above the surface line, total distance covered by transect, scanned area
- Environmental conditions: wind speed/direction, visibility, wave and swell (direction/height/period)

## 5. Concluding Remarks

Shipboard sighting survey for floating marine pollutants can provide valuable information on the density and types of marine debris. However, uncertainty in categories of items for floating debris stays as an unsettled problem yet. Careful consideration to survey design and standardization should be paid in order to develop robust estimates of floating marine pollutants density.

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