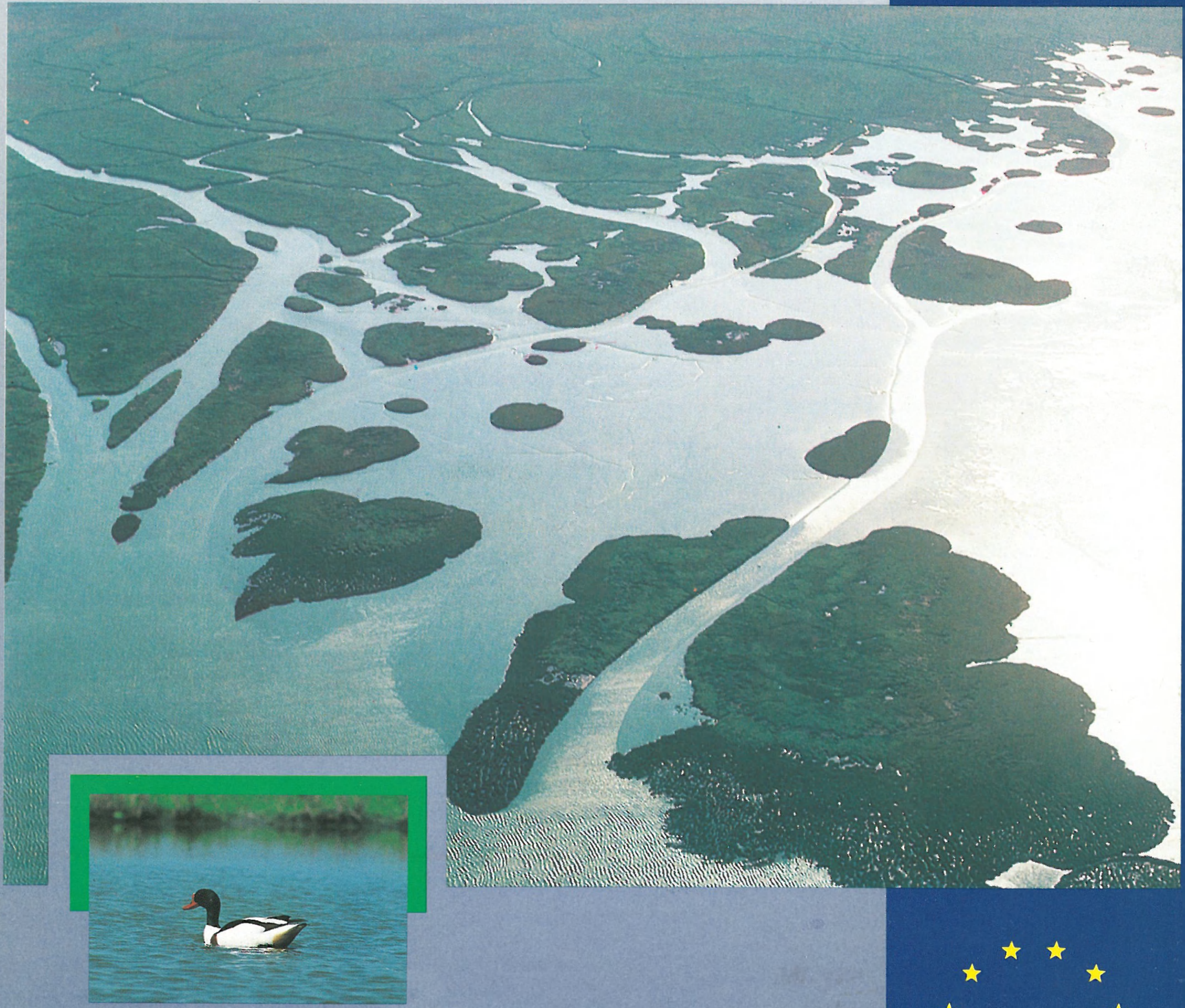
Jeep 92

Major Biological Processes in European Tidal Estuaries

Manual of sampling and analytical procedures for tidal estuaries



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Manual of sampling and analytical procedures for tidal estuaries

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Manual of sampling and analytical procedures for tidal estuaries

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Joint European Estuaries Project - JEEP92

JEEP92: A Joint European Estuarine Project
on
MAJOR BIOLOGICAL PROCESSES IN EUROPEAN TIDAL ESTUARIES

Project co-ordinator:

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Objectives:

To study the biological processes that influence the fate of organic matter in estuaries by:

a) **Comparison of the ecological structure of major European estuaries:**
   - co-ordinating the simultaneous determination of a selected set of state variables relevant to an ecosystem model in the Elbe, Ems, Scheldt, Somme, Gironde and Shannon;
   - providing a manual on approved methods to be used in the survey work;
   - compiling the results in a data base to make them available to participants and later to the scientific community.

b) **specific co-ordinated studies on the rates of major processes** on short and medium time scales in the field in the Scheldt, and Gironde,, and in laboratory experiments. Research will be conducted on occurrence and functional role of zooplankton and hyperbenthos, and on the effect of manipulations of food supply to the benthos.

c) **building mathematical ecosystem models describing the major biological processes** in the estuaries studied, which are based on appropriate hydrodynamical models, and incorporate field data and results from process studies.

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MICROPHYTOBENTHOS

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CONTRIBUTORS TO THIS MANUAL

Participants in the JEEP92 programme, the JEEP92 Arcachon and Plymouth workshops, and several others, have contributed to the contents of this manual through suggestions, discussions and advice. The authors want to express their gratitude to all for their stimulating support.

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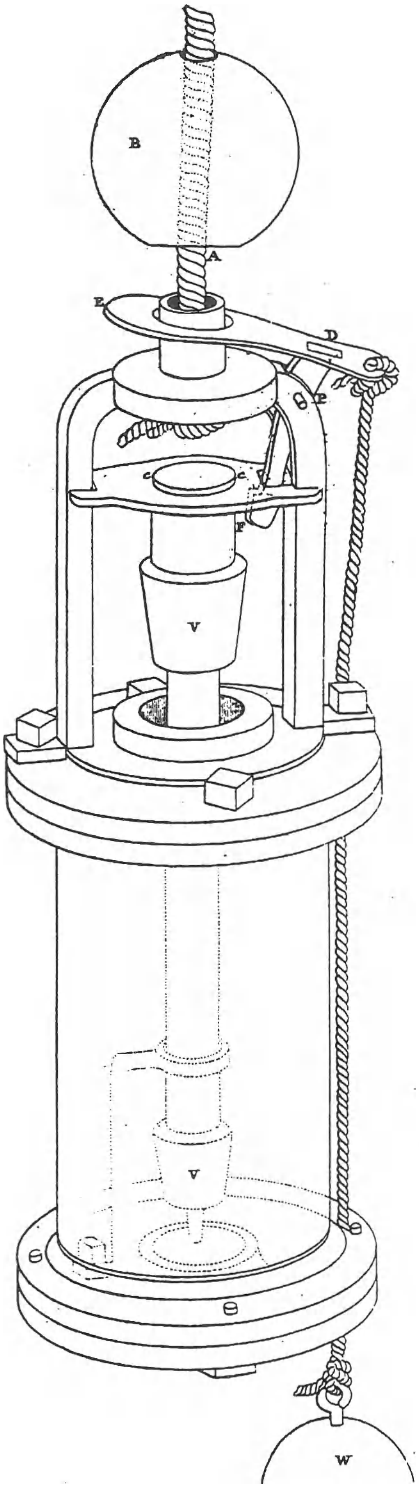

The past:
Improved water sampler of Marcet (Marcet, 1819)

EXECUTIVE SUMMARY

This "Manual" refers to a set of methodological descriptions of sampling strategies, samplingtools and methods for the study of chemical and biological variables in water and sediments of tidal estuarine environments. A detailed description of both the sampling strategy and procedures, as well as the analytical methods, are presented. Comparisons between methods are made in terms of their optimal value within the estuarine research context. The sampling methods and analytical techniques aim at the study of major biological processes in estuarine systems and involve the abiotic (e.g. organic matter, nutrients, S, T, pH, oxygen, light) and biotic compartments (bacteria, phyto- and zooplankton, micro- and macrophytobenthos, meio- and macrofauna). As not all variables are equally important to understand the various processes in the estuarine environment, a ranking (essential, desired and specialistic) has been introduced in the JEEP92 programme (table 5).

The set is based on descriptions in the literature, and involves minimum and optimum sampling schemes (frequencies and spatial distribution).

For reasons of easy comparison of results within the JEEP92 programme, preferred sampling techniques and analytical methods are proposed.

Minimum requirements within the JEEP92 project guarantee the establishment of a seasonal (pseudo)synoptic, structural and functional comparison of European tidal estuaries. Gradients and tidal variation of the ecosystems include only the main parameters at some selected seasonal situations.

The following set of sampling procedures is to be considered as the minimum programme within the JEEP92 project. Many aspects will be easily incorporated in already existing programmes, and several aspects may be combined into one operation. If logistics make it impossible to collect samples at the preferred period of the tidal cycle, the time (hours) after low water should be recorded.

JEEP92 minimum programme:

Preliminary survey (not obligatory)

(see section 4.2)

Aim:

to understand the general hydrodynamics of the estuary, especially the salinity distribution in relation to time and space . Other variables may be included (e.g. temperature, dissolved oxygen, fluorescence). The result of this survey will be the basis the selection of benthic sampling stations.

Sampling 1:

vertical profilesof salinity along the entire salinity range of the estuary (CTD or ST preferred);
minimal 3 samples per location (0.5 m, mid-depth and 1 m from the bottom), continuous recording is preferred.

Sampling 2:

at a minimum of 2-4 locations, based on expected salinity regimes, tidal variations in salinity are recorded over a full tidal cycle (13 h). This should be performed at minimum and maximum river discharge.

Effects of tidal cycles and river discharge

(see sections 4.3, 4.4 and 4.6)

Aim:

to collect samples that give an insight into the effects of tidal cycles, on a daily scale (high-low water cycle), on a fortnightly scale (spring-neap tide cycle) and on a seasonal scale (river discharge)

Locations:

one sampling station should be selected that falls within the salinity range 9 - 15 x 10^{-3}; a second option is the turbidity maximum.

Time:

samples should preferably be collected:

- once at the period of maximum river discharge, and once at the period of minimum river discharge;
- in the period half way between springand neap tide (additional samplingat two subsequent springand neap tide events is highly recommended);
- for at least 1 full tidal cycle, 13 h (25 h is preferred to detect also the diurnal cycle)
- at 1 hour intervals.

Sampling:

samplingat mid **water depth**.

Routine sampling, water, including seston and organisms

(see sections 4.5 and 4.6; tables 3 and 4)

Aim:
to collect water samples for the analysis of dissolved and particulate matter and of suspended biota , along the axis of the entire estuary;

Locations:
at locations that are determined by salinityof the water column, at salinities about every 3×10^{-3} (table 4);

Time:
each sample should preferably be collected

- throughout the year, with higher frequencies in the spring-summer period, and dependent on the variable under consideration (table 3);
- half way between spring tide and neap tide,
- at the period half way between high and low water, sampling: number of replicates in tables 3 and 4 collectionat mid-water depth.

Routine sampling, sediment, including organisms

(see sections 4.5 and 4.6; tables 3 and 4)

Aim:

to collect sediment samples and benthic biota at selected locations along the axis of the estuary.

Locations:

at fixed locations that are determined by the ranges in salinity, and based on the simplified Venice classification (tables 2 and 4);

Time:

the samples should be collected twice a year in spring and in autumn; sometimes higher frequencies are preferred, depending on the variable/ organism under consideration (table 3);

Sampling:

number of replicates in tables 3 and 4.

Sampling depth is set to:

- for physico-chemical characterization of (surface) sediments the top 2 cm,
- micro-phytobenthos is sampled in the top 0.5 cm,
- meiobenthos is collected in the upper 5 cm,
- for macro-zoobenthos the top 25 cm should be sampled.
- for hyperbenthos the lower 1 m of the water column during daylight hours

Analytical procedures **and priorities**

(see chapter 7)

A list of variables that are considered important for the JEEP92 programme is given in the table 5 with a priority 1. Priority 2 analyses are desirable and priority 3 analyses are for specialists.

Protocol

(see chapters 5 and 8)

A field data inventory form (table 6) has to be completed to provide all relevant background information on the sampling event (e.g. station number, sampling time, location, tidal stage, weather conditions, information about the samples and a list of samples that are collected). To allow comparison and interpretation of data a JEEP92 data base has been developed. Standard formats are therefore required. Units are given in table 5, JEEP92 data entry (sub)codes for sampling and analysis are summarized in Annexes II and III.

1. PREFACE

The objective of the EEC sponsored JEEP92 project (Joint European Estuaries research Project) is to study the major biological processes that influence the fate of organic matter in European tidal estuaries(Elbe, Ems, Scheldt, Somme, Gironde, Shannon, and others). This involves the simultaneous determination of a selected set of state variables relevant to these major biological processes, and which involve both chemical and biological data in the water column and in the sediment . The data set will be collected by the individual participants of the project, who carry out the data collection within their respective national programmes. This means that, in principle, the participants will apply their own proven methods and methodologies within their national estuarine programmes. As the JEEP92 project combines these separate researches in an intercomparison of the different estuarine ecosystems in a mathematical model, it is essential that the data are comparable in terms of collection(sampling time and location, strategy) and analysis. It appears that data collection and interpretation are not easy tasks in the estuarine environment, mainly due to inhomogeneity and temporal variation. Under these circumstances, in general, carefully designed multi-parameter sampling programmes are necessary. Especially careful design with respect to space and time is essential.

Because of the physical and hydrodynamical differences between the various estuaries which are often highly dynamic systems, and the logistic problems that occur in estuarine research, biological processes are usually only partly understood. Comparison between different estuariesmay help to solve this problem.

Ideally only those methods of sampling and/or analysis that give the same result are to be accepted, even if the methods have a different principle of methodology. For example, even though the calculation of salinityfrom the analysis of chlorinityand the determination of conductivityare basically different, the final result is very closely similar. Many methods for sampling and analysis are now standardized, sometimes for decades (e.g. chlorinity). Sometimes, however, several methods are in use that give non-identical results (e.g. DOC, POC). It seems simple to solve the problem by appointing one method as "the best" method, but it is often not clear what "the best" means. This is especially the case in sampling strategies and methods, and with new or improved analytical techniques.

Although this manual aims to establish identical methods and methodologies for application to all estuarine systems involved in the JEEP92 programme, the authors realize that not every participant will always have the technical means to apply each preferred method. In addition, it seems that where several methods are in use, every scientist is willing to cooperate to adopt a commonly accepted method in an international programme, but only provided that the selected method will be his own. Their is a strong need for intercalibration of the various techniques that aim at the same result.

This manual summarizes the samplingand analytical techniques that can be applied and proposes, in each case one or a few methods that should preferably be applied within the JEEP92programme, with a view to standardization. This manual tries to make an inventory of the different options and to guide estuarine scientists towards a common use of techniques and procedures , to enhance comparability of data.

It is not the intention of the authors to copy all handbooks either on physico/chemical and biological samplingtechniques in sea- or estuarine waters or on the different analyses. For detailed descriptions the reader is referred to these handbooks. Most laboratories will have at least some experience in many of the techniques described.

The use of names of manufacturers or brand names is sometimes unavoilable in a manual like this. It should be mentioned that the authors do not endorse these products.

The authors hope that, through this manual, more and more methods will become standardized throughout the estuarine project of JEEP92, and perhaps even more generally.

Collection of samples is usually the basis for marine and estuarine research. The proper method of collection has always intrigued scientists, many ideas have been formulated and ingenious instruments have been designed and tested. The present instrumentation in oceanography often finds its roots in these designs, which can be over one hundred years old.

Blank pages in this volume serve as a tribute to those inventors of instrumentation and techniques. Interesting graphs have been reproduced as non numbered figures. They originate from: Marcet (1819); Richard (1907); Rouch (1943); Thoulet (1890, 1912); Tornøe (1880).

The editors have tried to prepare this manual as clearly as possible, to ensure easy access to the information. We appologize if this has not always been successful. A manual like this will almost inevitably provoke discussions and comments. We are willing to receive any comments or corrections on the present volume, and welcome any suggestion for its further improvement.

2. INTRODUCTION

Estuaries are the areas where fresh and marine waters meet. They are consequently zones of transition, often with strong gradients and discontinuities. These are observed in the distribution of the chemical components, both major compounds like salt and suspended particulate matter (SPM or seston), and minor constituents, for example nutrients, organic matter , oxygen, etc. Estuaries are also highly dynamic zones where, for example, tidal effects exert a major influence on the influx and efflux of materials and the transport of dissolved and particulate materials within the estuarine boundaries. High turbidity zones (turbidity maxima), formed as a result of tidal circulation processes, are almost unique for estuarine environments, and are considered to be (bio-geo)chemically highly reactive.

These conditions have consequences for the estuary as a habitat for pelagic and benthic organisms. Water quality (especially temporal and lateral salinity distribution, but also nutrient regimes, oxygen concentrations etc.) and sediment quality (grain size distribution, carbon content, the stability of sediment etc.) will determine which species are present and in what (relative) amounts.

Each estuary will, because of its specific geological and geographic structure, its hydrodynamics, the amount and quality of inflowing fresh water, and many other factors, be very specific: it will in fact be unique. Due to climatological changes, interferences of the diurnal light cycle, changing tides and wind forces, etc., the specific situation in an estuary at any one time will always differ from situations measured before. Much estuarine research aims to investigate the system not only in a descriptive way, but also to define and quantify the typical processes that are biological, chemical and/or physical in nature. In theory the nature of these processes should not be limited to the estuary under consideration, but have a more universal character.

Comparison between different estuaries therefore seems a useful approach to investigating general estuarine processes. In practice it is highly preferable for the same group of scientists to collect the data in the various estuaries. Comparisons are difficult to perform if one has to rely on literature data only.

In a programme where data on different biological and (geo)chemical characteristics of tidal estuaries are to be collected and compared, it is essential that similar strategies and methodologies are used, and that through intercalibration the comparability of the data is tested, especially when the resulting data are to be used in mathematical modelling.

Additionally, the development of a consistent international approach and

harmonisation of methodologies may provide a training element to enhance transfer of knowledge to laboratories that are less experienced in one or more fields of inter-disciplinary interest. It will certainly stimulate a more integrated approach to the study of estuarine biogeochemical processes.
Much information on the methodology of the collection of physical data and of chemical and biological samples from the marine environment is at present available (see for example the COST 647 programme for biological sampling in coastal systems). These existing accounts are usually only relevant to specific programmes, and usually cover only a limited amount of methods and techniques, due to their specialised scope.
Additionally, estuaries provide very specific biogeochemical characteristics, that make sampling operations and procedures difficult to design. For example tidal currents, salinity, pH, oxygen, nutrients and organic matter concentrations, etc., show often steep gradients, which influence the species distribution and diversity even over small spatial scales. Tidal currents will influence the suspended matter concentration and the relative contribution of resuspended sediments. It is felt that the interpretation and comparison of many historical data sets from estuarine regions is hampered by random sampling procedures, ignoring physico-chemical variability.
In recommending the following set of procedures we have not simply advocated those which are theoretically the best but often cannot be realized, but instead we have adopted a more pragmatic approach which provides an optimum between applicability, availability and quality of the methods.

Wherever possible, internationally accepted procedures and strategies are the ones which are recommended in this manual.

This manual is based on the common experience of the authors and the participants in the JEEP92 programme and reflects the current knowledge of sampling strategies and analytical methods as found in the literature.
Examples of guidelines for the design of strategies and the performance of estuarine sampling programmes can be found for example in:

- Anon., 1990. Guidelines for the sampling and analysis of trace metals in seawater under the Joint Monitoring Programme (JMP). Annex to the recommendations at the fifteenth meeting of JMG, Lisbon, 1990.
- Baker, J.M. & W.J. Wolff (eds), 1987. Biological surveys of estuaries and coasts. EBSA Handbook, Cambridge Univ. Press, Cambridge, pp. 449
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- Jeffrey, D.W., J.G. Wilson, C.R. Harris & D.L. Tomlinson, 1985. A manual for the evaluation of estuarine quality, 2nd ed. Univ. of Dublin, pp. 161

- McIntyre, A.D., J.M. Elliot & D.V. Ellis, 1984. Introduction: design of sampling programmes . Methods for the study of marine benthos. N.A. Holme & A.D. McIntyre (eds). 2nd ed. Blackwell, Oxford, pp. 1-26
- Morris, A.W., 1990. Guidelines for monitoring estuarine waters and

suspended matter. UNEP Regional Seas Programme. Reference methods for Marine Pollution Studies. In press
- Sournia, A. (ed), 1978. Phytoplankton manual. Unesco, Paris, pp. 337
- Thistle, D. & J.W. Fleeger, 1988. Sampling strategies. Introduction to the study of meiofauna . R.P. Higgins & H. Thiel (eds). Smithsonian Inst. Press, Washington DC, pp. 126-133

More general works dealing with estuarine research are, for example:
- Aston, S.R., 1981. Estuarine chemistry. In: Chemical oceanography, 2nd ed. Vol. 7, J.P. Riley & R. Chester (eds). Academic Press, London, pp. 361-440
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- Olausson, E. & I. Cato (eds), 1980. Chemistry and biogeochemistry of estuaries. Wiley, Chichester, pp. 452
- Perkins, E.J., 1974. The biology of estuaries and coastal waters. Acad. Press, London
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- Zutic, V. (ed.), 1989. Physical, chemical and biological processes in stratified estuaries. Mar. Chem. 32 (2-4), special volume, pp. 111-390.

The present manual addresses the central theme of the JEEP92 project: the study of biological processes that influence the fate of organic matter in estuaries It will therefore be inevitable that not every possible compartment or compound will be treated.
We have set some limits to the present manual:

- the estuary is limited by its geographical borders as defined by Fairbridge (1980) (see chapter 3) which thus includes also part of the freshwaterenvironment and part of the coastal zone. This definition is vague with respect to the seaward boundary of the system, which will usually be chosen based on the coastline morphology. It must be realized, however, that many processes that are occurring in the estuary will also occur in the adjacent coastal area, and that for a proper understanding of many geochemical estuarine processes the open-sea endmember should be known. In other words, seawater samples of a water body that never reaches the estuarine environment should preferably be included in the sampling programme
- the description of the hydrodynamic pattern is only marginally treated; phenomena like fronts or eddies were considered beyond the scope of this project.
- the manual will only cover the aquatic system, including the intertidal zones, but excluding the salt marshes; it should be realized, however, that salt marshes may act as an important source (or sink) of organic matter and many compounds, and that they should be considered in this respect; inputs from the atmosphere are consequently not covered.
- the manual will not cover the sampling and analysis of toxic pollutants (trace metals, organic micropollutants);
- groups of organisms considered important for the present project are limited to bacteria, phytoplankton zooplankton, micro-phytobenthos, macro-phytobenthos, meiofauna and macrofauna. For the study of hyperbenthos no standard methods have been developed yet. In fact this is one of the objectives of the JEEP92 programme. The importance of other groups, like fish, birds and mammals is recognized. However, the present programme focuses on the study of the biological processes that influence the fate of organic matter in estuaries, with special emphasis on the compartments water and sediment . It was agreed that methods of sampling and analysis for these organisms does not fall within the scope of this manual.

3. ESTUARIES

3.1 General considerations

Pritchard (1967) defined estuaries as:

"a semi-enclosed coastal body of water which has a free connection with the open sea and within which sea water is measurably diluted with fresh water derived from land drainage".

This definition is rather vague, and could include many coastal areas that have no direct connection with river inputs . Therefore the definition by Fairbridge (1980) seems more suitable for the present project. It also includes part of the freshwater environment:

"an estuary is an inlet of the sea reaching into a river *valley as far as the upper limit of the tidal rise, normally being divisible into three sectors: (a) a marine or lower estuary, in free connection with the open sea; (b) a middle estuary, subject to strong salt and* freshwater *mixing; and (c) an upper or fluvial estuary, characterized by* freshwater *but subject to daily tidal action."*

The geographical boundaries between these sectors are not fixed. They are subject to the (spring) tidal movements, to seasonal variability (river discharge) and may vary according to water depth (type of estuary). In figure 1 a schematic representation of the various types of estuaries is presented. The estuary types range from those with a highly stratified salt-wedge and a sharp halocline in the vertical structure to well-mixed estuarieswhere a vertical homogeneity is almost reached. A summary of the major types is given in table 1, together with their main features. They are described in more detail by Morris (1983). Recognition of these hydrodynamic factors (i.e. mainly river discharge, wind stress and tidal stress) will affect the design of any sampling programme .

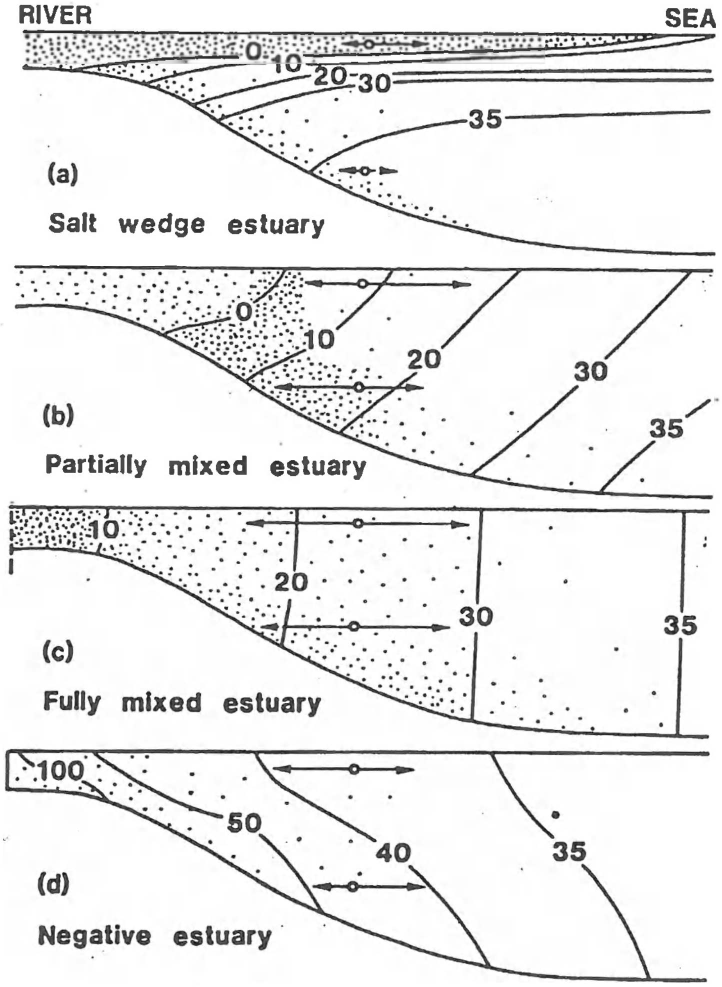


Figure 1
Schematic presentation of types of estuaries. (after Postma, 1980)

Pelagic organisms like phyto- and zooplankton dwell to some extent with the moving waterbody during estuarine circulation . The organisms and water samples may be collected at given preset salinities. Benthic organisms, however, and the sediments, experience the sometimes large and rapid variations in salinity at given locations.

For biological purposes the *Venice classification* (Caspers, 1959) has been designed to sub-divide the estuarine system into rather complicated salinity zones. Table 2 presents the full Venice classification . For practical purposes a simplified zonation is introduced for use in this manual.

Table 1.
Estuarine types according to mixing and circulation (after Pritchard, 1955; Dyer, 1979)

description	**mixing process**	**vertical structure**	**type**
highly stratified salt wedge	river flow dominates	very sharp halocline	A
fjord	river flow & entrainment	sharp halocline	
partly mixed	river flow & tidal mixing	increasing S with depth	B
well mixed	tidal mixing dominates	nearly vertical homogeneity	C or D (with lateral S gradient)

Table 2.
Venice system classification of estuarine waters into salinity zones (Caspers, 1959)

Venice zonation	**range (10^{-3})**	**salinity zonation**	**simplified**
Hyperhaline		> 40	
Euhaline		40 - 30)	Euhaline
(Mixo)euhaline		> 30 but < adjacent euhaline sea	
(Mixo)polyhaline		30 - 18	) Polyhaline
(Mixo)mesohaline		18 - 5	)
α-Mesohaline		18 - 10	) Mesohaline
β-Mesohaline		10 - 5	)
(Mixo)oligohaline		5 - 0.5	)
α-Oligohaline		5 - 3	) Oligohaline
β-Oligohaline		3 - 0.5	)
Freshwater		< 0.5	) Freshwater

This leads to the simplified scheme (in 10^{-3}) of freshwater ($S < 0.5$), oligohaline ($0.5 < S < 5$), mesohaline ($5 < S < 18$), polyhaline ($18 < S < 30$) and euhaline ($S > 30$). These simplified ranges correspond well with earlier findings, e.g. by Wolff (1973).

3.2 Characteristics of specific estuaries

Important features that characterize estuaries include the morphology, catchment area,river discharge and residence time (for various periods of the year), water volume, tidal amplitude, and general bathymetry. A description of the salinity distribution and the location of the turbidity maximum is required as well. In addition a characterization of the ecology of the estuary in question, subdivided into salinity related sub-regions should be given. The various groups of organisms are to be described in terms of total biomassand production, and should be completed by a short description. Within the JEEP92 programme, an "Inventory of the physical , chemical and biological characteristics of the major European tidal estuaries" will be published as JEEP92 report (De Winter, 1992b). In Annex I a synopsis of this inventory is given. It is subdivided into several sections:

- general description of the estuary, with its tributaries, the countries that interfere with the rivers and or estuaries, human activities, etc.
- economic use, especially related to industrial zones, (major) cities, harbour activities, fisheries, aqua-culture;
- hydrology, emphasizing the catchment area, river discharge, surface area and water volume, residence time, tidal amplitude and location of turbidity maximum, if applicable at different times of the year and at various locations;
- salinity zones, as defined by the biologically important sub-divisions (simplified Venice classification , see section 3.1): freshwater, oligohaline, mesohaline, polyhaline and euhaline. For each salinity zone the average surface area , length & width of the area, the water volume and the residual current should be given;
- bathymetry for the same salinity based sub-areas, where surface areas are subdivided according to water depth;
- biology, where each representative group is presented in terms of biomass and production, again for the different salinity based zones;
- physico-chemical parameters, indicating the most relevant variables.

Where applicable and available, additional maps (e.g. catchment area, estuary, sediment grain-size distribution), transects or time series (like river discharge, seston concentration, salinity, nutrient profiles) add to the value of the synopsis.

Clearly a complete data set will not be available for all estuaries. The JEEP92 project may fill most important gaps, however. For further details, the reader is referred to the synopsis (De Winter, 1992b).

4. SAMPLING STRATEGY

The objective of samplingis to collect a portion of material from an environmental compartment(either water, sediment or biota) small enough in volume to be transported conveniently and handled in the laboratory, while still accurately representing the part of the environment sampled.

This implies that the relative proportions or concentrations of the components of interest will be the same in the samples when they are being analyzed, as they were originally in the environment.

This is a most difficult task, however. Any system, and estuaries in particular, will differ from each other and in time. It seems impossible to set guidelines that are relevant to each system. It is certainly not the intention of the authors to provide a very strict scheme of obligatory procedures. The individual scientific knowledge of the system, the specific conditions at the location and day, will largely determine the sampling programme But, as already mentioned in the preface, some **minimum rules** have to be agreed upon if a comparison of such different systems is the objective. The minimum rules will in general not conflict with already existing programmes.

Several papers and books discuss and advise on sampling strategies in estuarine waters (Anon, 1990; Cairns & Pratt, 1986; Downing, 1989; Jeffrey *et al.*, 1985; McIntyre *et al.*, 1984; Morris, 1983, 1985, 1990; Thistle & Fleeger, 1988; Venrick, 1978). The scientific advice about the actual sampling in these references usually stops at the point where decisions have to be made for specific site selection, core diameter, etc., as different studies have different objectives and it is often considered an interference with individual scientific freedom.

Within this JEEP92 programme which aims at comparison, **standardisation** (to some extent) is considered essential. JEEP92aims at a standardized minimum set of data, sampled and analysed as much as possible according to preset rules. This almost automatically implies that no complicated procedures can be proposed. Minimum sampling strategies are given here, but it should be stressed here that sampling strategies involving more than the minimum set in space and/or time, will inevitably result in a better understanding of the estuarine system and the interrelating biogeochemical processes and dynamics. If large gradients in parameters are to be expected, like during a phytoplankton bloom event, the minimum sampling frequency will be too low, and a sampling frequencyevery one or two weeks is required to cover the environmental changes adequately. Likewise, the number of

locations may be increased.
In most estuaries it should be no problem to incorporate the minimum sampling programme into existing research projects. Scientists are encouraged to adapt (expand) the minimum requirements of place and time of sampling when the changes in the environmental system, or other scientific reasons, require such a step.
A discussion on some of these major constraints follows here.

4.1 Compartments

Each different compartment in an estuary has specific demands for sampling:

- **water**, with the dissolved compounds (by definition all material passing through a 0.45 µm filter);
- **suspended particulate matter** (SPM) or seston, which is an assemblage of living plankton, dead remains of organisms, and inorganic particles that may partly be derived from resuspension .
- **sediment** , which may vary in grain size considerably, even over short distances, and which is usually subdivided into a clayfraction (particles < 2 µm), a siltfraction (between 2 and 63 µm) and a sandfraction (> 63 µm). Sediment is frequently moved and transported by physical action: resuspension and (bio)turbation by (wind induced) waves and currents, by fluctuating tidal motion, and by organisms. Due to these transport phenomena, sorting of the particlesoccurs. In the tidal channels we will find the coarser material, while usually at the borders of the estuary and at the sides of the tidal flats the fine material is deposited.
  It should be realized that as for the SPM, the sediment consists of an organic (organisms, dead organic remains, organic coatings on particles) and an inorganic fraction (sand, silt, clay).
- **biota**, which is actually a large set of sub-compartments, each group of species being a separate sub-compartment.

A major distinction should be made between the organisms that are living in the water column and those in or on the sediment (benthos). The first group will generally float with the moving water (bacteria, phyto-plankton, zooplankton), and may therefore be subject to moderate differences in the water quality due to mixing of the salinity gradient. The benthic organisms on the other hand, have no or only limited possibilities to migrate, and are thus subject to the changing water quality regime under the tidal circulation influence (micro- and macrophytobenthos, meiofauna & macro-zoobenthos). Free, actively moving organisms form a group in between, which may actively search for their optimum environmental conditions (hyper-benthos, fish). These groups are not included in this manual, however.

JEEP92 strategy (compartments):
In order to be able to correlate the different variables that are collected, either from the same or from different compartments , it is considered very important that as many variables as possible are sampled together, if possible in one sample at the same spot and time.
Only then can the results be related to each other.

4.2 Preliminary surveys

The selection JEEP92 of stations and the period of sampling should be based on knowledge of the hydrographic, chemical and biological characteristics of the estuary. In many cases this information will, at least partly, be available from previous studies.
If this information is not available, a preliminary surveyshould be performed (Anon., 1990). The hydrodynamics (variation of the salinitydistribution in space and time) is an especially important aspect of the design of a sampling programme .

JEEP92 strategy (preliminary survey):
The description of the estuary in the preliminary survey is based on a study of salinity **profiles**, both vertically and along the axis of the estuary. It is recommended as a minimum requirement that the salinity profiles along the axis are measured just below the surface (0.5 m), at mid water depth and at 1 m from the bottom. Continuous *in situ* ST or CTD measurements are much preferred, however. The stations for such a survey should be selected over the entire salinity range at approximately equal salinity intervals.
As the understanding of the general hydrodynamics and salinity distribution is the basis for estuarine ecological modelling, some synoptic measurements could be interesting, since the local stratification can be a function of the tidal situation and also because some estuaries can present important cross-gradients. Profiles along cross-sections will be interesting when the estuary is not too large (they would take too much time) or too narrow (they would not be necessary).
For the preliminary survey, at selected locations a **tidal cycle** should be recorded to estimate the biologically important minimum and maximum salinity and temperature at that location. From three or four such tidal stations the salinity ranges for the other potential sampling locations can be estimated. At 2-4 fixed stations the water velocities should be incorporated in the measurement programme.
At least two such surveys should be performed, one at maximum river discharge, and one around minimal river discharge.

For the design of an effective sampling programme , tide tables and an indication of the river discharge over the year are essential.

4.3 Tidal effects

Tidal movement is one of the major driving forces in the (tidal) estuarine circulation . The most important movement in the estuary is usually along the horizontal axis. Particular attention should be paid to the difference in bottom and surface currents, particularly in salt-wedge type estuaries. Other water movements depend on the geography of the estuary, the river discharge and wind stress. The tidal movement will not only be visible in currents, but also in various major variables like salinity, turbidity, etc. (Fanger *et al.*, 1986). The currents will reach maximum speeds in either direction at mid-tide and a minimum of zero at about the change of the tides . This will affect the resuspension /deposition of the sediment and also the transport of pelagic organisms. Due to the tidal effects a more or less sinusoidal change in many constituents occurs in most estuaries. For example, a comparison between the concentration of chlorophyll *a* or of SPM will be hampered when the samples are taken at random periods during the tidal cycle, and the effects of resuspension /deposition are not accounted for.
On a daily scale, tidal effects are almost exclusively due to the M2 tidal cycle, a full tidal period covering just under 12.5 hours. This means that the tidal variation can be followed almost twice a day, which enables the additional monitoring of the diurnal cycle, the day-night rhythm, which is not a tidal but a biological cycle.
The spring-neap tide cycle, active on a fortnightly scale, often has an effect upon the concentration of the constituents in the water column. Large variations in suspended matter load have been observed, for example in the Tagus estuary between spring and neap tide situations (Vale & Sundby, 1987). The fluctuations caused by these events seem important enough to take into account.
Sampling of such complicated dynamic systems requires more or less complex sampling strategies . These strategies often involve the use of a large logistic input, which is not realistic within a JEEP92 programme.
Synoptic sampling, which means that all samples are collected at the same time involving as many sampling teams as stations, or samplingat the same (e.g.) mid-tide period at all locations is the best strategy, but will only be possible in a limited number of occasions within the logistic scope of the present programme.
A second best strategy is when all samples are collected at the same phase of the local tide, preferably around a mid-tide period (half way between the turn of the tides). This period should be calculated accurately for each location from the tide tables. Tide tables only provide information for certain defined locations and gravity influences only, however, deviations can be

significant even over a small spatial scale, or under only moderately changing wind pressure. Therefore, if the actual sampling locations are not mentioned in the tide tables, which will usually be the case, it is necessary to adjust for the difference, either by models or by measurements.
As a less reliable option, one may also decide ad-hoc by salinity or current measurements, the tide phase related sampling time. If logistic means make it impossible to collect samples at the preferred period of the tidal cycle, the time (hours) after low water should be recorded.
There may be a difference between the mid-tides during the flood and during the ebb (e.g. in current speed), but unless these differences reach unacceptable levels, they are, within the scope of this programme, neglected.
An alternative but less favourable approach involves sampling at the turn of the tides , either at high or low water, but the measured situation is certainly not representative of the average situation in the estuary.
For routine analysis, to avoid the dominating effects of spring-neap tide cycles, the samples should be collected in the (mid) period in between the spring tide and the neap tide, and on a daily scale in the mid-period in between the turn of the tides . This can be considered as some kind of average situation.
Additional sampling efforts to cover daily, diurnal and fortnightly processes is highly recommended. Therefore, at least one double tidal period should be covered, and preferably also the spring and the neap tidal situation. This should be combined with the sampling for river discharge phenomena.

4.4 Residence time, river discharge

The concept of residence time of an element is defined as: the ratio of the amount of a variable (dissolved or particulate) in a given environmental compartment (water, sediment or biota) over the amount of the compound supplied (or removed) per unit time:

$$\tau = A/(dA/dt)$$

where A is the total amount of the element in suspension or solution, and dA/dt is the input to the system (Barth, 1952). River discharge will be the most important driving force for the residence time.
Residence times vary between estuaries and within estuaries at different locations and during different periods of the year (seasonal variations), but also within the seasons major variations occur (see also De Winter , 1992b). Long term averages sometimes do not show the large variations that would be expected and may therefore not be used for data interpretation.
In some estuaries, the residence time is in the order of days (e.g. Rhine), and in others it may reach several months (Scheldt, Tagus). A water column sampling programme that takes several days will inevitably result in a set of

samples that have no or only limited relation to each other. The same water mass is not sampled, but a sequence of probably different water masses. A sampling strategy that is (pseudo)-synoptic is favoured here, or a scheme that collects the water samples within 1 to 2 days. This problem is ameliorated in estuaries with a longer residence time, where a collection of samples taking several subsequent days generally presents no problems of interpretation. In the latter programmes it is advisable to collect the samples in a sequence according to the longitudinal salinity profile.

In highly stratified estuaries the surface and bottom waters may behave differently, and have completely different residence times (Dyer, 1991). In these cases straightforward residence time calculation based on inputs of fresh water only is no longer valid. A method of computing the exchange time of freshwater and marine water in a stratified estuary has been described by Legovic (1991).

There are too many interfering processes that act on a seasonal scale. To characterize the changes in estuarine processes on an seasonal /annual scale, which are related to variations in river discharge the estuary should be sampled twice, during the minimum and during the maximum river discharge. These sampling occasions should conveniently be combined with the sampling for tidal effects.

4.5 Density & frequency

Sampling intervals are to be chosen on the basis of the expected frequency with which changes occur. This may vary from continuous recording or sampling every 5 minutes, to several hours or more. In situations where almost no major changes are expected, sampling once or twice a year is sufficient (e.g. benthic sampling).

The (minimum) number of independent samples that should be collected is dependent on the time-related processes one wants to investigate and the variation(s) in the relevant components that can be expected.

The statistical treatment (and requirements) of sampling in biological systems, which can also be applied to the non-biotic sampling, is given by Green (1979), while a discussion on the sample size and number of replicates is presented by Bros & Cowell (1987). The latter will in part depend on the kind of statistical analysis envisaged.

When (semi)-continuous monitoring can be performed (salinity, temperature, dissolved oxygen, pH etc), the sampling requency will inevitably be high. Continuous monitoring may facilitate the selection of sampling times or intervals, e.g. when a rapid change is observed, the frequency of sampling can be increased. Regular sampling intervals are possible, but not a prerequisite.

To follow the tidal effects, sampling every hour over a minimum of one entire tidal cycle (13 h) is necessary. The diurnal variations should be moni-

tored by sampling as a minimum a double set of tidal cycles (25 hours), to cover both the tidal variations and the biologically important day-night variations. The preferred procedure is, however, to sample a time series that covers 48-72 hours, at somewhat larger sampling intervals.
To investigate the tidal effects on a fortnightly scale at least one spring tide and one following neap tide should be sampled, both over one full tidal cycle, at hourly intervals.
Seasonal changes, especially related to the river discharge, require a minimal sampling programme that covers the maximum and minimum discharge periods, which should in principle be co-ordinated with the tidal stations discussed earlier.
In table 3 the frequency of routine sampling and minimum number of independent replicate samples per type of sampling activity, is indicated. These should be regarded as the JEEP92 minimum. It will be clear that the frequency is dependent on the expected variation in the compartment: in the water phase samples are collected at least at monthly intervals, while sediment (benthos) collection is basically carried out only two times per year: just before the start of the spawning season as a measure of survival over the winter period, and in the early autumn to estimate the recruitment (Essink & Beukema, 1991). Table 3 tries to combine practicality (logistics) and desired sampling frequency.

4.6 Sampling locations (salinity, distance)

One of the major characteristics of an estuarine system is the variation in the distribution of salinity. Many processes, either physical , chemical, or biological, are related to the salinity. It is therefore an acceptable procedure to design a sampling programme that is based on salinity rather than on location, like every 5 or 10 km. The latter method may result in a large number of samples within a limited salinity range, which do not really reflect the estuarine distribution of the compounds or biological assemblages in question. However, in the upper estuary, above the salt intrusion, the method of salinity based sampling is inappropriate and here a km scale might be used instead. An additional advantage of a sampling scheme based on salinity, rather that on location, is that processes can be compared in different estuaries on the same basis.
Salinity is generally accepted as the main index of mixing of seawater with water from the major river. The possible disturbing effects of tributaries, either small rivers, streams or discharge pipes and outlets, should however be recognized.

Table 3.
Minimum frequencyand month of sampling, together with the number of replicates (JEEP92 routine analysis)

	Months:												**replicates**
	1	2	3	4	5	6	7	8	9	10	11	12	
phys-chem parameters													
water	*		*	*	*	*	*		*		*		1
seston	*		*	*	*	*	*		*		*		1
sediments				*						*			2
bacteria	*		*	*	*	*	*		*		*		1
phytoplankton	*		*	*	*	*	*		*		*		3
zooplankton			*	*	*		*		*				3
microphytobenthos	*		*	*	*	*	*		*		*		3
macrophytobenthos				*						*			4
meiofauna				*						*			4
macrofauna				*						*			4
hyper	*		*	*	*	x	*		*		*		

Table 4.
Minimal sampling locations, coupled to salinity regimes (JEEP92 routine analysis)

	salinity (10^{-3}):												**replicates**
	0	3	6	9	12	15	18	21	24	27	30	33	
physico-chemical parameters													
water	*	*	*	*	*	*	*	*	*	*	*	*	1
seston	*	*	*	*	*	*	*	*	*	*	*	*	1
sediments		*			*				*				2
bacteria	*	*			*				*			*	3
phytoplankton	*	*			*				*			*	3
zooplankton	*	*			*				*			*	3
microphytobenthos	*	*			*				*			*	3
macrophytobenthos		*			*				*				4
meiofauna		*			*				*				4
macrofauna		*			*				*				4
hyper	x	*			*				*				

classification:
I : fresh water ($S < 0.5$);
II : oligohaline, ($0.5 < S < 5$);
III : mesohaline ($5 < S < 18$);
IV : polyhaline ($18 < S < 30$);
V : euhaline ($S > 30$)

The distribution of organisms in an estuary, both in the water phase and in the sediment , is largely determined by the (extremes in) salinity (see also section 3.1; Wolff, 1973; Perkins, 1974). Biological sampling based on salinity is therefore very appropriate, and the simplified Venice classification (see table 2) can be used for guidance.

At different locations the tidal ranges of salinity will vary. From experience and/or from surveys (section 4.2) these ranges can be estimated. A very interesting situation occurs where the salinity ranges of subsequent sampling locations overlap (high water salinity station A $\simeq$ low water salinity station B), thus building a complete coverage of the estuarine salinity regime, with a limited number of locations (but involving tidal stations with e.g. 13 hourly samples) (Wollast & Duinker, 1982; Duinker *et al.* 1983). An example of such an application is given in figure 2.

Instead of this laborious but rewarding method the simpler strategy of sampling of the water compartment, including seston and organisms, at locations that are based on the salinity distribution can be used. In this way the sampling positions are not connected to fixed locations, but to the water parameter salinity. As the water movement will not be the same each time a sampling programme is carried out, this is a better approach to cover the entire salinity regime of the estuary.

For benthic sampling the situation is slightly different, as the sediment is a sedentary compartment that will be influenced by the moving water, with its variable salinities, with tidal and seasonal time scales. It is essential to sample at least the biologically important salinity zones as defined in chapter 3.1 and table 2. For sediment sampling and sampling of the benthos the locations, once selected, should be maintained over the time series to observe trends.

For both the water sampling locations and the benthic stations an indication of the variabilityin the local salinity regime over a tidal period (in the dry season and during high river discharge) will facilitate future interpretation of the data.

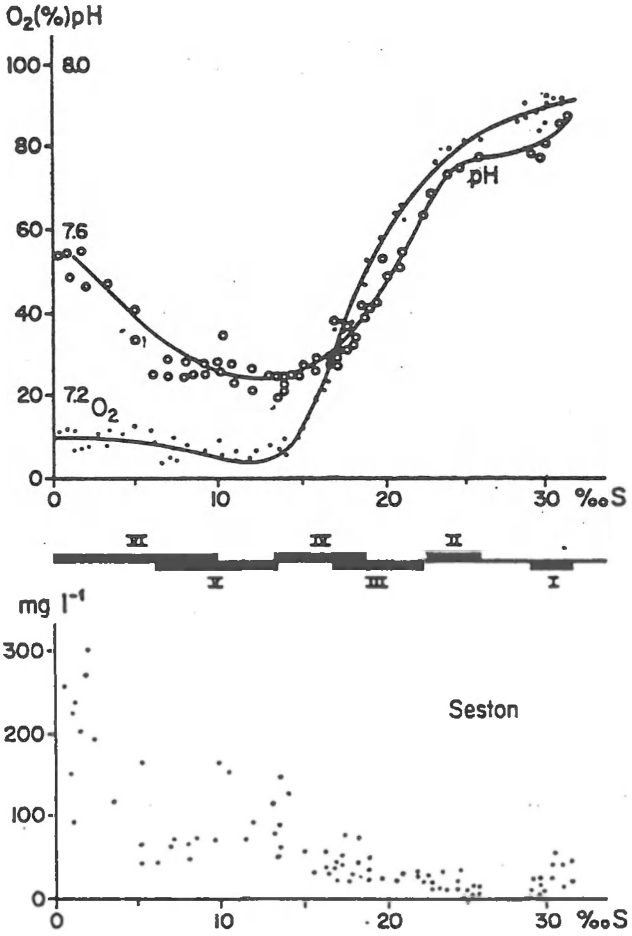


Figure 2
Data on dissolved oxygen concentration, pH and SPM in relation to salinity, obtained in the Scheldt estuary. The salinity limits of each of the six tidal stations (roman numbers) is indicated (from: Duinker et al., 1982)

JEEP92 strategy (tides, river discharge, locations and frequency):

The following strategy combines the minimum requirements for routine sampling of the estuary, and to estimate the effects of the daily tidal processes, diurnal variability , spring -neap tide cycles, and seasonal (river discharge) effects.

Routine sampling (water, including seston and organisms)

Each sample should preferably be collected:

- at locations that are determined by salinity of the water-column, at salinities about every 3 x 10^{-3} (table 4), thus totalling 8-10 stations; the location can be selected by sailing with the tide until the proper salinity is found at the correct tidal period,
- at mid-water depth,
- at the period half way between high and low water,
- half way between spring tide and neap tide,
- throughout the year, with higher frequencies in the spring -summer period, and dependent on the parameter under consideration (table 3).

  The number of replicates is given in tables 3 and 4.

Routine sampling (sediment, including organisms)

Sampling should occur at 5 fixed locations that are determined by the ranges in salinity , and based on the simplified Venice classification ; in other words, they should completely fit into the freshwater , oligohaline,, mesohaline, polyhaline and euhaline zones, preferably even under different seasonal conditions (tables 2 and 4).

The samples should be collected twice a year in spring and in autumn. Sometimes higher frequencies are preferred, depending on the variable/organism under consideration (table 3).

The number of replicates is presented in the tables 3 and 4.

Effects of tidal cycles and river discharge

Samples should preferably be collected:

- at one sampling station that is selected within the salinity range 9 - 15 x 10^{-3}; a second option is the turbidity maximum,
- at mid water depth,
- at 1 hour intervals,
- for at least 1 full tidal cycle, 13 h (25 h is preferred to detect also the diurnal cycle),
- in the period half way between spring and neap tide, (additional sampling - tidal stations, 13 hourly samples - at two subsequent spring and neap tide events, is highly recommended),
- one sampling event should fall in the period of maximum river discharge, a second operation at the period of minimum river discharge.

4.7 Selection of variables

Of the long list of parameters or variables that can be studied in an estuarine system, a selection of those which are important has to be made for each programme. A priority list with a list of all variables considered in the JEEP92 project has been prepared (table 5). The priorities are an indication whether the variable is essential for the interpretation of the estuarine data set or not. Where priority 1 is indicated the variable is considered as essential information that should be present in all data sets, priority 2 is a desirable variable, while priority 3 means that the information is relevant for or can be collected by specialists only.

Table 5.
Variable list, JEEP sampling and analysis-codes, symbols, units and priority status

Physico-chemical variables: water (S-6.1)						
variable	**code**	**symbol**	**unit**	**priority**		
				1	**2**	**3**
salinity	A-7.1	S	10^{-3}	*		
chlorinity	A-7.2	Cl	10^{-3}		*	
temperature	A-7.3	Temp	°C	*		
light penetration (Secchi depth)	A-7.4	-	m	*		
turbidity	A-7.4	-	%		*	
dissolved oxygen	A-7.5	O_2	mg/l	*		
pH	A-7.6	-		*		
total alkalinity	A-7.7	Alk	mmol/l		*	
nutrient concentrations						
nitrate	A-7.8	NO_3^-	µM	*		
nitrite	A-7.9	NO_2^-	µM	*		
ammonia	A-7.10	NH_4	µM	*		
phosphate	A-7.11	PO_4^{3-}	µM	*		
silicate	A-7.12	H_4SiO_4	µM	*		
sulphide	A-7.13	S^{2-}	µM			*
sulphate	A-7.14	SO_4^{2-}	µM			*
dissolved organic carbon	A-7.15	DOC	mg/l	*		
dissolved organic nitrogen	A-7.16	DON	mg/l			*
dissolved total carbohydrates	A-7.17	-	µgC/l			*
dissolved mono- & oligo saccharides	A-7.18	-	µgC/l			*
dissolved total amino acids	A-7.19	-	µgN/l			*
dissolved proteins	A-7.20	-	µgN/l			*
dissolved lipids	A-7.21	-	µgC/l			*

Table 5 (cont).
Variable list, JEEP sampling and analysis-codes, symbols, units and priority status

Physico-chemical variables: seston (S-6.2)

variable	code	symbol	unit	priority		
				1	2	3
suspended particulate matter	A-7.22	SPM	mg/l	*		
particle size (per size class)	A-7.23	-	n*10^3/ml	*		
pigments						
chlorophyll *a*	A-7.24	Chl.a	mg/l	*		
chlorophyll b	A-7.24	Chl.b	mg/l	*		
chlorophyll c	A-7.24	Chl.c	mg/l	*		
other pigments	A-7.24		mg/l		*	
% degradation	A-7.24		%		*	
particulate organic carbon	A-7.25	POC	mg/kg	*		
particulate organic nitrogen	A-7.26	PON	mg/kg			*
particulate organic phosphorus	A-7.27	POP	mg/kg			*
particulate total carbohydrates	A-7.17	-	µgC/l			*
particulate mono- & oligo saccharides	A-7.18	-	µgC/l			*
particulate total amino acids	A-7.19	-	µgN/l			*
particulate proteins	A-7.20	-	µgN/l			*
particulate lipids	A-7.21	-	µgC/l			*

Physico-chemical variables: sediment (S-6.3)

variable	code	symbol	unit	priority		
				1	2	3
grain size distribution						
< 2 µm, clay	A-7.28	-	%		*	
< 63 µm, silt	A-7.29	-	%	*		
63-125 µm	A-7.30	-	%		*	
125-250 µm	A-7.30	-	%		*	
250-500 µm	A-7.30	-	%		*	
500-1000 µm	A-7.30	-	%		*	
> 1000 µm	A-7.30	-	%		*	
particulate organic carbon	A-7.25	POC	mg/kg	*		
particulate organic nitrogen	A-7.26	PON	mg/kg			*
particulate organic phosphorus	A-7.27	POP	mg/kg			*

Table 5 (cont).
Variable list, JEEP sampling and analysis-codes, symbols, units and priority status

Biological variables: water

variable	code	symbol	unit	priority		
				1	2	3
bacteria (S-6.4)						
numbers	A-7.31		n*10^6/ml		*	
production	A-7.32		g/m^3			*
phytoplankton (S-6.5)						
species abundance	A-7.33		n/ml		*	
production	A-7.34		gC/m^3.d		*	
biomass	A-7.35		g/m^3		*	
zooplankton (S-6.6)						
species abundance	A-7.36		n/m^3	*		
stage distribution			n/m^3		*	
(key species only)	A-7.37					
indiv. stage weights			g		*	
(Copepods only)	A-7.38					
biomass	A-7.39		g/m^3		*	

Biological variables: sediment

variable	code	symbol	unit	priority		
				1	2	3
micro-phytobenthos (S-6.7)						
species abundance	A-7.40		n/m^2		*	
production	A-7.41		gC/m^2.d			*
biomass	A-7.42		g/m^2		*	
macro-phytobenthos (S-6.8)						
species abundance	A-7.43		n/m^2	*		
biomass	A-7.44		g/m^2	*		
meiobenthos (S-6.9)						
species abundance	A-7.45		n/m^2	*		
biomass	A-7.46		g/m^2	*		
macro-zoobenthos (S-6.10)						
species abundance	A-7.47		n/m^2	*		
age distribution			n/class	*		
(selected species)	A-7.48					
biomass	A-7.49		g/m^2	*		

4.8 Sample type

Different types of samples can be collected:

a ***spot samples,***(also called catch samples), where only one sample is taken at a given location, depth and time. This will be the most applied method of sampling, and be a default entry in the database. Examples are a surface sample by filling a bottle, the use of a watersampler, a sediment core.

b ***composite samples***. In most cases, these samples refer to a mixture of spot samples collected at different times (or places). This method of collection reduces the number of analyses, because e.g. daily variations are averaged out in one analysis. If, however, the series of spot samples are not mixed but analyzed individually, information on the variability and of the analytical accuracy can also be obtained, and afterwards the average might be computed. Examples are the collection of a series of sediment cores at one location, the hourly sampling over a tidal cycle, etc.

Sometimes 'time-composite' is used to distinguish from 'location-composite' sampling. Time-composite sampling representing a 13-hour period (one full tidal cycle) is often used, and the time interval between sampling events is 1-2 hours for many determinations.

For variables that will change after collection, and that can not be preserved, in-situ determinations should be applied if possible (see later). If preservatives are to be added, add them to each individual sample and not in the end to the composite sample.

c ***Integrated samples***. Sometimes samples are collected at the same location but, due to horizontal or vertical variation in the composition of the estuary, they are continuously collected over (part of) a vertical or a horizontal section. Examples are sampling using a pumping system, lowering an open sample bottle, a haul with a plankton net.

To evaluate the average composition in terms of total load or mass balance, integrated samples may be collected in relation to the current velocity at that location. This method is often applied in sampling for effluent control purposes.

d ***In-situ measurements.*** A number of variables can be measured *in situ*, with the advantage that a direct reading may give information on the structure of the water column, or the variation with time. *In situ* measurements therefore present a valuable tool in the selection of sampling locations and times. The most important variables that can be analyzed *in situ* are salinity, pH, dissolved oxygen concentration, temperature, conductivity , turbidity and fluorescence.

For the proper interpretation of the data, and for comparison with data sets from other estuaries, a record should be made of whether the data are derived from either spot, composite, integrated or *in situ* measurements. This is therefore indicated by a special sub-code in the sampling identification code (see annex II).

In the chapter on sampling methods (chapter 6) a distinction has been made between the various types of samples; the JEEP92 preference is thus indicated. In-situ analyses are covered in chapter 7, and Annex II.

4.9 Sampling depth & vertical profiles

Sampling depth, both in water and in sediment, can have a large influence on the analytical results in estuaries. The physico-chemical characteristics may change completely over 10-20 cm in the water column, and centimetres or even millimetres in the sediment. To what accuracy the samplingdepth has to be known will depend on the objective of the sampling programme . In some stratified estuaries, the vertical gradient in salinity is so large that in a standard watersampler, with an internal height of ca. 0.8 m, a salinity range from $10\text{-}37 \times 10^{-3}$ can be collected (see e.g. in the Krka estuary, Legovic *et al.*, 1991). Such a sharp interface will not only be visible in the salinity profile, but also affect the distribution of other variables, like organic matter (Zutic & Legovic, 1987). Especially in these situations, where large S gradients exist, the salinity has to be measured in the sample that is collected for other variables, rather than derived from a combination of (CTD) salinity profile and (approximate) sampling depth (Dyer, 1991).
Each estuary, and each location within the estuary may require a different approach in sampling. For comparative purposes (for the JEEP92 programme), some minimum requirements have to be specified; more samples are, of course, never a problem.
In estuariesit is very important to have an idea of the vertical structure of the watercolumn, before any sampling starts. The use of a CTD or TS meter is almost obligatory. Vertical profiles of other variables, such as dissolved oxygen or turbidity, are helpful for the interpretation of biological, hydrodynamical and (geo)chemical processes.
From the brief preliminary survey the sampling depths can be determined.

JEEP92 strategy (sampling depth, profiles):

In principle, in case of a non-stratified estuary, a minimum of one sample should be collected at mid-water depth.

When stratification occurs, the minimum requirement is that samples are collected at the sub-surface (0.5 m, avoiding the surface micro-layer) and at 1 m above the sediment. If the water depth is more than 5 m, an additional sample has to be collected at mid water depth; above a water depth of 12 m more samples may be collected. If the vertical salinity profile indicates a strong gradient at or around one of the intended sampling depths, it is recommended that samples are collected at either side of the interface.

For physico-chemical characterization of (surface) sediments the top 2 cm should be considered as it will contain the most recent and thus relevant information. Micro-phytobenthos is sampled in the top 0.5 or 0.5 and 0.5-2 cm, meiobenthos is collected in the upper 5 cm and for macro-zoobenthos the top 25 cm should be sampled.

It does not seem possible to store all the *in situ* measured vertical profiles in the JEEP92 data-base, and a selection has to be made. Every 1 m a characterisation should be given; where a strong gradient is observed a 0.5 m internal can be applied.

For sediments vertical profiles are possible, e.g. using micro-electrodes, but this seems beyond the scope of the present programme.

5. FIELD DATA INVENTORY

During each sampling event a minimum standard set of sampling characteristics should be recorded to facilitate completion of the database (see chapter 8), and to keep a record of the sampling activities. For this purpose an example Field Data Inventory is introduced here, that may be expanded if desired.

Table 6.
Estuarine sampling field data inventory (JEEP92)

Institute :
Estuary :
Vessel :

Responsible scientist :

Year/Month/Day :
Time (24 h) :

Location (N) :
(+E/-W) :
Water depth :
Sampling cruise code :
Sampling station code :

Sampling activities (tick:)

** physico-chemical parameters:*
☐ salinity ☐ temperature ☐ pH ☐ DOC ☐ DON
☐ oxygen ☐ nutrients ☐ sulphide ☐ sulphate ☐ fluoresc.
☐ Secchi ☐ turbidity ☐ current speed ☐ current direction ☐ other

** seston:*
☐ SPM ☐ part. size ☐ pigments ☐ POC ☐ PON
☐ POP ☐ other

** bacteria*
☐ numbers ☐ production ☐ other

** phytoplankton*
☐ abundance ☐ biomass ☐ production ☐ other

** zooplankton*
☐ abundance ☐ biomass ☐ stage distr. ☐ stage weight ☐ other

** sediment :*
☐ grain size ☐ POC ☐ PON ☐ POP ☐ other

** micro-phytobenthos*
☐ abundance ☐ biomass ☐ production ☐ other

** macro-phytobenthos*
☐ abundance ☐ biomass ☐ other

** meiobenthos*
☐ abundance ☐ biomass ☐ other

** macro-zoobenthos*
☐ abundance ☐ biomass ☐ age distr. ☐ other

6. SAMPLING METHODS

In this chapter the sampling procedures are documented to emphasize the differences that may exist between the various methods, and also the possibility or impossibility of using a given method for a specific variable. No attempt has been made to include all existing sampling methods. Methods have been selected in view of optimum applicability for the estuarine environment, keeping in mind that not all participants have access to specially designed or the newest available sampling techniques or sophisticated (automated) instruments.

All sampling procedures in this manual have been given a **S-number code** for easy reference. These numbers are used throughout the volume.

In each sampling procedure the compartment is given, and the **variables** that fall under the presented sampling method(s).

The list of sampling methods is sub-divided into different compartments and sub-compartments :

sampling water (S-6.1)
sampling seston (S-6.2)
sampling sediments(S-6.3)
sampling biota (S-6.4):
bacteria, phytoplankton, zooplankton, micro-phytobenthos, macro-phytobenthos, meiobenthos and macro-zoobenthos, hyperbenthos.

A short introduction presents the different possible sampling methods for the given compartment and variables for estuarine sampling.

Often the final results of different sampling techniques do not compare well. This can be a problem for intercomparison . In these cases the methods will have different numbers: Method #1, Method #2, etc. Unless stated otherwise, Method #1 is preferred over Method #2, etc. Where more techniques or methods are possible that give (nearly) identical results, this is indicated using suffixes: Method #1a, Method #1b.

Participants (within the JEEP92 programme) are strongly encouraged to apply the first sampling method given (thus Method #1, #1a or #1b, ...) as much as possible. The only reason why other sampling methods are treated here is to provide the possibility of including old data sets in the data base, and of separating data sets that are based on different sampling methods in the data treatment.

The number of individual replicate samples that are to be collected has been given in table 3.

In situ measurements, e.g. of salinity, temperature, turbidity, dissolved oxygen content, pH and others, are found in the chapter on analytical procedures (chapter 7).

An overview of the various sampling methods, together with their sampling codes and sub-codes for additional information in the JEEP92 data base is given in Annex II. This list can be used as a quick reference guide.

A short description of each procedure follows. References will be provided where applicable. For further details on sampling techniques, refer to the following references, handbooks and standard works, which contain valuable chapters on sampling methodology:

- Grasshoff, K., M. Ehrhardt & K. Kremling (eds), 1983. Methods of seawater analysis. 2nd ed. Verlag Chemie, Weinheim
- Head, P.C. (ed), 1985. Practical estuarine chemistry. Cambridge Univ. Press, Cambridge , pp. 337
- Higgins, R.P. & H. Thiel, 1988. Introduction to the study of Meiofauna. Smithsonian Institution Press, Washington DC
- Holme, N.A. & A.D. McIntyre (eds), 1984. Methods for the study of marine benthos. Blackwell Sci. Publ., Oxford, pp. 387
- Morris, A.W. (ed), 1983. Practical procedures for estuarine studies. A handbook prepared by the Estuarine Ecology Group of the Institute for Marine Environmental Research. IMER, Plymouth, pp. 262
- Sournia, A. (ed), 1978. Phytoplankton manual. Unesco, Paris, pp. 337
- Tranter, D.J. & J.H. Fraser (eds), 1968. Zooplankton sampling. Unesco Monographs on oceanographic methodology, 2. Unesco, Paris, pp. 174

S-6.1

Compartment: water

Variable(s): (salinity), chlorinity, pH, nutrients, alkalinity, DOC, seston

Introduction:
Sampling of water for physico-chemical analyses is one of the most common sampling events. For estuarine work several special considerations are to be considered (inhomogeneity , sampling depth, etc), as discussed in chapter 4. Possible techniques involve: sampling directly in a sample bottle, or using a bucket, water samplers, or a pumping system. Both spot samples and integrated samples can be obtained. When sample bottles are filled, take care to clean them, including the caps, with water from the sample location at least twice.

Sampling methods:
Method #1a. Water-sampler. The water-sampler is much preferred as it can be operated much more reproducibly and at any depth desired. Before actually using the sampler it should be checked for proper functioning, and cleaned from shipborn dust by moving it several times up and down in the water. Mounting and operation of water-samplers is model specific, and one is referred to the individual manual.
Most samplers operate in a vertical position, and will thus collect a vertical segment of water, depending on the size of the sampler up to 1 m, that may cross boundaries between different water types. Sampling of the surface microlayer should be avoided.
The sampler is mounted to a weighted hydrowire, and closed at the desired depth by a dropping weight. In case of strong currents when the ship is at anchor, the sampler may be used sliding along a separate hydrowire that is kept almost vertical using a heavy weight or better, a type of 'depressor' as used in zooplankton catches in the open sea. In all cases the actual depth should be estimated as accurately as possible using a graduated line. Assume the middle of the sampler as the actual sampling depth and correct for deviation from the vertical .
Method #1b. Sample bottle. Collection of water samples direct into a sample bottle, or the use of such bottles as samplers has been used for many years. This method prevents contamination of the sample by using a sampler. In addition, samples can be collected directly in the preferred type of container (material). It is most simple to lower the bottle by hand below the surface of the water and let it fill. Sampling of the surface microlayer should be avoided. For non-surface, or better sub-surface samples, many constructions have been designed which either lower the bottle in the open position, or open it at the desired depth. The latter may be of the displacement type, that allows a steady flow into the bottle. In the open sea the method is limited because the pressure difference does not allow sampling at a depth of more than 20-30 m, depending on the type of bottle. In estuarine waters this is usually not a limitation. Lowering in the open position collects samples not at discrete depths but some sort of integrated sample. However, collection is not even over the sampling path, because of pressure differences. An active opening procedure is therefore preferred. The bottle(s) are lowered attached to a graduated polythene line in a suitable weighted enclosure and opened at the desired depth.

Method #2. Pump. Pumping of water for collection has become popular for use when a ship is under way, or for large volumes of water. Several types of pumping system are used, both *in situ*, peristaltic, bellow, etc. pumps or using a vacuum behind the collection bottle. Most pumps will provide sufficient pressure, provided they don't have to suck the water to a considerable height first. Mounting of the pump close to the surface (or under water) is essential. The practical sampling depth is up to about 25 m, although larger depths have been sampled. The draw back of the method may be settlement and separation of particles when the water flow is inadequate. Because of the large length of tubing that may be required, contamination may become a problem. Polythene or teflon is very useful. Flush the tubing with at least two times its content with water from the sample location before collection. Because of the pressure already building up by the pump, on line filtration is a possibility. Beware of contamination when using in line filters, because of their relatively large surface to volume ratio. The tubing may be lowered along a hydrowire or polythene line until the inlet is at the desired depth. Integrated sampling is easy. Strong currents may give problems in the sampling operation.

Method #3. Bucket. This type of sampling is apt to contamination, even though the bucket is cleaned before operation. Also, as sub-samples (bottles) are filled from the bucket, settlement of the particulate material may occur. The bucket can only collect surface samples, the surface microlayer cannot be avoided.

Remarks:

Certain materials from which samplers are made may not be appropriate for specific analyses. Check with the analytical procedures. Neither a free filling bottle or a pumping system is suitable for the collection of samples intended for the analysis of gasses. For sampling for dissolved oxygen and hydrogen sulphide see A-7.5 or A-7.13 of the analytical section.

References:

Grasshoff (1976); Venrick (1978); Brockmann & Hentzschel (1983); Grasshoff *et al.* (1983); Leatherland (1985); Gomez-Parra *et al.* (1987)

S-6.2

Compartment: seston

Variable(s): physico-chemical characteristics

Introduction:

Especially in (shallow) estuaries, affected by relatively high tidal currents, the inhomogeneity of the amount of suspended particulate matter (SPM or seston) will usually be large. Here special attention should be given to the sampling, as discussed in chapter 4. Possible techniques involve: sampling directly in the sample bottle, or using a bucket, water samplers, or pumping system. Both spot samples and integrated samples can be obtained.

How much water should be collected for the collection of SPM, will strongly depend on the amount of seston (and the particle size). In most estuaries 0.3 - 1 litre will be sufficient for a filter of 50 mm diameter. For a high yield of seston, continuous centrifuge techniques may be applied, which enable the collection of seston from 1 m^3 and more. As this technique uses the density of particles as the driving mechanism (and not the arbitrarily chosen 0.45 μm pore size), an underestimation of the lighter fractions may occur.

The methods of sampling relate to a large extent to those of water sampling (ad S-6.1) and to the sampling of phytoplankton (S-6.5). The use of a sample bottle or a pumping system may lead to settlement of the SPM, and thus to incorrect results.

Sampling methods:

Method #1a. Water-sampler. The water-sampler is much preferred as it can be operated much more reproducibly and at any depth desired. Because of the (rapid) settling of seston, it is recommended to use a small volume sampler that is emptied completely (thus collecting all seston in the sampler). If a larger sampler is used, collection of the water from the sampler should be under regular shaking of the sampler to keep the seston in suspension. In highly turbid waters the best option for seston sampling is the use of a horizontally operated sampler, constructed like a tube with constant diameter, and which is directed into the current with a vane.

Method #1b. Sample bottle. It is simplest to lower the bottle by hand below the surface of the water and let it fill.

For sub-surface samples, a number of constructions have been designed, which either lower the bottle in the open position, or open it at the desired depth (see e.g. Brockmann & Hentzschel, 1983). The system may be of the displacement type, that allows a steady flow into the bottle. An active opening procedure is preferred. The bottle(s) are lowered attached to a graduated polythene line in a suitable weighted enclosure and opened at the desired depth.

Method #2. Pump. As for sampling water, pumping for the collection of seston has become popular for use when a ship is under way, or for large volumes of water. Several types of pumping system are used, both *in situ*, peristaltic, bellow, etc. pumps or using a vacuum behind the collection bottle. A disadvantage of the method may be the settlement of particles when the water flow is inadequate. Because of the pressure already built up by the pump, on-line filtration is a possibility.

Method #3. Bucket. As sub-samples (bottles) are filled from the bucket, settlement of the particulate material, even under continuous movement of the bucket while sub-sampling, may occur. The bucket can only collect surface samples. This method is therefore not recommended.

Remarks:

References:
Eleftheriou & Holme (1984); Fleeger *et al.* (1988)

S-6.3

Compartment: sediment

Variable(s): physico-chemical characteristics

Introduction:
Sediment is usually heterogeneous in nature. The collection of one sample for a physical -chemical characterization will almost certainly not be characteristic for the area. Multiple sampling and analysis offers better possibilities. Separate samples may be pooled (homogenized) if necessary, but this will destroy any information on the structural differences. Often samples will be collected in conjunction with the collection of benthic biota , where the combination of samples is out of the question. The interrelation between sediment characteristics and biological results is considered valuable. A review of various sampling devices can be found in Fleeger *et al.* (1988), Eleftheriou & Holme (1984). For sediment analysis undisturbed samples are more easy to interpret. Cores will provide such samples, the actual depth of sampling (sediment thickness) can be measured, and even sub-sampled. Grab samplers collect disturbed samples, with an undefined, but rather limited sediment depth.

Sampling methods:
Method #1a. Handcorers.
Method #1b. Gravity corers.
Method #1c. Piston corers.
Method #1e. Vibro corers.
Several types of corers are available: gravity-corers, piston-corers, vibro-corers. In shallow waters and in the intertidal zone a simple perspex or PVC tube will be a very useful corer. For sediment analysis the size is not really important. If multiple analyses are to be performed sufficient sample material has to be collected, thus a tube diameter of approximately 8 cm is recommended. When composite samples are collected, about 10 samples (2 cm Ø corers) of defined length are mixed (homogenised) in a wide-mouth polythene bottle. Collect the top 2 cm of the sediment . This is not the sediment depth that is collected for the various organisms (either 5 or 25 cm, see S-6.7, S-6.9, S-6.10), but this sediment layer is considered representative for the analysis of the physico-chemical characteristics.
Method #1d. Box-corer. Box-corers are only useful in large estuaries, where a sufficiently large ship can manoeuvre. In addition their use may be limited to low (tidal) current situations under estuarine conditions. Box-corers are specialised equipment, of considerable size and weight. Once they are retrieved, sub-samples can be collected by sub-coring using plastic tubes. The major advantage of box-corers is that they collect undisturbed samples, and contain sufficient material for all types of analysis, both of the sediment and biota . The results will therefore be better linked than when separate corers are applied. Collect the top 2 cm for physico-chemical characteristics.

Method #2. Grab-sampler. Various types of grab-samplers are currently in use, e.g. several versions of the Van Veen Grab, the Petersen-, Smith-McIntyre- and Day-samplers. Riddle (1989) compared the sampling efficiency of these samplers, including their 'bite' profiles and depth of sampling. For sediment collection an undisturbed sample is much preferred, together with a reasonable sampling depth (at least 5 cm). The chain-rigged Van Veen and the Petersen grabs are not ideal for this purpose.

Most grab-samplers are self-operating: when lowered to the sediment , they will close automatically when lifted. It is likely that not all samples will have the desired quality, because e.g. a stone is trapped between the jaws, the sampler didn't penetrate far enough into the sediment , etc. Collection of a new sample is strongly advised when this occurs.

Samples of the sediment should be collected in such a way that the vertical structure is preserved as much as possible. A small plastic corer can prove a useful tool. Contamination of the sample should be prevented.

Remarks:

References:
Eleftheriou & Holme (1984); Fleeger *et al.* (1988)

S-6.4

Compartment: water

Variable(s): bacteria

Introduction:

Two major approaches are followed when sampling for bacteria: filling the (sterilized) sample bottle directly with the sample, using a pumping system or using a sterilized water sampler. In both cases precautions should be taken to prevent contamination of the sample. For simple counting work, extreme precautions (sterilizing all equipment) are not essential, but the sample bottles should still be sterilized.

Sample bottles for the collection of bacteria should not be prewashed with water from the field. To overcome patchines in the field, especially in case aggregates - with adhering bacteria - are present, it is recommended to sample a number of times (≈ 20), and pool the sample before analysis.

Sampling methods:

Method #1. Sample bottle. Direct sampling in the bottle can provide an effective, reliable and cheap method of collection. When the sample is taken by hand only surface waters may be collected, using a mounting device as discussed under S-I.1b, only the version that is lowered in the closed condition can be used. The maximum depth of sampling is then limited, but this is usually not a problem in estuarine waters. Care should be taken not to contaminate the sample with material that comes from the hands or gear. Preferably open the bottle under water. For sub-surface sampling a set-up has been described by Brockmann & Hentzschel (1983).

Method #2. Pumping system. Lowering the tubing of a pumping system to the desired depth provides a useful means of collection. To prevent contamination from previous samples, the tubing and pump should be cleaned by flushing with water from the sampling location for at least three minutes.

The sample is collected in sterilized sample bottles which have not been rinsed with water from the field. The sample bottle should be filled in a clean area, preferably in a laminar flow clean bench with UV lighting.

Method #3. Sterile water sampler. Special water samplers that can be sterilized have been developed for the collection of micro organisms (Grasshoff, 1976). They are considered specialist equipment and the user is referred to their instruction manual. They are all of the type that they can be opened at the desired water depth. General remarks on the use of water samplers have been given under S-6.1. The sampler should be emptied in a clean area, preferably in a laminar flow clean bench with UV lighting.

Remarks:

References:

Grasshoff (1976)

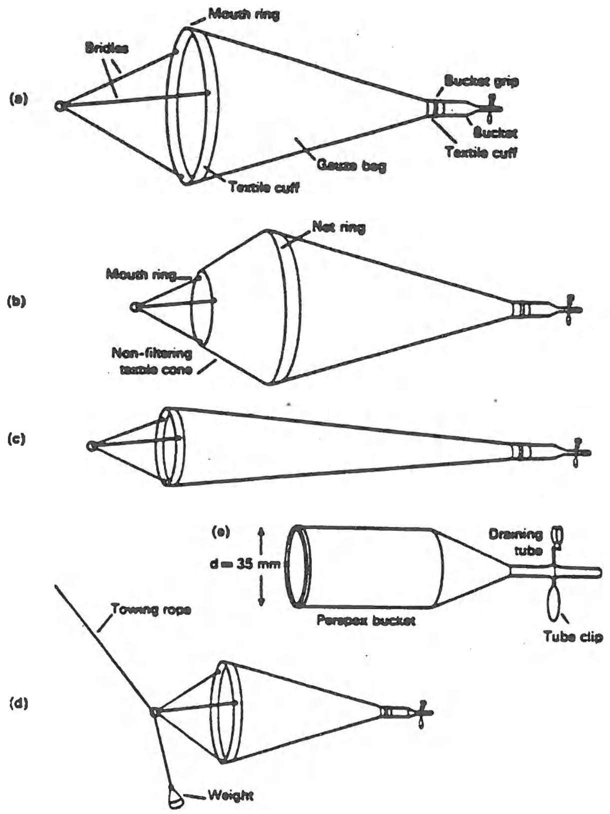


Figure 3
Examples of various types of plankton nets (from: Sournia, 1978)

S-6.5

Compartment: water

Variable(s): phytoplankton

Introduction:
The collection methods for the study of phytoplankton will to some extent affect the diversity of the species . Water depth (spot samples) or water column collection (integrated samples) will almost always give different results. The use of a water sampler or the direct filling of a sample bottle seems the simplest method to standardize for estuarine work, as it is commonly used and a technique that will be available in all laboratories. Pump systems and nets are useful for large quantities of material, as they offer possibilities for concentration of the phytoplankton. Water samplers, sample bottles and pumping systems may collect spot samples , while pump systems and nets may give integrated results. The use of Plankton Recorder systems (e.g. Robinson & Hiby, 1978) is considered beyond the scope of routine estuarine sampling programme.
One should be aware of the usually large patchiness of plankton in the water column (Duston & Pinckney, 1989).

Sampling methods:
Method #1a. Sample bottle. Collection of water samples direct into a sample bottle, or using these bottles as a sampler and transferring the sample later, is an adequate method. The most simple method is to lower the bottle by hand below the surface of the water and let it fill. Sampling of the surface microlayer should be avoided.
For sub-surface samples, constructions have been designed which either lower the bottle in open position, or open it at the desired depth. The latter may be of the displacement type, that allows a steady flow into the bottle. In estuarine waters the depth limitation of the method is usually not a problem.
Lowering in the open position collects samples not at discrete depths but some sort of integrated sample. However, collection is not regular over the sampling path, because of the pressure difference. An active opening procedure is therefore preferred. The bottle(s) are lowered attached to a graduated polythene line in a suitable weighted enclosure and opened at the desired depth.
Method #1b. Water sampler. Methods for the collection of samples for dissolved compounds or for seston, including phytoplankton, are the same. Any water sampler can be used. However, sampling is best performed using relatively small water samplers (1-2 litres), of which the total water content can be used. As part of the phytoplankton may have a tendency to be deposited, an overestimation of some species may occur if only part of the sampler content is collected. If this is not possible, the sample in sampler should be homogenised (reversing the sampler) before sub-sampling.
General remarks on the use of water samplers are given in S-6.1. The sampler is mounted on a weighted hydrowire, and closed at the desired depth by a dropping weight. In case of strong currents when the ship is at anchor, the sampler may be used sliding along a separate hydrowire that is kept almost vertical using a type of 'depressor' as used in zooplankton catches in open sea. In all cases the actual depth should be estimated as accurately as possible using a graduated line. Assume the middle of the sampler is the actual sampling depth and correct for deviation from the vertical .

Method #2. Pumping system. Several types of pumping system are used, both peristaltic, bellow, membrane, etc. pumps or using a vacuum behind the collection bottle. Centrifugal pumps are not to be recommended because then may be highly damaging to the plankton. Most pumps will provide sufficient pressure, provided they don't have to suck the water to a considerable height first (static suction head). The practical sampling depth is to about 25 m, although larger depths have been sampled. A disadvantage of the method may be the settlement of particles when the water flow is inadequate. Also, fragile species might be physically damaged. Flush the tubing with at least two times its volume of water from the field site before collection. In between operations the tubing may get coated on the inside with organisms, which can be a serious source of contamination. A larger diameter tube will have less total frictional loss than thin tubing given a constant discharge. The tubing may be lowered along a hydrowire or polythene line until the inlet is at the desired depth. Strong currents may give problems in the sampling operation. The volume that has been filtered should be recorded and for statistical reasons should not vary between sampling events. A standard volume of 50 litres is proposed for estuarine work.

Both spot sampling and integrated sampling are easy to perform.

Method #3. Nets. The major advantage of nets is the relative ease of filtering large volumes of water and the resulting concentration of species . The use in small estuaries may be limited, however. A major drawback of the method is the selectivity of the nets for species size and form, which is dependent on the mesh-size and the clogging of the net during collection.

Because of this, information on the net gauze and the volume of water filtered has to be presented together with the results.

For phytoplankton studies in estuaries standard small nets (mouth diameter 15 cm, length 110 cm; Tangen, 1978) with a gauze of 10 µm are proposed. Vertical hauls collect material from the entire water column. The weighted net is lowered from the drifting ship to the bottom, and withdrawn slowly. When this is performed from a moving ship (oblique haul) the total amount of material collected is larger, but in small estuaries the chance of mixing different phytoplankton assemblages, because of changing physico-chemical conditions, is evident. Towing speed should not exceed 1 m/s (ca 2 knots). In estuarine waters clogging of the nets can be a major problem (figure 3).

Remarks:

References:

Beers (1978); Sournia (1978); Throndsen (1978)

S-6.6

Compartment: water

Variable(s): zooplankton

Introduction:
The large diversity in groups of organisms, their size and life-stage make it difficult to establish one generally accepted method for sampling. Again several methods are given that are not very comparable, but which are commonly used and require equipment which is not too complicated. Independent of the collection method a mesh-size has to be defined. Proposed here as a preferred standard method for zooplankton sampling is a mesh of 55 or 63 µm.
The Methods #1, #4 and #5 sample 100 litres water which is poured through a conical net as a standard procedure, while the other methods require large volumes of water, the organisms being collected directly in the nets. A volume of 5 m^3 should be the preferred volume. For estuarine work these are considered less practical.
The nets are all sprayed from the outside with water from the field, to allow the zooplankton on the inside of the net to be collected in the funnel at the bottom. A wash bottle is a convenient tool here.
One sample per location is a minimum requirement, which should be collected at mid water depth.

Sampling methods:
Method #1. Pump & net. Several types of pumping system are used, especially bellow, membrane pumps. Centrifugal pumps are not recommended because they may be highly damaging to the zooplankton. Actual speed of pumping is very important, to avoid missing fast swimming plankton. One drawback of the method is, that it is not known to what extent more active organisms can avoid the mouth of the pump. Also, fragile species or life stages might be physically damaged. A larger diameter tube will have less total frictional loss than thin tubing given a constant discharge. The tubing may be lowered along a hydrowire or polythene line until the inlet is at the desired depth. Strong currents may give problems in the sampling operation. The volume that has been filtered should be recorded and for statistical reasons should not vary between sampling events. A standard volume of 50 litres is proposed for estuarine work. Both spot sampling and integrated sampling by moving the tube entrance vertically through the watercolumn, is easy to perform.
Method #2. Towing nets. For large amounts of zooplankton, or very low densities, this procedure has advantages. In estuaries there is probably no need for this procedure. A drawback of this method is the difficulty of estimating the volume of water passed through the net. Clogging may, depending on the mesh-size, be a problem in estuaries. Sampling is performed from a moving ship; in small estuaries there is a strong chance of mixing different zooplankton assemblages, because of changing physico-chemical conditions. Towing speed is critical for the quantitative collection of the plankton (figure 3).

Method #3. High-speed samplers. High-speed samplers are not very useful for estuarine work for the reasons already mentioned in Method #4.
Method #4. Collection tube & net. For the integrated collection of a vertical cross section of the upper ca 4 metres of the water column a tube sampler has been used. The design involves a PVC tube of about 12 cm diameter. At one end a ball valve is mounted. When the tube, with the valve at the base, is quickly inserted vertically into the water, a water-column can be collected. The contents are then poured through the net. By repeating this procedure the desired 50 litres can be filtered. The handling of a sizeable length of the tube is limited to about 4 - 5 m, but this may be sufficient for estuarine work. Fast moving organisms probably escape from the tube mouth, but will be collected more quantitatively than by using a pumping system.
Method #5. Bucket & net. For the collection of surface water zooplankton the use of a bucket which is emptied in the conical net offers a simple and effective means of collection. A 10 litres graduated bucket thus requires 5 samples to be filtered.

Remarks:

References:
Tranter & Fraser (1968); Raymont (1983); Tett (1987)

S-6.7

Compartment: sediment

Variable(s): micro-phytobenthos

Introduction:
Micro-phytobenthos significantly contributes to the primary production of estuarine areas and is present not only at the very surface of sediments, but also in deeper layers (Cadée, 1974; De Jonge, 1992). Therefore the top 0.5 cm should be collected for mass production and the top 2 cm for determining biomass. This 2 cm should be subdivided into 0 - 0.5 and 0.5 - 2 cm instead of the very surface layer.
Composite samples should be collected to avoid the influence of supposed heterogeneous micro-distribution of the micro-flora. A minimum of 20 individual samples should be collected along one (or more) pre-described transects at 1 m distance intervals. For optimal work 50 to 100 samples should be collected and pooled.
Due to the turbidity of most estuarine waters, the micro-phytobenthos will be most important in the intertidal areas.

Sampling methods:
Method #1. Hand corer, ***2.5 cm Ø.*** Hand operated corers are almost exclusively used. A plastic tube of the proper diameter is introduced into the sediment (see figure 4). A sawn-off syringe is very useful, as it also provides a plunger that keeps the sediment in place when the tube is withdrawn. The excess sediment is easily removed by pressing the plunger, so the desired length of sediment (0.5 or 2 cm) is retained.
Method #2. Box corer, ***2.5 cm Ø (sub-samples).*** For subtidal areas box core samples can be used, where sub-samples are collected using the syringe described under Method #1. A minimum of 5 box cores should be collected, and from each 4 replicate samples can be taken and homogenized into a composite sample.

Remarks:

References:
Cadée & Hegeman (1974; 1977); De Jonge (1992)

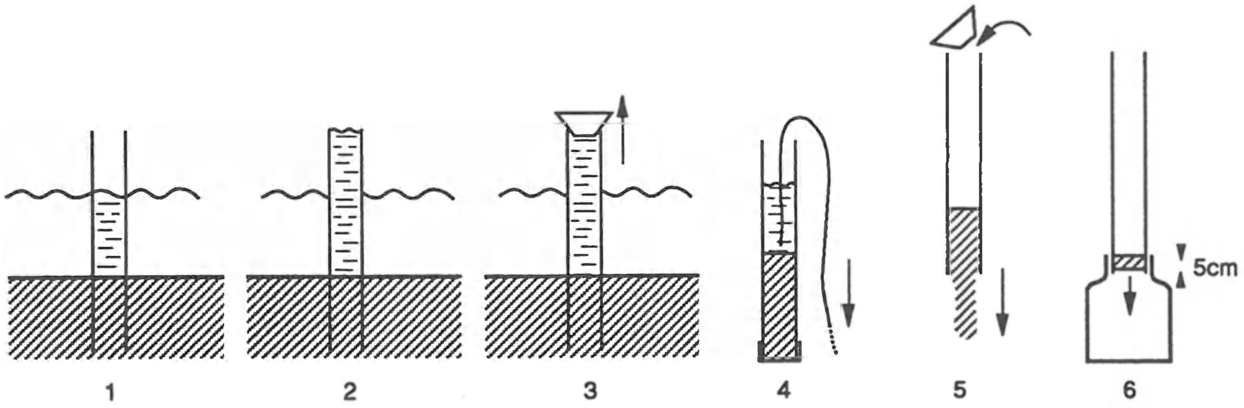


Figure 4
Sequence of operation with a hand corer on tidal flats or in shallow waters

S-6.8

Compartment: sediment

Variable(s): macro-phytobenthos

Introduction:

Macro-phytobenthos is not of importance in all estuaries For the JEEP92 project the dominant species , those that exhibit a relatively large portion of the biomass, will be of interest. As stated in the preface, salt marsh species are not considered here, and the study is limited to intertidal macrophytes. Most important in this respect are the macro-algae *(Fucus* spp., *Ulva* spp., *Enteromorpha* spp.) and seagrasses (*Zostera* spp.).

Apart from specificity for certain salinity regimes, macrophytes are rather sensitive to bathymetric gradients. Therefore species abundance and biomass should be related to this variable.

In areas where there is a large variation in sediment type or (bathymetric) gradient, only those areas should be sampled that cover a relatively large proportion of the intertidal area in the salinity regime that has to be investigated. Sampling of non-representative areas should be avoided.

Sfriso *et al.* (1991) studied the effect of different sampling strategies in the shallow waters of the Venice Lagoon. They tested the effect (on average biomass) of the number of sub-samples that should be collected. Assuming that 20 sub-samples would represent a good representation of the area studied, they found that usually 4-8 subsamples were to be considered representative. Of the two sampling methods given, the first is usually applied in intertidal areas. The second on the other hand, serves well in subtidal substrates.

Sampling methods:

Method #1. Selected 10 x 10 cm samples. A minimum of four 1 m^2 quadrats should be selected in the area of investigation, on a transect perpendicular to the bathymetric gradient. These squares are to be selected randomly, but must be representative of the intertidal macro-phytobenthos in the salinity regime area. Within these square metres four sub-areas of 10 x 10 cm are randomly selected (using randomization tables, making 16 samples all together) and all macrophytes that are contained in this surface area (at the base of the plant) are collected by cutting. As the roots (if available) are difficult to collect quantitatively, they are not collected. Other surface areas may be desirable, when thin densities are observed.

***Method #2. Selected 100 x 100 cm* sampling.** At representative locations an area of 15 x 15 m is selected. Within these areas a minimum of four (but eight is preferred) randomly selected 1 m^2 squares are selected. An aluminium box of 1 x 1 x 0.7 m is efficient for this purpose. All macro-algal material is collected within this box by cutting. As the roots (if available) are difficult to collect quantitatively, they are not collected.

Remarks:

References:

Polderman (1978); Sfriso *et al.* (1991)

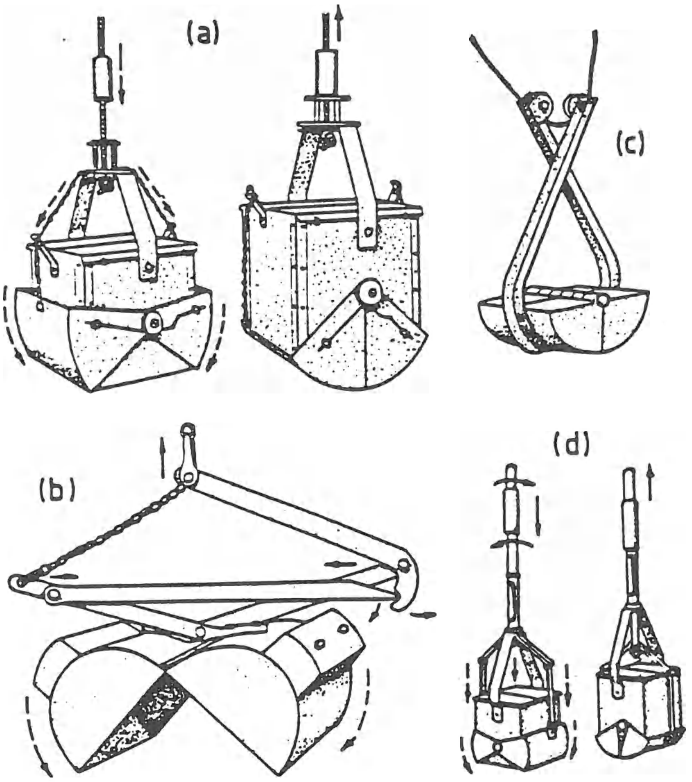


Figure 5.
Examples of grab samplers (from: Holme & McIntyre, 1984)

S-6.9

Compartment: sediment

Variable(s): meiofauna

Introduction:
Where the meiobenthos is concerned there is a large variation between various locations and different types of sediment . For practical reasons it is not adequate to prescribe one single method of collection. There is no optimum between surface area (corer diameter) and number of organisms per sample (underestimation vs counting problem). In practice, experienced scientists have arrived at a procedure that involves somewhat larger size samples in sandy sediment , and smaller in muddy sediment . As the ultimate goal is (among others) a estimation of the abundance of the different species , the different core sizes for the different sediments is not necessarily a problem. Therefore two corer sizes are recommended here.
For subtidal work the use of a box-corer is much preferred, from which subsamples of the appropriate diameter can be collected. The major problem of box-corers is their availability and size. Various gravity or piston cores can be used instead. Grab-samplers will often be used for subtidal sediment sampling because of availability, but a rather disturbed sample will result, and much of the fine surface material will be washed away by the bow-wave of the grab, loosing a high proportion of certain taxa, especially copepods.
Intertidal areas are easier to reach and to sample. Hand operated corers are then preferred.
A minimum of four individual replicate samples should be collected on a transect, at about 10 m intervals.
Standard sampling depth is 5 cm. In case deeper samples are required, 15 cm becomes the second standard (especially in clean sand where the meiofauna penetrate deeper).
If the density of the organisms is expected to be the too high for the given tube diameter, the sample should be split once or twice over the length. Take care to apply a separation parallel to the sides of the core, weigh the subsamples for corrections in surface area . This method may be called inaccurate, and it is preferable to split the homogenised preserved sediment sample with a special sample splitter (Pfannkuche and Theil, 1988).
(Sediment) samples should be stored after addition of 4 % formalin in warm (60°C) seawater solution, in polythene bottles prior to analysis. A warm solution of formalin is advised, to prevent nematodes rolling up, which will make identification nearly impossible.
Because of the different logistical problems encountered in sampling intertidal and subtidal regions, different approaches are given here.

Sampling methods (subtidal):
Method #1a. Box-corer, 2 cm Ø sub-samples (mud). Box-corers are specialised equipment, of considerable size and weight, offering optimal possibilities for collecting undisturbed sediment samples. Once they are retrieved, subsamples can be collected by corer using plastic tubes of the desired diameter. Another advantage of this method of collection is that the cores con-tain sufficient material for all types of analysis. The results will therefore be better linked (e.g. to the physico-chemical characterization of the sediment)
than when separate corers are applied.

Method #1b. Box-corer, 8 cm Ø sub-samples (sand). This method only differs from the previous one by the diameter of the sub-sample collection tube. In stead of withdrawing the corer, it may be dug out.

Method #2a. Hand operated corer, divers, 2 cm Ø (mud). Divers are able to position (small) coring tubes with care and can push the corer slowly into the sediment . This has the added advantage that the exact sampling spot can be visually inspected. A sawn-off syringe is very useful, as it also provides a plunger that keeps the sediment in place when the tube is withdrawn (see figure 3). For longer corers plastic tubes serve well.

Method #2b. Hand operated corer, divers, 8 cm Ø (sand). This method is identical to method #2a except for the diameter of the coring tube.

Method #3. Bow wave free corers. A special type of subtidal corer has been developed that eliminates the shock or bow wave that develops in front of the free falling or pole corers (see Method #4). Good design features include slow sediment penetration, large flow-through tubes and a trip mechanism that does not interfere with the water flow through the corer tube or disturb the sediment before penetration (Fleeger *et al.*, 1988). Examples that are useful in estuarine waters include the Craib-sampler.

Method #4. Corers. For water depths up to about 4 m pole-samplers can be used. The consist of corers attached to a pole and can be operated manually from a boat. For deeper subtidal collection of sediment , remote samplers like piston- or gravity-corers can be used. They all suffer, however, from the shock wave that builds up in front of the opening of the corer, and which may blow away the meiobenthos that is present at the very surface of the sediment . This will result in an underestimation of (surface dwelling) meiofauna species .

Method #5. Grab sampler. Grab samples differ very much from the previous methods. Various types of grab-samplers are currently in use, e.g. several versions of the Van Veen Grab, the Petersen-, Smith-McIntyre- and Day-samplers. Riddle (1989) compared the sampling efficiency of these samplers, including their 'bite' profiles and depth of sampling. For sediment collection an undisturbed sample is much preferred, together with a reasonable sampling depth (5 cm). The chain-rigged Van Veen and the Petersen grab do not seem suitable for the purpose. Grab samplers also suffer from the bow wave effect. Most grab-samplers are self-trigerring: when lowered to the sediment , they will close automatically when lifted (figure 5).

It may be that not all samples will be of the desired quality, because e.g. a stone is trapped between the jaws, the sampler didn't penetrate far enough into the sediment , etc. A new sample should be collected when this occurs.

Sub-samples of the sediment should be collected in such a way that the vertical structure is preserved as much as possible. A small plastic corer (sawn off syringe) can prove a useful tool.

Sampling methods (intertidal):

Method #1a. Hand-corer, 2 cm Ø (mud). Because of the easy access to intertidal areas, hand operated corers are almost exclusively used. A plastic tube of the appropriate diameter is pushed into the sediment . A sawn-off syringe is very useful, as it provides also a plunger that keeps the sediment in place when the tube is withdrawn (see figure 3). The excess sediment is easily extruded by pressing the plunger so that the desired length of sediment (5 cm) is retained.

Method #1b. Hand-corer, 8 cm Ø (sand). A PVC or perspex tube is inserted in the sediment. The corer should preferably have a piston, which facilitates the withdrawal of the sediment . Another solution to overcome the impossibility to retrieve the sediment core, is to insert the tube, fill the tube with local water to the top, place a rubber stopper on the tube. Extract the corer from the sediment, place a stopper at the bottom end. Then remove the top stopper, and finally siphon the water off. Retain a sample of 5 cm length. Instead of withdrawing the corer, the corer may be dug out.

Remarks:

References:
Craib (1965); Warwick (1984); Higgins & Thiel (1988); Fleeger *et al.* (1988); MacIntyre & Pfannkuche & Thiel (1988)

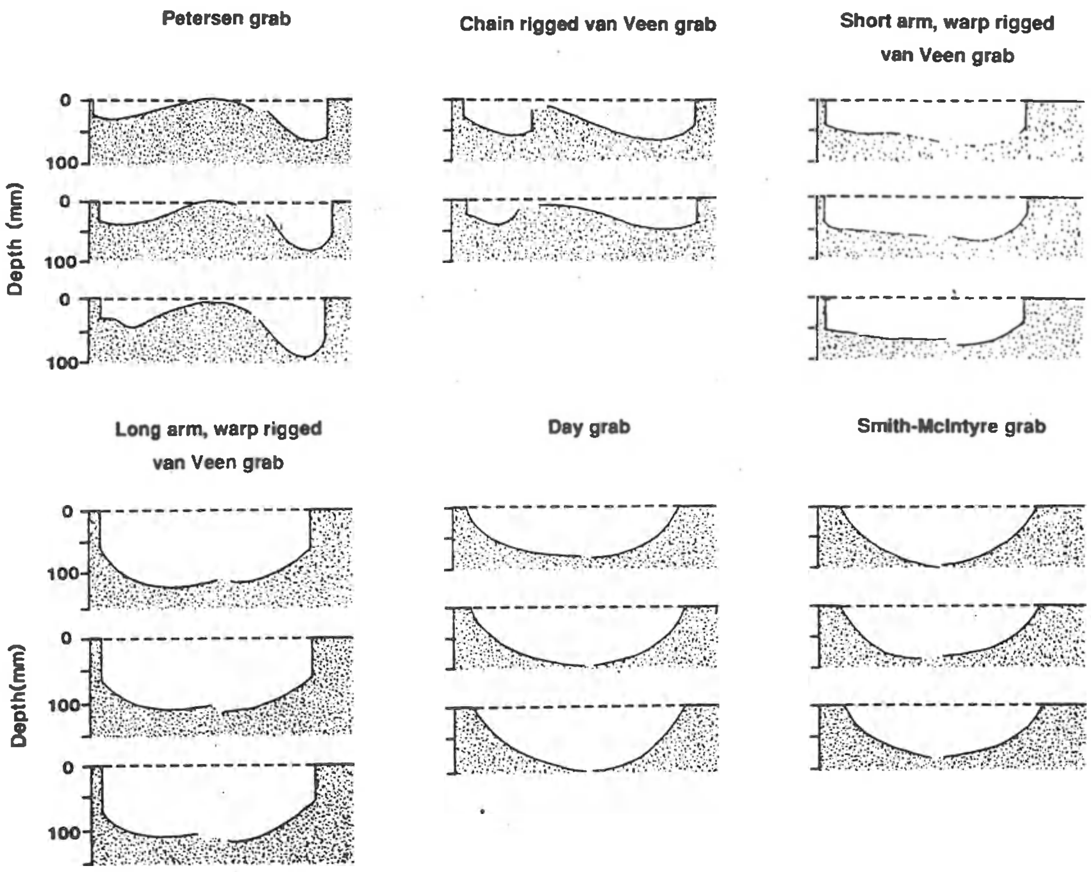


Figure 6.
Differences in bite profiles of six grab samplers (from: Riddle, 1989)

S-6.10

Compartment: sediment

Variable(s): macro-zoobenthos

Introduction:
In areas where there is a large variation in sediment type, only those sediment types that cover a relatively large proportion of the subtidal area in the salinity regime that has to be investigated should be sampled.
Preferably sampling should be conducted in the shallow subtidal and the intertidal zones. In the deeper subtidal areas the abundance of the macro-zoobenthos will be poorer, especially when the deeper parts are formed by tidal channels. Due to high current velocities few species /numbers are able to live there. This is not considered a typical estuarine habitat.
For subtidal work the use of a box-corer is much preferred. The major problem of box-corers are their availability and size. Therefore, grab-samplers will be often used for subtidalsediment sampling. For intertidal work hand corers of different sizes are almost exclusively used. One standard size is proposed, as differences may occur when collection is carried out with different core diameters.
The standard sampling depth is 25 cm. This can be achieved using a boxcorer or handcorer, but may be more difficult when using a grab sampler.
A minimum of 4 individual replicate samples should be collected either randomly or along a pre-described transect at 10 m distance intervals, in order to get representative information on the subtidal macro-zoobenthos. Sampling depth will also be dependent on the type of grab sampler used (Riddle, 1989b) (figure 6).
The organisms should be extracted from the sediment using a sieve with a 1 mm mesh. In fine sediments containing mainly oligochaetes sieving over a 0.5 mm screen may be more appropriate.
The samples should be stored separately in polythene bags or bottles.
Add 6 % formaldehyde in seawater to the core residues for preservation and storage. For biomass estimations, the use of glutaraldehyde is preferred, however (5%).

Sampling methods (subtidal):
Method #1. Boxcorer. A boxcorer must be operated from a relatively large research vessel. The samples should be collected either randomly or along a pre-described transect at 10 m distance intervals, in order to get representative information on the subtidal macro-zoobenthos. The minimum number of samples that should be per station/area is 5 - 10 boxcores.
Method #2. Grab sampler (0.2 m^2). Grab samples differ very much from the boxcoring method and the coring methods used in intertidal areas. Various types of grab-sampler are currently in use, e.g. several versions of the Van Veen Grab, the Petersen-, Smith-McIntyre- and Day-samplers. Riddle (1989b) compared the sampling efficiency of these samplers, including their 'bite' profiles and depth of sampling. For sediment collection an undisturbed sample is much preferred, together with a reasonable sampling depth. The chain-rigged Van Veen and the Petersen grab do not seem suitable for the purpose.

Most grab-sampler are self-trigering: when lowered to the sediment, they will close automatically when lifted. It may be that not all samples are of the desired quality, because e.g. a stone is trapped between the jaws, the sampler didn't penetrate far enough in the sediment, etc. A new sample should be collected when this occurs.
The 5 - 10 replicate samples should be collected on a transect, at about 10 m intervals.

Sampling methods **(intertidal):**
Method #1. Handcorer (200 cm^2). A hand operated corer consists of a PVC or stainless steel tube of ca. 16 cm Ø It should be possible to close the top (reduced) opening with a rubber stopper. In some sediments the air above the sediment during collection prevents collection of the sample due to expansion. In those cases, fill the space on top of the core completely with water, close the top opening and extraction will be easier.
The corer is pushed into the sediment, taking care not to sample an area that has been disturbed by human action.
Method #2. Handcorer (other size). Other diameters of hand corers may be in use. One should be aware of the differences that may be the result from the deviating diameters (Elliot, 1977).
For operation the conditions are equal to Method #1.

Remarks:

References:
Holme (1964); Elliot (1977); Downing (1979, 1989); Morin (1985); Salonen & Sarvala (1985); Hartley & Dicks (1987); Wolff (1987); Vézina (1988); Riddle (1989a,b); Rumohr (1990); Essink & Kleef, (1991)

7. ANALYTICAL PROCEDURES AND METHODS

In this chapter the most important physical , chemical and biological analyses that are important for a description of the estuarine environment are summarized. It is not the intention of the authors to reproduce all the details in handbooks on analytical chemical and biological methods in seawater. Neither are all the possible variables included, nor all possible methods per variable. Methods have been selected in view of optimum applicability for the estuarine environment, keeping in mind that not all participants have access to the newest techniques or sophisticated (automated) instrumentation. Another criterion for the selection of any analytical technique was the possibility of a direct measurement of the variable. A number of variables cannot be quantified by measurements, but are derived by calculation from other variables. These calculations, e.g. the conversion of particulate organic carbon data to biomass, is based on conversion factors derived from the literature. If analytical data have to be stored in a data base, as is the case with the JEEP92 programme it is more sensible to store the raw data in the data base, and leave it to the users of the data base to perform the conversions. The data will thus be more easily useable if, for example, the conversion factor changes in the future.

Where several possible techniques or methods give identical results, this is indicated using suffixes: Method #1a, Method #1b. Sometimes the results of different techniques do not compare well in intercalibration exercises. In these cases the methods will have different numbers: Method #1, Method #2. Unless stated otherwise, Method #1 is preferred over Method #2, etc. Participants within the JEEP92 project are strongly encouraged to apply the first analytical method indicated, thus Method #1, #1a or #1b.

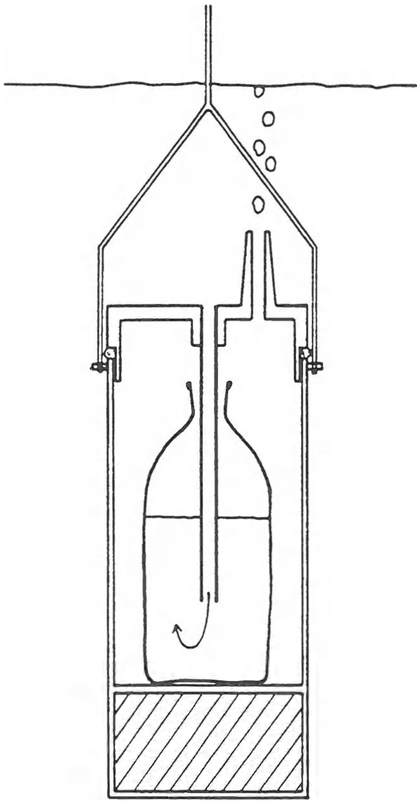
For each method the units in which the results should be expressed are given. These may be different from traditional units (as for example in the case of nutrients), but international guidelines are followed here: SI rules are used (Unesco, 1985). Though the expression of a concentration should preferably by per kg (instead of per litre, or dm^3), in this manual the "per litre" is used throughout for practical reasons. In table 5 an overview of the various analyses together with the symbols, units and JEEP codes is presented for easy reference. Annex III lists all analytical methods considered in this manual, with the (sub)method codes intended for use in the JEEP92 data base.

Each analytical procedure has the same lay-out. The meaning of the unique JEEP92 **analysis code** (A-7.xx number) for easy reference and used throughout the volume, the **variable** with symbol and the **unit** in which the results should be expressed, and the compartment are marked in the heading of each procedure. The **JEEP database** refers to the code that is used in the JEEP92 database: JEEP92 (De Winter, 1992a), which employs a maximum of four capital letters as abbreviation (see also Annex IV).
In the **Introduction** to each method some general information is presented, together with information on other techniques where applicable.
Sampling, Sample volume, Sample treatment and **Storage** are only briefly mentioned, and the reader is referred to the sections on sampling procedures (chapter 6).
As samples are not always collected by the most specialized personnel, relatively detailed information is given when necessary on the field activities (sampling, sample treatment, preservation and storage).
In the **Analytical** methods section the method(s) of analysis are outlined, in most cases the reader is referred to a detailed description of the method in the literature.
Several standard handbooks are available on seawater and estuarine analysis, which are considered essential. For detailed information the reader is referred to these handbooks and the original sources given therein, and to the **Method(s) references** given at the end of each analytical procedure.
Useful handbooks on marine analytical chemistry and on marine (and estuarine) biology include:

- Grasshoff, K., M. Ehrhardt & K. Kremling (eds), 1983. Methods of seawater analysis. 2nd ed. Verlag Chemie, Weinheim
- Higgins, R.P. & H. Thiel, 1988. Introduction to the study of Meiofauna. Smithsonian Institution Press, Washington DC, pp. 488.
- Morris, A.W. (ed), 1983. Practical procedures for estuarine studies. A handbook prepared by the Estuarine Ecology Group of the Institute for Marine Environmental Research. IMER, Plymouth, pp. 262
- Parsons, T.R., Y. Maita & C.M. Lalli, 1984. A manual of chemical and biological methods for seawater analysis. Pergamon, Oxford, pp. 173
- Rodier, J. (ed), 1984. L'analyse de l'eau: eaux naturelles, eaux résiduaires, eau de mer. 7th Ed., Dunod, Paris, pp. 1365
- Sournia, A. (ed), 1978. Phytoplankton manual. Unesco, Paris, 337
- Strickland, J.D.H. & T.R. Parsons, 1968. A practical handbook of seawater analysis. Fish. Res. Bd. Can. Bulletin 167, Ottawa, 311; ibid. 1972, 2nd edition.
- Unesco, 1985. The International System of Units (SI) in oceanography. Technical Papers in Marine Science, no. 45, pp. 124

The list of analytical procedures is sub-divided into different sections:
analyses in water (A-7.1 - A-7.21)
analyses in seston (A-7.22 - A-7.27)
analyses in sediment (A-7.28 - A-7.30)
analyses of biota :

bacteria (A-7.31-A-7.32)
phytoplankton (A-7.33 - A-7.35)
zooplankton (A-7.36 - A-7.39)
micro-phytobenthos (A-7.40 - A-7.42)
macro-phytobenthos (A-7.43 - A-7.44)
meiofauna (A-7.45 - A-7.46)
macro-zoobenthos (A-7.47 - A-7.49)
hyperbenthos



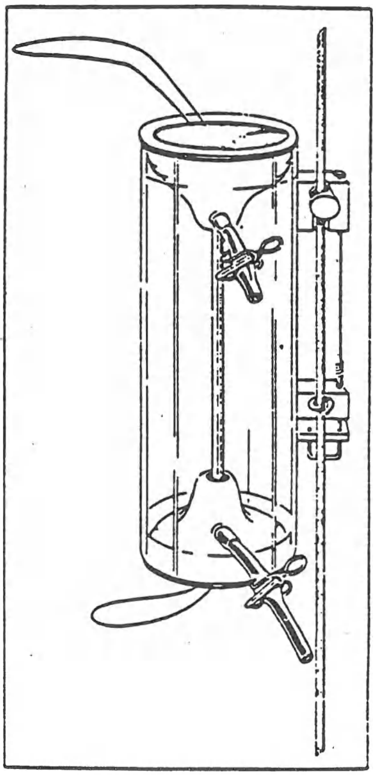


Figure 7.
Examples of water samplers. A simple water bottle in a holder for shallow depths (l) and the Van Dorn bottle (r) (from: Head, 1985b and Van Dorn, 1962 respectively)

A-7.1

Variable: **salinity**

S

Unit: **10^{-3} 1)**

JEEP database: **S**

Compartment: **water**

Introduction:
Salinity is one of the earliest defined variables in the marine environment (Grasshoff *et al.*, 1983; Mamayev, 1975). It constitutes one of the most fundamental variables in estuarine research, and great care should be given to its determination and precision, and thus calibration.
Absolute Salinity is defined as the ratio of mass of dissolved material in seawater to the mass of seawater. As this quantity cannot be measured directly a Practical Salinity is defined as a polynomial equation, which is, however, only valid between $2 \leq S \leq 42$ (Unesco, 1985). For practical reasons we use Salinity, which implies that there is a constant relationship between the major constituents in seawater. There are several methods of determining the salinity of sea- and estuarine waters. The most important and practical are:

- Conductivity. As this relationship is dependent on the fixed ratio between major seawater constituents, the method is not reliable for $S < 2 \times 10^{-3}$. This has serious implications for use in the upper estuarine regions.
- calculation based on chlorinity, the amount of chlorine 2). In the upper regions of the estuary this method is more reliable than the conductivity method, but one should beware of riverine water with relatively high chloride content 3). Essentially chlorinity is a separate, independent property, but for practical reasons and within the JEEP92 project, we may use Cl to calculate the salinity.

Calibration of the salinity measurement should be performed regularly by using Standard Seawater (obtained from IAPSO Standard Sea Water Service).

Sampling:

- in-situ measurement of conductivity, using a CTD probe;
- collection of water directly in a bottle, using a water sampler or a pump.

The measurement of profiles is encouraged before the actual sampling starts, to enable detection of possible haloclines. The salinity should be measured in relation to the other samples collected, as rapid salinity changes are likely to occur in estuarine environments 4).

Sample volume:
for in-situ measurement non relevant; others: 1 litre

Sample treatment:
none

Storage:
samples can be stored for several weeks preferably at low temperatures (but never < 0 °C) in borosilicate glass bottles with screw caps. No ground glass stoppers, and no high-pressure polythene should be used.

Analytical methods:
Method #1a. Conductivity, is a measurement of a physical property of the seawater and determined either as a conductivity ratio (calibrated against standard seawater) or by direct measurement of conductivity, both of which are corrected for temperature. The conductivity-density-salinity-chlorinity relationships are defined in Unesco (1981). Usually the measurement is performed in-situ using a Conductivity-Temperature-Depth (CTD) probe, or a more simple ST -meter; samples are collected for calibration in a salinometer.
Method #1b. Determination of chlorinity by titration of the chlorine with silver nitrate (Mohr-Knudsen titration), which is recalculated into salinity according to the definition : $S\ 10^{-3} = 1.80655\ Cl\ 10^{-3}$ (Unesco, 1985).

Remarks:

^{1}) According to the new definition of salinity the units are not expressed in 10^{-3}, but as 1 (the 10^{-3} is then ommitted). (Unesco, 1985). Generally the 10^{-3} is still in use, however.

^{2}) See method for chlorinity

^{3}) Millero (1984) discussed the conductivity-density-salinity-chlorinity relationships for estuarine waters. He concluded that if errors in salinity of ±0.04 are acceptable, the UNESCO definition may be applied without corrections for the ionic composition of the estuary, even in the freshwater part.

^{4}) A commonly used procedure where salinity profiles are recorded and afterwards samples for various variables are collected should be avoided. If large variations in salinity occur, the salinity should be determined for each sample taken, either during sampling (conductivity probe) or by separate analysis.

Method(s) references:
Strickland & Parsons (1968; 1972); Grasshoff *et al.* (1983); Rodier (1984); Leatherland (1985);

A-7.2

Variable: **chlorinity**

Cl

Unit: **10^{-3}**

JEEP database: **CL**

Compartment: **water**

Introduction:
Chlorinity is still used in estuarine research. The measurement is based on the constant ratio between the major constituents in seawater, including chloride. As chloride is usually not important in river water, this measurement is a better indication of mixing of fresh- and seawater, especially in the upper part of the estuary (at $S < 2 \times 10^{-3}$), where the interfering ionic composition of fresh water hampers the conductivity measurement.

Sampling:
Collection of water direct in a bottle, using a water sampler or a pump.

Sample volume:
1 litre

Sample treatment:
None

Storage:
Samples can be stored for several weeks preferably at reduced temperature in borosilicate glass bottles with screw caps (but not < 0 °C, as during freezing precipitation will partly occur due to formation of micelles of different densities).

Analytical methods:
Method #1. Determination of chlorinity by titration of the chlorine with silver nitrate (Mohr-Knudsen titration) [1])

Remarks:
[1]) From chlorinitythe salinity can be calculated according to the definition:
$S\ 10^{-3} = 1.80655\ Cl\ 10^{-3}$ (UNESCO, 1985).
Calibration of the chlorinity measurement should be performed regularly by using Standard Seawater (obtained from IAPSO Standard Sea Water Service).

Method(s) references:
Strickland & Parsons (1968; 1972); Grasshoff *et al.* (1983); Rodier (1984)

A-7.3

Variable: **temperature**

T

Unit: **°C**

JEEP database: **TEMP**

Compartment: **water**

Introduction:
Temperature affects the rates of all chemical and biological processes. It also acts as a tracer for studies of the dispersion of thermal effluents.
Today either the semiconductor thermistor or the platinum resistance thermometer are most commonly used. Thermistors are cheaper but have an accuracy in the order of 0.1 °C, while a resistance thermometer has an accuracy of about 0.01 °C.
The mercury filled (deep-sea) thermometers are not often used in estuarine work, nor are the very accurate quartz crystal thermometers (Uncles *et al.*, 1983).
In-situ measurements are usually made by combined S and T (and depth) sensors in one probe. Vertical profiles are easily obtained and may indicate inhomogeneity or stratification in the water column.

Sampling:
in situ measurement aty selected depths or along profiles whatever probe is used, regular calibration with high-precision calibrated thermometers in a stirred waterbath at different tempera-tures is necessary.

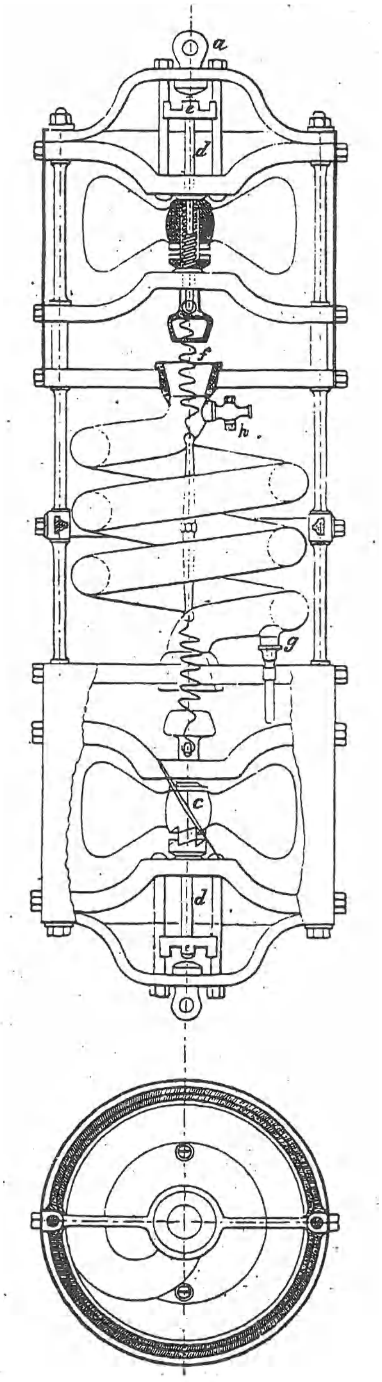
Sample volume:
non relevant

Sample treatment:
none

Storage:
non relevant

Analytical methods:
Method #1a. Thermistor
Method #1b. Resistance thermometer.
Usually the measurement is performed in-situ using a Conductivity-Temperature-Depth (CTD) probe, or a more simple ST -meter, where one of the two sensor types is incorporated. Allow sufficient time to equilibrate at selected depths (5 sec), or lower the probe slowly (< 30 cm/s) when recording continuously.
Method #2. Thermometer. Traditional thermometers, filled with mercury or alcohol are known for their slow response. At least 30 sec should be allowed for equilibration. The oceanographic reversing thermometers seem to be a good option, but are rather tricky to use in shallow estuarine waters.

Method(s) references:



The past:
Water sampler of Wille (Tornøe, 1880)

A-7.4

Variable: **turbidity**

Secchi depth, SD, Transmission, T_r

Unit: **Secchi disc: m, Transmission: %**

JEEP database: **TURB, SECH**

Compartment: **water**

Introduction:

The transmission of light is affected by absorption and scattering Because of the relatively high amount of suspended particulate matter, scattering is the most important process.
Determination of the 'Secchi depth' is a useful and simple way of providing an indication of the transparency of the (surface) water. More elaborate techniques involve the measurement of the transmittance of light over a given length (beam transmissometers) which can used either in-situ or on board the sampling platform using a pumping system and flow through cell.
A more sophisticated type of instrument is the nephelometer, consisting of a collimator and a photocell set at an angle (usually 90°) to the light path and which measures directly the light scattered at a specific angle. *In situ* measurement is possible.
All types of measurements suffer from the problem that different types of suspensions scatter varying amounts of light, which means that estimation of the amount of suspended matter requires empirical calibration in the field (in space and time) by collection of samples 1).
Since turbidity is highly dependent on current speed, *in situ* measurements should be combined with current measurements. When a multi-sensor probe can be used, discrepancy between collection of data with several devices, and consequently measurement of different water masses, can be prevented (Fanger *et al.*, 1990).

Sampling:

Either *in situ* measurement (Secchi disc, Transmissometer, Nephelometer) or on-board detection in a flow through cell under continuous pumping.
Both transmissimeter and nephelometer allow the measurement of vertical profiles.

Sample volume:

not relevant

Sample treatment:

none

Storage:

not relevant

Analytical method(s):
All methods give specific results that cannot be compared between techniques; no ranking is implied here.
Method #1: Secchi depth'. A white disc, 30 cm Ø, is lowered into the water. The depth at which it is no longer visible is the Secchi depth' (SD). Disappearing and re-appearing should be checked.
Method #2. Beam transmission. The transmissometer is usually specific in design, with a fixed cell length (for estuarine waters between 1 and 10 cm). The instrument gives a reading in percentage of full transmission (transmission in air or particle free water).
Method #3. Nephelometer. Either *in situ* or in the laboratory. As the scattering measured is measured under a 90° angle, nepholometry is more suitable for the detection of particles in low turbidity waters (transmittance > 90 %).

Remarks:
[1]) see the determination of the amount suspended particulate matter (SPM), procedure A-7.22.

Method(s) references:
Morris (1983); Leatherland (1985); Instrument specific manuals

A-7.5

Variable: **oxygen**

O_2

Unit: **mg/l** [1)]

JEEP database: **O2**

Compartment: **water**

Introduction:
The chemical determination of oxygen in seawater is traditionally done by the Winkler titration, which is accurate and sensitive, and is used to check on other methods of measurement.
New methods involve the use of oxygen sensing electrodes which are generally of two types: galvanic or polarographic. These techniques measure the in-situ partial pressure of oxygen which is dependent on temperature, pressure and salinity. Thus meters calibrated in % oxygen saturation will give accurate values in both saline and freshwater, while those instruments with a concentration readout will only be accurate in saline waters if a salinity correction has been built-in in the instrument. Calibration thus becomes important; procedures are given by HMSO (1980).
Other methods have been described (e.g. by mass spectrometry, gas-chromatography, amperometry) but they have no practical use in estuarine research.

Sampling:
In-situ analysis when applying electrodes , allowing ample time for equilibration (> 30 sec). Vertical profiles are possible.
Collection of water using a water-sampler. Sub-samples for oxygen should be collected as soon as possible after recovery of the sampler. Care must be taken that no air is mixed with the water sample. This can be achieved by mounting a clear plastic tube to the outlet of the sampler, long enough to reach the bottom of the storage bottle. Take care that no air is trapped in the tube and flush the bottle with sample water (at least twice the volume), avoiding a too high turbulence; gently withdraw the tube while the sample is still running. The bottle is completely filled. Check for air bubbles sticking to the wall, and remove them by gently tapping. Before closure, reagents are added (see below).

Sample volume:
Typical sample volumes are in the order of 50-55 ml. Special, volume calibrated glass (oxygen) bottles should be used, with special glass stoppers. Because of calibration, the bottle and stopper should be numbered as they belong together.

Sample treatment:
For the Winkler titration the sample should be preserved immediately after collection. This is performed by adding manganese(II) chloride and alkaline iodide using a dispenser reaching almost the bottom of the flask and slowly withdrawn when the chemicals are added. The stopper is then inserted displacing about 5 ml of air-contaminated sample. Shake the bottles vigorously for one minute.

Storage:
During transport the stoppers may be secured with a rubber band. Ideally the analysis should be performed within 10-12 h of sampling. The bottles should be stored in the dark under even temperature conditions, preferably under water to minimize possible air exchange.

Analytical method(s):
Both Winkler titrations and the careful use of oxygen electrodes should yield the same order of accuracy.
Method #1a. Winkler titration. The dissolved oxygen is chemically bound by manganese(II) hydroxide in a strong alkaline medium. Mn(II) is oxidised to Mn(III) consuming the oxygen. Before titration the precipitated hydroxides are dissolved by acidification with sulphuric acid to $pH < 2.5$. Mn(III) oxidises iodide ions in an acidic medium to iodine, which is titrated with thiosulphate, using starch as an indicator (i.e. a colorimetric detection).
Method #1b. Oxygen electrode. For a detailed description of the working of the electrodes the manufacturers instructions should be followed. It should be noted that the slope will change over time and that calibration before each set of measurements is essential. Electrode membranes will have to be replaced from time to time, requiring full calibration afterwards. The electrodes should be stored under moist conditions. Lack of sufficient equilibration of the electrode in the sample is the most common source of error.

Remarks:
[1] both the units mg/l and % saturation are important. For the interpretation of estuarine processes the latter can be calculated if salinity and temperature are known.

Method(s) references:
Strickland & Parsons (1967; 1972); Grasshoff *et al.* (1983); Parsons *et al.* (1984); Rodier (1984); Head (1985a)

A-7.6

Variable: pH

Unit: -

JEEP database: PH

Compartment: water

Introduction:

The pH, or the negative logarithm of the hydrogen ion activity (- log $a_{H}+$) is today exclusively measured by an ion specific electrode (glass electrode), either as a glass electrode with a separate reference electrode, or a combined electrode device. The electrode is connected to a pH meter.

In-situ electrode systems are available, but often with a poor performance so far. Temperature and pressure affect the reading and care should be taken to correct for them. There is also a salt effect, but this is considered unimportant.

The care necessary for correct determination of pH is often underestimated, due to the false simpleness of the analysis (insert a stick in the sample, stir somewhat and read three digits from the digital display).

The pH is one of the measurements that should preferably be made at the sampling location.

Sampling:

Because interaction with the CO_2 in the atmosphere will affect the pH in the sample, for very precise analysis the sample should be collected with the same care as for the oxygen measurements. When the samples cannot be analyzed directly, this procedure is essential. The subsampling from the water-sampler should be similar to the subsampling of oxygen. It involves mounting a clear plastic tube onto the outlet of the sampler, long enough to reach the bottom of the storage bottle. Take care that no air is trapped in the tube and flush the bottle with ample water (at least twice the volume), avoiding a too high turbulence; gently withdraw the tube while the sample is still running. The bottle is completely filled. Check for air bubbles sticking to the wall, and remove them by gently tapping.

Sample volume:

Glass bottles with glass stoppers, 50-100 ml should be used.

Sample treatment:

None

Storage:

Storage at reduced temperature, in the dark prior to measurement, which should be within 1 hour of sampling. Biological activity will seriously affect the pH.

Analytical method(s):

Method #1. Potentiometric determination of the hydrogen ion activity. Measurement involves calibration with at least two buffer solutions, and at controlled or at least recorded temperature. From the temperature during measurement the pH in-situ can be calculated (the field temperature should be known).

Remarks:

Method(s) references:
Strickland & Parsons (1968; 1972); Grasshoff *et al.* (1983); Rodier (1984)

A-7.7

Variable: **total alkalinity**

Alk_t

Unit: **mmol/l**

JEEP database: **ALK**

Compartment: **water**

Introduction:
The alkalinity may be defined as the excess of anions of weak acids in seawater. The alkalinity can be titrated and total alkalinity or titration alkalinity corresponds to the amount of strong acid required to neutralize 1 kg of seawater. Three different methods can be applied: the pH method, the back titration method and the potentiometric titration method. Their results are not considered equally accurate (precision respectively ±1, 0.2 and 0.8 %).

Sampling:
Samples can be obtained from any sampling device

Sample volume:
Depending on the method and equipment used 100-500 ml in (brown) glass bottles that have preferably been aged with hydrochloric acid for months. Bottles should be very clean to prevent/minimize bubble formation on the walls around 'nuclei'.

Sample treatment:
If the samples cannot be analyzed soon after collection an exact amount of hydrochloric acid should be added to the sample as preservative.

Storage:
Although exchange of CO_2 with the atmosphere does not hamper the analysis, the loss of CO_2 by precipitation or biological activity does influence the analysis. Analysis should therefore be performed as soon as possible after collection. See under sample treatment.

Analytical method(s):
Method #1. The pH method is a single point potentiometric titration of seawater. The sample is acidified to about pH 3.5. The pH is measured with a high precision pH meter and the alkalinity is calculated from the difference between the amount of acid added and the excess acid present.
Method #2. The back titration method according to Gripenberg involves acidification with hydrochloric acid to pH 3.5. Total CO_2 is driven off by boiling. The solution is then back titrated with sodium hydroxide to pH 6 using an indicator and under purging with CO_2-free air.
Method #3. The potentiometric titration method is more complicated and involves iterative calculations and Gran plots. It requires the use of a computer for calculation. It involves the use of a pH electrode and a closed titration cell of accurately determined volume, under controlled or recorded temperature conditions.

Remarks:

Method(s) references:
Strickland & Parsons (1967; 1972); Grasshoff *et al.* (1983); Parsons *et al.* (1984); Rodier (1984)

A-7.8

Variable: **nitrate**

NO_3^-

Unit: **µmol/l**

JEEP database: **NO3**

Compartment: **water**

Introduction:
Nitrate is the final oxidation product of nitrogen compounds in seawater and is considered the thermodynamically stable oxidation level under aerobic conditions. Nitrate is considered one of the most important nutrients controlling primary production. In estuaries large variations may occur, due to input and conversion in primary production. If the oxygen content becomes depleted as a result of microbial mineralization processes, nitrate may be used as an alternative electron acceptor. This denitrification happens in organic rich sediments and sometimes in estuarine waters (e.g. the Scheldt).
In the most frequently applied method, nitrate is reduced to nitrite, and the nitrite already present is corrected for. Manual and automated analytical methods (Autoanalyzer etc.) have been developed based on the same principle.

Sampling:
Any sampling device is acceptable.

Sample volume:
If all nutrients are sampled together, 0.5-1 l is more than sufficient. For nitrate the sample should be collected in glass bottles, or transferred to glass bottles as soon as possible (within 1 hour after sampling).

Sample treatment:
No treatment is necessary, unless the samples have to be stored for a longer period of time. In that case ammonium chloride buffer or mercury chloride (0.01 % w/w) should be added as preservative.

Storage:
Samples should be analyzed within 5 hours and, when no preservative is added, stored in the dark in a refrigerator. They may be stored deep frozen (- 20 °C) for several weeks. Chemically preserved samples should be stored dark and cool without refrigeration.

Analytical method(s):
Method #1. Nitrate reduction method. For this method the nitrate ions in the estuarine water, after buffering with ammonium chloride, pass over a reductor (copper coated cadmium granules), and are quantitatively reduced to nitrite . Detection is similar to the detection of nitrite through an azo dye, which is spectrophotometrically detected (see A-7.9). The original nitrite should be corrected for.

Remarks:

Method(s) references:
Strickland & Parsons (1967; 1972); Grasshoff *et al.* (1983); Parsons *et al.* (1984); Rodier (1984); Head (1985a); Kirkwood *et al.* (1991)

A-7.9

Variable: **nitrite**

NO_2^-

Unit: µmol/l

JEEP database: **NO2**

Compartment: **water**

Introduction:
Nitrite forms the intermediate between reduction of nitrate or the oxidation of ammonia in sea- and estuarine water. Usually the concentrations are rather low, except in transition zones between oxic and anoxic conditions. Especially in polluted estuaries it can become an important aspect of the nitrogen cycle.
Only one spectrophotometric detection method is commonly used.
Manual and automated analytical methods (Autoanalyzer etc.) have been developed based on the same principle.

Sampling:
Any sampling device is acceptable.

Sample volume:
If all nutrients are sampled together, 0.5-1 l is more than sufficient.

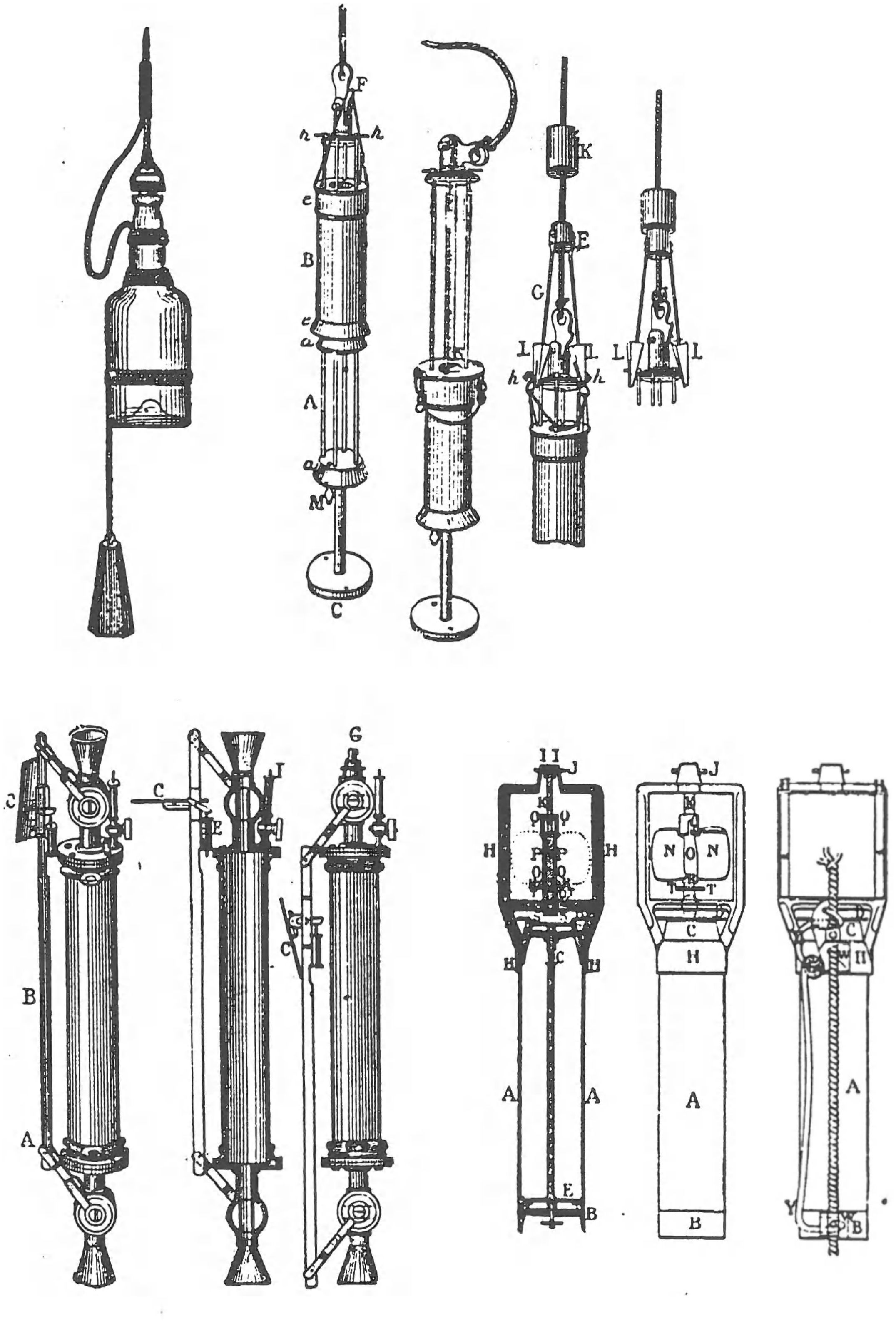
Sample treatment:
In turbid waters filtration over a prewashed polycarbonate 0.4 µm Nuclepore or 0.45 Ø membrane filter is necessary. Because of biological (bacterial) activity, analysis should be performed within 30 min of sampling, or at least the reagents should have been added. Mercury chloride (0.01 % w/w) may be added as preservative.

Storage:
Samples (with the reagents added) should be analyzed within 5 hours. Direct sunlight should be avoided. Samples may be stored deep frozen (- 20 °C), though prolonged storage is not recommended.
Chemically preserved samples should be stored dark and cool without refrigeration.

Analytical method(s):
Method #1. The nitrite in sea- and estuarine water is allowed to react with sulphanilamide in an acid solution. The resulting diazo compound reacts with N-(1-naphthyl)-ethylenediamine and forms a highly coloured dye. The extinction is measured spectrophotometrically at 540 nm.

Remarks:

Method(s) references:
Strickland & Parsons (1967; 1972); Grasshoff *et al.* (1983); Parsons *et al.* (1984); Rodier (1984); Head (1985a)



The past:
Water sampling bottles of 1) the 'Kiel commission' (1873); 2) of Meyer (1889); 3) of Buchanan (1893) and 4) of Sigsbee (1880)(from: Thoulet, 1890)

A-7.10

Variable: **ammonia**

NH_4

Unit: **µmol/l**

JEEP database: **NH4**

Compartment: **water**

Introduction:
In all methods the sum of NH_3 and NH_4^+ is determined, which is not unrealistic because of the acid-base pair that exists in nature (NH_4^+-NH_3).
Two major methods are used which, however, measure different amounts of nitrogen. The oxidation method determines not only the ammonia but also a considerable fraction of the amino acids (which may not be undesirable in production studies, as the amino acids will be used as an N-source as well). The alternative more preferable method determines ammonia only. More methods can be found in the literature, however. Manual and automated analytical methods (Autoanalyzer etc.) have been developed based on the same principle.

Sampling:
Any sampling device is acceptable.

Sample volume:
If all nutrients are sampled together, 0.5-1 l is more than sufficient.

Sample treatment:
No treatment required. Filtration is not recommended because of the serious chance of contamination by the filters.

Storage:
Samples for the determination of ammonia should be analyzed as soon as possible after collection. Storage in a refrigerator is possible up to 3 hours. Samples can be stored for up to 2 weeks after addition of phenol as a preservative, or freezing at - 20 °C.

Analytical method(s):
Method #1. Ammonia. The sample is treated in an alkaline citrate medium with sodium hypochlorite and phenol in the presence of sodium nitroprusside which acts as a catalyst. The blue indophenol colour formed with ammonia is measured spectrophotometrically at 640 nm.
Method #2. Oxidation method. The ammonia (and some other amino compounds) in sea and estuarine water is oxidized to nitrite by alkaline hypochlorite at room temperature and the excess oxidant destroyed by the addition of arsenite. The nitrite is determined spectrophotometrically by the procedure given under A-7.9.

Remarks:
Avoid smoking and other air-borne contamination during sample handling.

Method(s) references:
Strickland & Parsons (1967; 1972); Grasshoff *et al.* (1983); Parsons *et al.* (1984); Rodier (1984)

A-7.11

Variable: **phosphate**

PO_4^{3-}

Unit: **µmol/l**

JEEP database: **PO4**

Compartment: **water**

Introduction:
Phosphorus is one of the most important nutrients. Inorganic phosphate is present in seawater in the form of ions of (ortho) phosphoric acid, PO_4^{3-} and HPO_4^{2-}. In addition to these (and other possible) inorganic forms, organic phosphorus compounds are present. Speciation into various phosphorus species is possible, as is the determination of total phosphorus, but this is beyond the scope of this manual. Generally only one method is used for the detection of inorganic phosphate .
Manual and automated analytical methods (Autoanalyzer etc.) have been developed based on the same principle.

Sampling:
Any sampling device is acceptable.

Sample volume:
If all nutrients are sampled together, 0.5-1 l is more than sufficient. For phosphate the sample can be collected in polythene or glass bottles, or transferred to glass bottles.

Sample treatment:
Filtration can cause serious errors, because of contamination of the sample.
The use of 0.4 µm Nuclepore filters has been recommended, after washing with part of the sample water.

Storage:
Preferably samples are analyzed directly after sampling, within 2 hours, but if necessary may be stored for a short period in a cool dark place . Several methods have been suggested for long term storage: deep frozen (at - 20 °C), addition of sulphuric acid or of chloroform. Mercury chloride (0.01 % w/w) has been suggested as preservative. There is conflicting evidence on the effects of these storage methods, however.

Analytical method(s):
Method #1. The sample is allowed to react with a composite reagent containing molybdic acid, ascorbic acid and trivalent antimony. The resulting complex acid is reduced *in situ*, and measured spectrophotometrically at 885 nm.

Remarks:

Method(s) references:
Strickland & Parsons (1967; 1972); Grasshoff *et al.* (1983); Parsons *et al.* (1984); Rodier (1984); Head (1985a)

A-7.12

Variable: **silicate**

H_4SiO_4

Unit: **µmol/l**

JEEP database: **SiO4**

Compartment: **water**

Introduction:
Silicon is an essential element especially for diatoms. It is present in seawater in the form of orthosilicic acid $Si(OH)_4$. Usually a method involving a silicomolybdic complex is applied which, however, only measures the so called "reactive" silicate. Several routes can be followed.Manual and automated analytical methods (Autoanalyzer etc.) have been developed based on the same principle.

Sampling:
Any sampling device can be used.

Sample volume:
If all nutrients are sampled together, 0.5-1 l is more than sufficient. For silicates the sample should never be collected in glass bottles, but in polythene or PVC bottles.

Sample treatment:
Filtration over a 0.45 µm membrane filter is recommended in estuarine waters as the (re)suspended silicate rich particulate matter will overestimate the dissolved silicate content. No further treatment is required, unless prolongued storage is espected. Preservation by addition of sulphuric acid to pH 2.5, or addition of mercury chloride (0.01 % w/w) as preservative is then recommended.

Storage:
Storage of a sample in the refrigerator in the dark does not change the silicate content significantly. Polymerisation of orthosilicate has been reported for freshwater and may be important in the upper estuary. Storage at - 20 °C can be used provided that the samples are left for at least 3 hours after thawing.

Analytical method(s):
Method #1a. The estuarine water sample is allowed to react with molybdate under conditions which result in the formation of silicomolybdate, phosphomolybdate and arsenomolybdate. A metol and oxalic acid containing solution is added which reduces the silicomolybdate complex (or to give a blue colour (detection at 810 nm) and at the same time decomposes the other two molybdates.
Method #1b. The acidified sample is treated with a molybdate solution to form yellow coloured silicomolybdic acid, which can be detected at 390 nm (ß-isomeric form) after addition of oxalic acid and ascorbic acid, the latter as reductant.

Remarks:

Method(s) references:
Strickland & Parsons (1967; 1972); Grasshoff *et al.* (1983); Parsons *et al.* (1984); Rodier (1984); Head (1985a); Eberlein & Kattner (1987)

A-7.13

Variable: **hydrogen sulphide**

H_2S

Unit: **µmol/l**

JEEP database: **H2S**

Compartment: **water**

Introduction:

When the oxygen is depleted by bacterial mineralisation processes, sulphate is used as an electron donor and consequently sulphide is produced. In these anoxic waters, that may also occur in estuaries when a high load of organic matter (sewage) is introduced in the system, the sulphide is dissolved mainly as hydrogen sulphide (H_2S). Sulphides (S^{2-}) are particularly reactive towards (heavy) metals.

Two colorimetric methods, the methylene blue method and Lauth's violet method, are used. A titrimetric method has also been described, but is less accurate. The latter method is not exclusive for the detection of H_2S and will also include effects of other reductants.

Sampling:

The oxygen in the air will readily oxidize the hydrogen sulphide. Therefore similarly careful sampling as in the case of dissolved oxygen has to be applied. Plastic or glass water samplers should be used as metal parts influence the sulphide content.

Sub-samples for hydrogen sulphide should be collected as soon as possible after recovery of the sampler. Care must be taken that no air is mixed with the water sample. This can be performed by mounting a clear plastic tube onto the outlet of the sampler, long enough to reach the bottom of the storage bottle. Take care that no air is trapped in the tube and flush the bottle with sample water (at least twice the volume), avoiding a too high turbulence; gently withdraw the tube while the sample is still running. The bottle is completely filled. Check for air bubbles sticking to the wall, and remove them by gently tapping.

Sample volume:

Typical sample volumes are in the order of 50-55 ml. Special glass (oxygen) bottles should be used with special glass stoppers.

Sample treatment:

No treatment is required (see under storage)

Storage:

Samples may be preserved by addition of zinc acetate or-chloride, which results in the precipitation of ZnS. When kept in the dark the samples can thus be stored for a long time.

Analytical method(s):

Method #1a. Addition of dimethyl-p-phenylene diamine and ferric chloride results in the formation of methylene blue which is detected spectrophotometrically at 670 nm.

Method #1b. To the acidified sample p-phenylene diamine and ferric chloride are added, resulting in the formation of Lauth's violet, which is detected spectrophotometrically at 600 nm.

Method #2. The hydrogen sulphide in the sample is precipitated with manganous sulphate reagent and alkaline potassium iodide (see Winkler titration, in A-7.5) Manganous sulphide is formed and precipitated with the hydroxide. Addition of potassium iodate and sulphuric acid, and titration with thiosulphate using starch as an indicator.

Remarks:

Method(s) references:

Strickland & Parsons (1967; 1972); Grasshoff *et al.* (1983); Rodier (1984)

A-7.14

Variable: **sulphate**

SO_4^{2-}

Unit: µmol/l

JEEP database: **SO4**

Compartment: **water**

Introduction:
Sulphate is abbundant in the marine environment, and in seawater a constant sulphate/chlorinity ratio can be found. Per kg standard seawater of salinity 35 x 10^{-3} 2.773 g (0.02889 M) sulphate is present. In estuaries anoxic water conditions are not uncommon, due to the mineralisation of organic matter . Under these conditions sulphate is reduced to sulphide(s) by sulphate reducing bacteria. Thus in estuaries the constant ratio may change.
Analysis can be performed in various ways, the most important include gravimetric and turbidimetric methods. Also the methylene blue method that is used for the determination of H_2S (see A-7.13) has been applied, after reduction of sulphate. This method has been adapted for use in an Autoanalyzer, involving methylthymol blue. Radiochemical (use of ^{131}Ba or ^{133}Ba), atomic absorption (AAS, detection of Ba) and ion chromatographic methods exist, but they will not be treated here.

Sampling:
Any sampling device is acceptable.

Sample volume:
If all nutrients are sampled together, 0.5-1 l is more than sufficient. The sample can be collected in polythene or glass bottles.

Sample treatment:
The filtration of the watersample over a 0.2 µm filter is recommended to remove bacteria that may interfere (see storage).

Storage:
In the presence of organic matter bacteria may reduce sulphate to sulphide. To prevent this the samples should be stored deep froozen at - 20 °C.

Analytical method(s):
Method #1. Tubidimetric determination. The sulphate in the sample is quantitatively precipitated as small, uniformly sized crystals of $BaSO_4$ in a suspension medium containing HCl and gelatin of which the turbidity is determined on a colorimeter fitted with a blue filter.
Method #2. The gravimetric method also involves the quantitative precipitation of sulphate in the sample as $BaSO_4$ that is formed after addition of an excess of barium chloride. The precipitate is washed, dried and weighed.

Method #3. Barium sulphate is formed by the reaction of the SO_4^{2-} with barium chloride at low pH. At high pH excess barium reacts with methylthymol blue to produce a blue chelate. The uncomplexed methylthymol blue is gray. The detection of this uncomplexed indicator is by colorymetry using a 460 nm filter. ***Method #4.*** After reduction of the sulphate to sulphide, the sulphide is colorimetrically detected by the methylene blue method (see A-7.13).

Remarks:
One should be aware of the processes that may lead to the transformation of either sulphate into sulphide by bacteria, or of sulphide into sulphate due to (rapid oxidation in air), especially in organic matter rich estuaries.

Method(s) *references:*
Johnson & Nishita (1952); Berglund & Sörbo (1960); Morris & Riley (1966); Tabatabai (1974); APHA *et al.* (1985)

A-7.15

Variable: **dissolved organic carbon**

DOC

Unit: **mg/l**

JEEP database: **DOC**

Compartment: **water**

Introduction:

Carbon is present in sea- and estuarine waters in organic and inorganic forms. The latter occur in concentrations that are orders of magnitude higher than the DOC. Particulate and dissolved organic carbon play a very important role in the estuarine system, as they contribute to a large extent to the oxic/anoxic conditions, the biological activity etc. (Duursma & Dawson, 1981).
Several methods have been described and are used, all based on the quantitative destruction of the DOC and subsequent measurement.
The methods involve either chemical wet oxidation, high temperature combustion (with or without a catalyst), and photo-oxidation.
Unfortunately there is strong evidence that not all methods break down the DOC quantitatively, and some refractory material remains undetected (Sugimura & Suzuki, 1988). How far this discrepancy is important for estuarine work is as not clear yet. As long as there is no general agreement upon the use of one technique, it seems unrealistic to prescribe one method as the preferential choice. Nevertheless the better the breakdown of organic matter , the better the technique, despite the possibility that some refractory material is not available for organisms. The sequence of the methods listed here follow more or less a decrease in their ability to destroy dissolved organic matter .
In some methods there may be serious interference from the chlorine that develops during destruction. Moreover, it is necessary to avoid the interference of inorganic carbon. The methods used for this step still cause many problems.
Manual and automated analytical methods (Autoanalyzer etc.) have been developed based on some of these principles.
Specialized instruments have been produced.

Sampling:

Because of the very low DOC concentrations in seawater great care must be taken to avoid contamination. Oil and grease from sampling gear is a major source of error. Plastic water samplers or glass samplers can be used. For surface water sampling, care must be taken not to collect the organic rich surface micro layer.

Sample volume:

The collection of 1 litre water in (dichromate/sulphuric acid) cleaned glass bottles with glass stoppers allows later filtration and collection of particulate organic matter . A 100 ml sample is sufficient for DOC analysis only.

Sample treatment:

In estuarine waters it is essential to filter the samples prior to analysis. Preignited (450 °C overnight) glass fibre filters should be used (GF/C or GF/F).

Storage:

The method of storage will partly depend on the method of analysis. Biological activity must be prevented. This has traditionally been performed by addition of mercury chloride (0.01 % w/w) as a preservative; this is a serious toxic agent, however, and is banned in several institutes. It may also interfere with the analysis, especially where catalysts are involved. A minimum requirement is cold storage in the dark.

Addition of phosphoric acid to pH < 4.5 has been proposed, but suffers from potential contamination in waters with a low DOC concentration.

In methods which use ampoules, the samples, once sealed can be stored for a long time, especially after they have been autoclaved.

Analytical method(s):

Method #1. High temp. catalytic oxidation. The oxidation is carried out on a platinum catalyst at 680 °C under an oxygen atmosphere after the sample has been freed of inorganic carbon. The concentration of the CO_2 is measured with a non-dispersive IR gas analyser.

Method #2. High temp. catalytic oxidation. The nitric acid acidified sample is freed from inorganic and CO_2 volatile organic compounds. The liquid phase is introduced in the copper filled oxidation reactor maintained at 880 °C. Collected CO_2 is desorbed, mixed with hydrogen and passed through a methane reactor containing a nickel catalyst. The methane formed is detected using a flame ionisation detector (FID).

Method #3. UV oxidation. The acidified sample is purged with nitrogen to remove carbonates. After a persulphate/UV destruction of the DOC in a quartz coil the CO_2 is dialysed into a (bi)carbonate-phenolphthalein colour reagent and photometrically detected at 550 nm.

Method #4. Dry combustion. The method involves the evaporation of the water and burning the residue in a combustion tube. The method suffers from high blanks.

Method #5. Wet chemical oxidation. Phosphoric acid and $K_2S_2O_8$ (persulphate) are added to the sea-water, the inorganic CO_2 is purged out with a CO_2-free gas and the sample is sealed in ampoules. The DOC is oxidized by heating the ampoules in an autoclave, the resulting carbon dioxide measured by infrared analyzer.

Remarks:

Method(s) references:

Gershey *et al.* (1979); MacKinnon (1981); Grasshoff *et al.* (1983); Rodier (1984); LeB-Williams (1985); Sugimura & Suzuki (1988); Specific instrument manuals

A-7.16

Parameter: **dissolved organic nitrogen**

DON

Unit : **mg N/l**

JEEP database: **DON**

Compartment: **water**

Introduction:
Few methods have been described and used, all are based on the quantitative destruction of the DON to nitrate and its subsequent measurement.
The methods described here involves chemical wet oxidation. Other methods involve the Kjeldahl method or a photo destruction with ultra violet light. The latter usually suffers from still more incomplete oxidation of the organic nitrogen compounds than the wet oxidation (see also the discussion under DOC, A-7.15), and is thus not recommended.

Sampling:
Because of the very low DON concentrations in seawater great care must be taken to avoid contamination. Plastic water samplers or glass samplers can be used. For surface water sampling, care must be taken not to collect the organic rich surface micro layer.

Sample volume:
The collection of 1 litre water in (dichromate/sulphuric acid) cleaned glass bottles with glass stoppers allows later filtration and collection of particulate organic matter . A 100 ml sample is sufficient for DON analysis.

Sample treatment:
In estuarine waters it is essential to filter the samples prior to analysis. Pre-ignited (450 °C overnight) glass fibre filters should be used (GF/C or GF/F).

Storage:
Preferably, wet-oxidized samples should be stored. If this is not possible, biological activity must be prevented. This has traditionally been performed by addition of mercury chloride (0.01 % w/w) as a preservative; this is a serious toxic agent, however, and is banned in several institutes. A minimum requirement is cold storage in the dark.

Analytical method(s):
Method #1. Persulphate method. DON will be analyzed after oxidation with potassium peroxidisulphate (persulphate) with boric acid and sodium hydroxide at 125 °C under pressure in a destruction bomb. A pressure cooker may be used instead. After cooling the organic nitrogen has been converted into nitrate which is analyzed according to the method for nitrate as given under A-7.8.
Method #2. micro-Kjeldahl method. Total Kjeldahl nitrogen in the sample is determined by digestion of the organic nitrogen in an aqueous sulphuric acid solution. The ammonia produced is determined in the neutralized digest by a colorimetric method.

Method #3. UV oxidation. The sample, contained in quartz glass tubes (or coil) is treated with UV light from a mercury arc tube to decompose the organic matter . The nitrogen compounds are oxidized to nitrate which is measured according to A-7.8.

Remarks:

Method(s) references:
Parsons *et al.* (1984)

A-7.17

Variable: **total carbohydrates**

Unit: **μg C/l or μg C/g**

JEEP database: **DTCH, PTCH**

Compartment: **water, seston, sediment**

Introduction:
Carbohydrates, or better total carbohydrates, are part of the menu of estuarine bacteria and plankton, and constitute of an important, easily digestable carbon source. There are indications that carbohydrates are converted passing the estuary and that they are essential for the formation of particles and the flocculation process.
Analysis can be separated into the dissolved and total fraction, of which the analysis, apart from filtration is the same.
Automated analysis has been developed.

Sampling:
As for water (S-6.1). Care should be taken not to contaminate the sample. Cleaning procedures as for DOC (A-7.15). Avoid sampling of the surface micro-layer.

Sample volume:
One litre is more than sufficient.

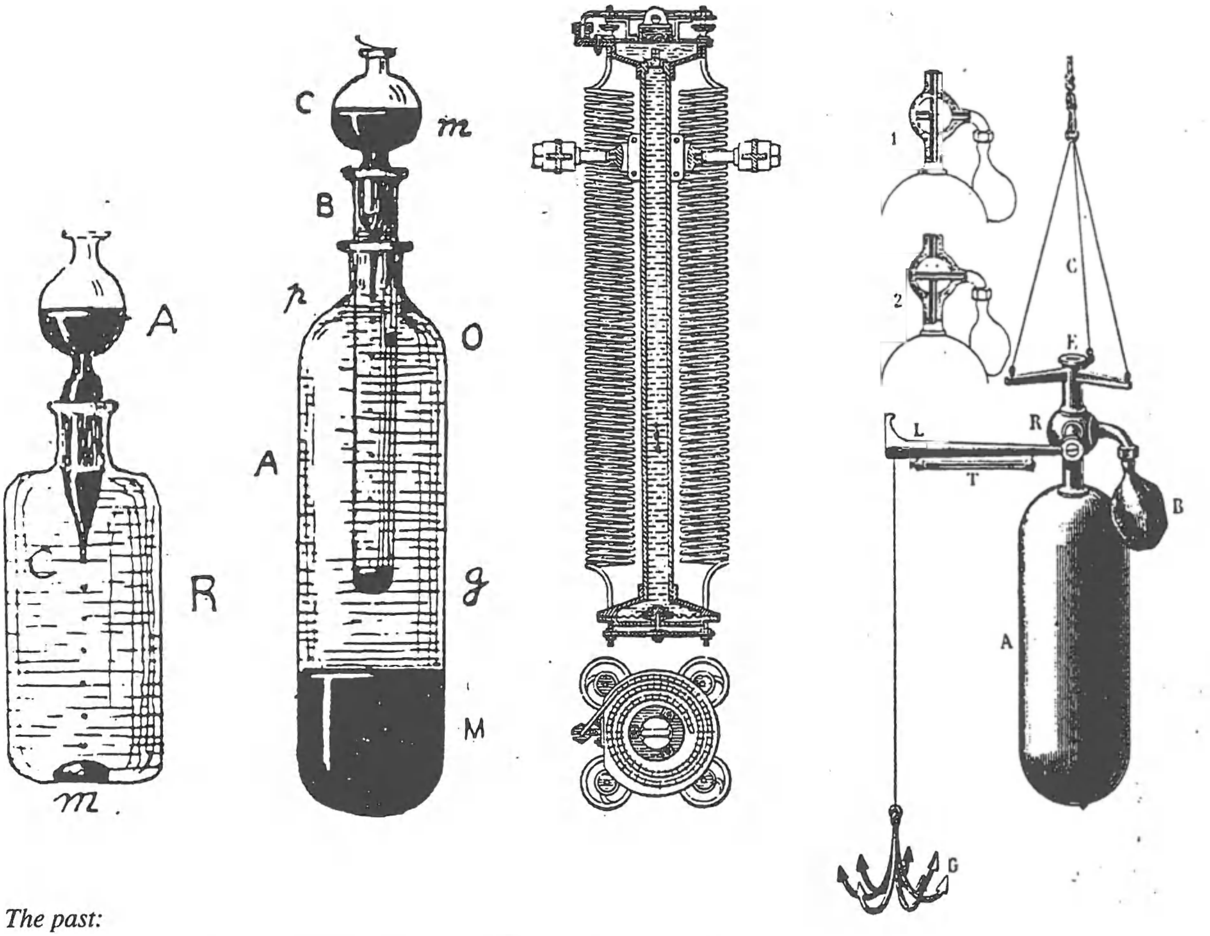
Sample treatment:
To separate the dissolved from the particulate carbohydrates, the sample is filtered over a pretreated GF/C or GF/F glass fiber filters in precleaned glassware.

Storage:
Storage in the refrigerator at 4 °C after addition of mercury chloride (0.01% w/w) for conservation is recommended.

Analytical method(s):
Method #1. After filtration the filter is treated with 10 ml 1 M sulphuric acid at 100 °C. Either the dissolved fraction or the (treated) particulate phase is mixed with a reagent containing tryptophan, boric acid and sulphuric acid, and heated for 15 min at 100 °C. The resulting violet coloured complex is measured photometrically at 520 nm. Nitrate gives a brownish complex and may at high concentrations disturb the carbohydrate analysis.

Remarks:

Method(s) references:
Eberlein & Hammer (1980); Dawson & Liebezeit (1981); Eberlein & Schütt (1986)



The past:
Sounding instruments of 1) Fol (l) and of Berget (r), operating with mercury dropping in the lower chamber under pressure; 2) bathometer of Siemens (1876); 3) bathomètre of Regnard (from: Rouch, 1943)

A-7.18

Variable: **individual carbohydrates**

Unit: **µg C/l or µg C/g**

JEEP database: **DMOS, PMOS**

Compartment: **water, seston, sediment**

Introduction:
For the analysis of mono- and oligosaccharides the procedures for detection in the dissolved phase or in the seston the same, except for the filtration step. Methods include the identification of individual (mono)saccharides (Dawson & Liebezeit, 1981).

Sampling:
As for water (S-6.1). Care should be taken not to contaminate the sample. Cleaning procedures as for DOC (A-7.15). Avoid sampling of the surface micro-layer. Collection in cleaned glass containers.

Sample volume:
One litre.

Sample treatment:
To separate the dissolved fraction from the seston, the sample is filtered over a GF/C or GF/F glass fiber filter.

Storage:
Preservation by addition of mercury chloride (0.01 % w/w)

Analytical method(s):
Method #1. For the preparation of the particulate sample, the filter is hydrolysed using 2 M HCl in a sealed glass tube for 3.5 h at 210 °C.
Depending on the salt content of the filtered sample, the salt is removed by electrodialysis for 2-6 h using an ion exchange membrane and 60-200 V DC. The dialysed sample is reduced in volume by a rotation evaporator.
The thus purified sample is eluted by HPLC using a highly alkaline anion exchange resin in a basic borate buffer. The individual mono-/oligomeres are detected as ethylene diamine complexes using a fluorimeter (excitation 320 nm, emission 460 nm).
Method #2. Free monosaccharides are reduced to sugar alcohols with KBH_4, periodate oxidation and subsequent spectrometric determination of the liberated formaldehyde with 3-methyl-2-benzothiazolinone hydrazone hydrochloride. This gives the monosaccharide content. The poly-saccharide content is determimined after a hydrolysis step using 0.1 M HCl.

Remarks:

Method(s) references:
Johnson & Sieburth (1977); Mopper (1977, 1978); Dawson & Liebezeit (1981)

A-7.19

Variable: **total amino acids**

Unit: **µg N/l or µg N/g**

JEEP database: **DTAA, PTAA**

Compartment: **water, seston, sediment**

Introduction:
Dissolved amino acids pass, by definition , a 0.45 µm filter; all amino acids together that pass through are the total amino acids.
The method of fluorimetric analysis reported here determines total amino acids in the water phase , rather than single amino acids. Individual amino acids can be determined after chromatographic separation.

Sampling:
As for water (S-6.1). Care should be taken not to contaminate the sample. Cleaning procedures as for DOC (A-7.15). Avoid sampling of the surface micro-layer.

Sample volume:
One litre.

Sample treatment:
The sample is filtered over a precleaned GF/C or GF/F glass fiber filter.

Storage:
Storage in the refrigerator at 4 °C after addition of mercury chloride (0.01% w/w) for conservation.

Analytical method(s):
Method #1. Fluorimetry. After addition of a reagent consisting of o-phtalaldehyde/mercaptophenol in an alkaline borate buffer, the filtered sample is incubated at room temperature. The resulting fluorescent complex is measured fluorimetricaly (excitation 356 nm, emission 450 nm).

Remarks:
Proteins, urea and/or ammonia at higher concentrations disturb the analysis, which might be eliminated by gel filtration prior to further analysis.

Method(s) references:
Dawson & Liebezeit (1981); Hammer & Luck (1987)

A-7.20

Variable: **proteins**

Unit: μg N/l or μg N/g

JEEP database: **DPRO, PPRO**

Compartment: **water, seston, sediment**

Introduction:
Particulate and dissolved proteins follow the same analytical procedure, with the exception of the filtration step.

Sampling:
As for water (S-6.1). Glass or plastic samplers can be used. Care should be taken not to contaminate the sample. Cleaning procedures as for DOC (A-7.15). Avoid sampling of the surface micro-layer. Collection in cleaned glass containers.

Sample volume:
One litre.

Sample treatment:
No treatment is performed for the dissolved proteins.
To separate the dissolved phase from the suspended particulate matter, a precleaned GF/C or GF/F glass fiber filter is used.

Storage:
Storage in the refrigerator at 4 °C after addition of mercury chloride (0.01% w/w) for conservation.

Analytical method(s):
Method #1. The filter is hydrolysed using 2 M NaOH, which is followed by centrifugation.
The 'extract' or the filtered water are measured photometrically as brilliant-blue complex in a phosphoacidic-methanolic solution at 625 nm, using bovine protein as a standard.
Method #2. After gel-filtration , separating the low molecular weight substances, the same fluorimetric method is applied that is described for the analysis of amino acids (A-7.19).

Remarks:
Higher concentrations of amino acids may disturb the analysis; a gel filtration separation step must then be included in the sample treatment. Care must be taken to avoid adsorption of proteins to surfaces.

Method(s) references:
Dawson & Liebezeit (1981); Hammer & Nagel (1986)

A-7.21

Variable: **lipids**

Unit: **µg C/l or µg C/g**

JEEP database: **DLIP, PLIP**

Compartment: **water, seston, sediment**

Introduction:
Dissolved and particulate lipids follow the same analytical procedure, except for the filtration step.

Sampling:
As for water (S-6.1). Care should be taken not to contaminate the sample. Cleaning procedures as for DOC (A-7.15). Avoid sampling of the surface micro-layer. Collection in cleaned glass containers.

Sample volume:
One litre is sufficient.

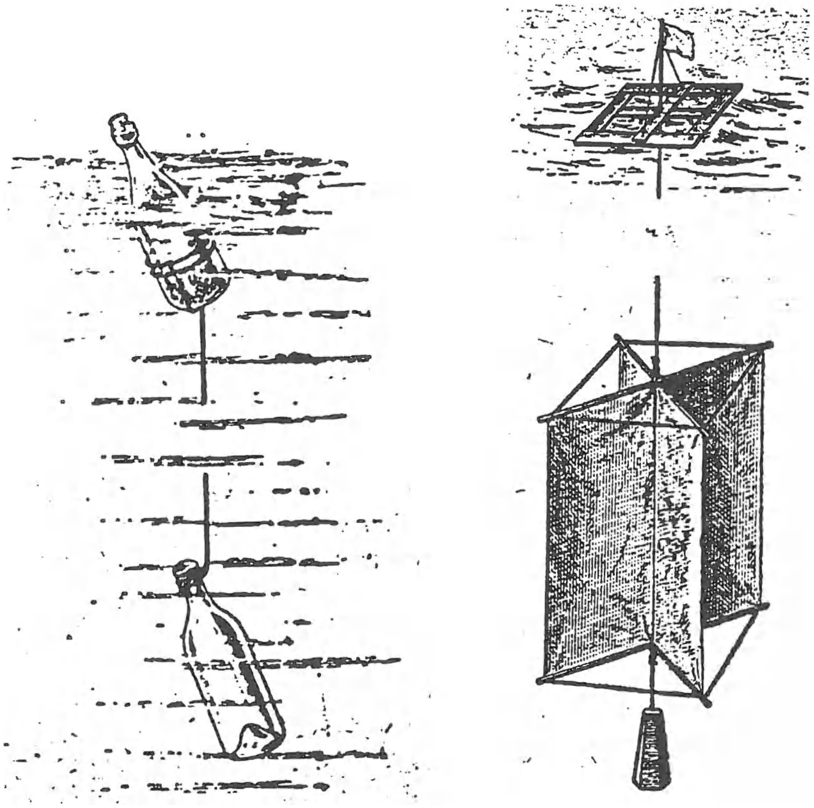
Sample treatment:
Filtration is performed over cleaned GF/C or GF/F glass fiber filters.

Storage:
Store in a cool place (4 °C). Addition of chloroform or dichloromethane is recommended.

Analytical method(s):
Method #1. The particulate phase is extracted in a Soxhlet apparatus using a mixture of chloroform/methanol/water, while the filtered water phase is extracted with a mixture of chloroform and hydrochloric acid.
A preseparation step in phospholipids, glycolipids and neutral lipids involves column chromatography under sequential elution with methanol, acetone and chloroform.
After hydrolysis and trans-esterification the resulting fatty acid methylesters are purified by thin layer chromatography (TLC).
Identification and quantification are performed by GCMS.

Remarks:

Method(s) references:
Dawson & Liebezeit (1981); Saliot *et al.* (1988); Heemstma *et al.* (1990)



The past:
Droques/floats for current measurements: 1) bottles of Hautreux; 2) design used by the Challenger (1873-1876) (from: Thoulet, 1912)

A-7.22

Parameter: **suspended particulate matter**

SPM

Unit : mg/l

JEEP data set: SPM

Compartment: seston

Introduction:

Suspended particulate matter (SPM) or seston consists of living particulate matter (plankton, etc), particulate organic matter derived from dead organisms, condensates of organic matter , flocks, etc., and inorganic material such as sand, silt and clay. To a large extent biological production and hydrodynamic conditions (currents, density; hence influencing sedimentation, resuspension) determine the nature and abundance of the seston. High variations in the total content and the relative composition, both in time and place (along the estuary but also in the water column) will occur. SPM and phytoplankton samples should be collected together from the same sample.

SPM is usually measured by back weighing of a filter after filtration . When a large amount of suspended particulate matter is required, flow-through centrifugation has been used. By (empirical) definition SPM consists of all material that is retained by a 0.45 µm filter. The material collected by centrifugation is not the same as that collected by filtration as the density of the particles is of major importance; also the salt content cannot be eliminated. Other methods for the estimation of the amount of suspended particulate matter involve the various methods that are considered under the determination of turbidity (A-7.4). (*In situ*) turbidity meters will give an estimate of the distribution of particulate matter, but these methods have to be calibrated by collection of samples as the measurements are dependent on the type of particulate matter (size, form).

Sampling:

Sampling is best performed using relatively small water samplers (max. 1-2 litres), in which the total water content can be sampled. As the particulate matter has a tendency to be deposited, an overestimation may occur if only part of the sampler content is collected. This will particularly occur if the filter is mounted directly on the sampler because of the time necessary for filtration . Shaking of the sampler is essential to keep the SPM in suspension and homogeneously distributed. Horizontally mounted samplers, that enable undisturbed passage when open, are considered the best option in high turbid waters. Practical experience will be necessary to estimate the amount of water that can pass a filter before it is clogged by the seston.

Collection directly in a sample bottle or by a pumping system may segregate the particles and give incorrect results.

For the collection of large volumes for flow-through centrifugation, the centrifuge may act as a pumping system. Otherwise an additional pump can be used to bring the water to the centrifuge.

Sample volume:
Usually 1 litre is sufficient to determine the amount SPM; in waters of low turbidity more water may be required (2-5 l). The samples are collected in a narrow mouth bottle of sufficient size.
For centrifugation volumes of 100 - 1000 litres are not uncommon. To be able to calculate the SPM content, the volume passing the centrifuge must be determined.

Sample treatment:
The filtration step for collection of the SPM over 0.45 µm filters (either membrane or glass fibre types) should be terminated by adding distilled water to remove the salt, taking care not to extract the organic matter . The amount of distilled water should preferably be in the order of 20 - 50 ml, but in high turbidity waters this amount may not pass the filter. At least a few ml should be applied in any case. Filters are air dried at 60 °C.

Storage:
Biological activity may change the SPM content. The filtration should preferably be carried out directly after sampling. In between, the samples should be stored cool in the dark.
Dry filters are best stored in petri dishes of convenient size. While still wet, prevent the filter sticking to the glass, by leaving it on the rim of the petri dish for a while.
The SPM collected by centrifuge may be stored deep frozen (- 18 °C or better) for several months without major alteration.

Analytical method(s):
Method #1. Filters (0.45 µm, polycarbonate membrane, 0.4 µm Nuclepore, or glass fibre types, e.g. GF/F), are pre-weighed after drying in an desiccator. The sample is thoroughly homogenised and a (pre-)defined amount (depending on the estimated amount SPM, 0.1 - 1 litre for filters of 50 mm ø) of suspension is filtered under vacuum or under moderate pressure (nitrogen or filtered air, about 100 kPa). The filter is then washed with distilled water to remove salt. The filter is air dried and stored. Before back-weighing the filters are placed in a desiccator until the weight is constant.
Method #2. Flow-through centrifugation. For large amounts of SPM tens to hundreds of litres of water are pumped through a convenient flow-through centrifuge either from a storage tank or directly from the water column. The optimum flow rate in terms of retaining the particles should be based on test experiments. The flow rate should be checked regularly and the amount of water that passed the centrifuge should be determined.
Method #3. Calibrated turbidity. Transmission is measured in discrete samples or in-situ. The method should be calibrated by filtering the SPM over a 0.45 µm filter (see Method #1). See also A-7.4.

Remarks:

Method(s) references:
Duinker *et al.* (1979); Reuter (1980); Bewers *et al.* (1985)

A-7.23

Parameter: **particle size**

Unit : **$n*10^3$/ml (per size class)**

JEEP database: **PART**

Compartment: **seston, (sediment)**

Introduction:

In estuarine environments large variations may occur in the size of particulate matter. From the very small colloidal material that mainly exists in the low salinity parts, the size may extend to an excess of 400 µm due to aggregation of small particles to flocculates. These flocculates are difficult to sample, as they are extremely fragile and easily break up into smaller parts (Gibbs, 1982; Gibbs & Konwar, 1983; Eisma, 1986). For flock size determination the use of an *in situ* camera technique offers minimum disruption of the flocks (Eisma *et al.*, 1990). Within the scope of the JEEP92 project the sampling of flocks is not considered of prime importance, however. It is taken for granted that in the determination of particle size the large particles are underestimated.

For the interpretation of transects not only the size of particles in the water column, but also of the very sediment surface would be helpful. The silt fraction of sediments (< 63 µm) can conveniently be analyzed by the methods described below.

Electronic counting and sizing of particles by conductometric methods (e.g. Coulter Counter) has become wide spread in marine environmental studies. These techniques involves the suction of a given sample volume through one or more counting tubes with specific aperture; particles displace an amount of water proportional to their size and number, this is detected in the aperture by a change in current.

Another technique involves laser diffraction (e.g. Malvern Autosizer). A light beam (laser) is directed towards the water sample. The particles scatter the light, and the angle of the scattered light and its intensity are a measure of the size of the particle. The method requires no calibration.

The resulting data sets usually involve a large number of data points, depending on the instrument, that cover the spectrum in size classes (even up to 256). For the JEEP92 programme and the JEEP database it is not considered useful to use all the available information, (although this may be obtained from the original source). For practical reasons, a reduction to a limited number of size classes is required. The maximum number of size classes for reporting in the JEEP programme is 20.

The classes which are defined will be dependent on the instrument and technique, hence a standard cannot be put forward here. The dataset should include those data that make a reconstruction of the frequency/size plot possible and thus size class and number of particles. The volume can be calculated from the diameter, assuming a spherical shape.

Sampling:

Sampling is best performed using relatively small water samplers (1-2 litres), of which the total water content can be sampled. As the particulate matter has a tendency to be deposited, an overestimation may occur if only part of the sampler content is collected. Collection directly in a sample bottle or by a pumping system may segregate the particles and may give incorrect results.

Sample volume:
One litre is more than sufficient, and should be collected in a plastic or glass bottle.

Sample treatment:
None. The use of fixatives should be avoided. Dilution of samples to facilitate measurement is discouraged.

Storage:
Samples can not be stored and should be analyzed as soon as possible after collection (within 6 - 12 hours). Biological activity will change the size/density spectrum of the samples. Before analysis the samples should be stored cool (as close as possible to the *in situ* temperature) and in the dark.

Analytical method(s):
Method #1. Conductometric analysis (e.g. Coulter Counter). To cover the entire bulk of the estuarine suspended particulate matter (seston), a size range of about 3 to 100 spheric equivalent diameter (SED) should be detected. Experience suggests that at least three counting tubes are necessary: 100 µm, 280 µm and 560 µm. Generally the following conditions are used:

SED (µm)	tube considered	size classes	counting volume
1.55 - 6.17	100	2 - 8	0.5 ml
6.17 - 24.55	280	4 - 10	2 ml
24.55 - 97.68	560	6 - 12	20 ml

Testing of the performance of each tube in natural estuarine water conditions is advised. To adequately sample the largest particles (> 50 µm SED) counting of substantial volumes will be necessary (e.g. 20 ml). This can be achieved by counting on 'time' and calibrating the volume sampled.
Blocking of the orifice, especially of the smallest tube, at high particle concentrations can be avoided by reducing the counting volume to 0.05 ml.
Method #2. Laser diffraction. A laser light beam is directed towards the water sample in a flow-through or discrete volume cell. The suspended particles in the sample scatter the light, the angle of the scattered light and its intensity are measured and interpreted in terms of size of the particle. A size spectrum can thus be calculated.

Remarks:

Method(s) references:
Parsons *et al.*, 1984; Individual instrument manuals

A-7.24

Parameter: **chlorophyll *a* , *b*, *c*; other pigments**

Chl a, b, c

Unit : µg/l

JEEP database: **CHLA, CHLB, CHLC, CHLX; PHAE**

Compartment: **seston**

Introduction:
Determination of particulate chlorophyll serves as an indicator of phytoplankton biomass.

The standard analytical methods for chlorophylls are based on spectrophotometry or fluorometry (Strickland & Parsons, 1968, 1972), and involve extraction in acetone/water or methanol/water mixtures. It should be understood that under the terminology 'chlorophyll' a series of pigments (chlorophylls, carote-noid pigments) and degradation products are covered. The spectrophotometric and fluorometric methods, despite their refinement, do not always provide the information needed on these various pigments found in natural waters.

In recent years, high-performance liquid chromatography (HPLC) methods have been developed that distinguish between the various pigment forms. These methods are more precise and may give additional insight into species composition and stage of plankton blooms through the pigment/degradation product spectrum. For these reasons the HPLC method is recommended. For low accuracy chlorophyll determinations, i.e. when ± 20% of the true chlorophyll *a* concentration is acceptable, the standard spectrophotometric or fluorometric methods may be used.

In situ fluorometric detection of chlorophyll has become available.

Several solvents are used to extract pigments from the particulate matter. Most common are acetone/water or ethanol/water (90%). The efficiency of ethanol (and methanol) is claimed to be better, especially when green algae or less readily extractable plankton species are present (Nusch, 1980). In an estuary, the salinity gradient and a distinct change in species composition may cause difficulties when using acetone as solvent. The ethanol/water mixture is therefore preferred.

For the JEEP92 project primary importance should be given to the determination of chlorophyll *a* , *b* and *c*, and of phaeophytin.

Sampling:
Any sampling device can be used.

Sample volume:
Depending on the density of the phytoplankton, between 0.5 and 5 litres of estuarine water are sampled. Usually 1 litre is collected. Due to the patchiness of phytoplankton it is strongly suggested that the various physico-chemical analyses and the chlorophyll determination are made from the same water sample.

Sample treatment:
Prior to filtration the water samples must be pre-filtered through 200 µm mesh to remove zooplankton and large debris. Filtration over a 0.5 µm glass fibre (e.g. Whatmann GF/F) or a 0.45 µm membrane filter of 25 to 50 mm ø, should be performed as soon as possible after collection (< 4 hours). To do this a known volume of sample (for estuaries usually between 200 and 1000 ml for a 47 mm GF/F) is filtered, and some magnesium carbonate is added to the sample while filtering to prevent acidity of the filter.
Filters should either be immediately extracted or stored under controlled conditions.

Storage:
Biological activity will seriously interfere with the chlorophyll analysis. Therefore storage of the water sample is not possible. Until analysis the samples have to be stored cool and in the dark. Once collected on the filter, the filters have to be stored immediately deep frozen at - 20 °C or lower (up to 30 days); storage in liquid nitrogen (- 196 °C) is preferred.

Analytical method(s):
Filters should be completely ground in either ca 5 ml methanol containing 2% ammonium acetate, or 5 ml acetone/water 90% using either an ice- or CO_2-cooled tissue homogenizer under subdued light. The extract should be analyzed as soon as possible (within 6 hours) by HPLC, spectrometry or fluorometry.
Method #1. Reverse phase HPLC is used to separate the different components in the extract. Detection after separation is usually performed by a fluorescence ($\lambda_{ex} = 430 \pm 20$ nm, $\lambda_{em} = 665 \pm 20$ nm) and/or absorbence detector. In the chromatogram the different peaks are identified and quantified. Identification of HPLC separated pigments should be based on coelution of standards and on spectral matching of the UV-visible spectra between 350-700 nm for the chlorophylls (and their break-down products).
Method #2. Spectrophotometric analysis of the extract in a spectrophotometer. The extinction is measured at 750, 664, 647, 630, 510 and 480 nm. The extinction at 750 nm is used to correct for the turbidity blank.
Method #3. Fluorometric analysis. chlorophylls and degradation products are fluorescing compounds and can therefore be detected fluorimetrically by a (spectro)fluorimeter. The excitation at 431 nm is used together with an emission of 667 nm.
In situ fluorimeters have been designed for the continuous measurement of longitudinal or vertical profiles.

Remarks:

Method(s) references:
Holm Hansen *et al.* (1965); Lorenzen (1967); Jeffreys & Humphrey (1975); Abayachi & Riley (1979); Nusch (1980); Gieskes & Kraay (1983); Mantoura & Llwellin (1983); Parsons *et al.* (1984); Murray *et al.* (1986)

A-7.25

Parameter: **particulate organic carbon**

POC

Unit : **mg/kg**

JEEP database: **POCW, POCB**

Compartment: **seston, sediment**

Introduction:
Seston consists of inorganic particles (silt, clay) and of organic matter , derived from e.g. living organisms (phyto- and zooplankton, bacteria), (pseudo)-faeces, and from their remains (lysed cell walls), or organic matter that is transported to the estuary from the basin slopes either by rivers or from the atmosphere .
Flocculated or aggregated organic matter , for example humic substances will also be part of the seston that will be characterized as particulate organic carbon. Organic colloids will usually not be trapped by the normally applied filtration techniques. POC represents, by its nature, a sum parameter that does not discriminate between the multitude of different organic components that can be present in the seston. POC is the main component of organic matter ; the latter is commonly calculated by multiplying POC with a factor of two.
Rapid determination of POC became available with the persulphate oxidation method. More recently, dry combustion techniques were developed and these seems to be the universal method today. Care should be taken not to decompose the carbonates, that may be present on the filter, during combustion. This is usually performed by the inclusion of an acid treatment step prior to combustion. When this step is neglected, the combustion temperature should not exceed 500 °C. The spectrophotometric detection method, based on the wet oxidation of carbon by acid dichromate, usually gives higher results than those obtained by the persulphate method due to the oxidation of highly reduced compounds such as lipids.
Studies to evaluate the particulate organic matter content by microscopic observation give non reproducible results that are not comparable with the chemical methods.
The dry combustion method involving combustion of the filter in a muffle furnace and subsequent analysis based on the weight loss should not be used for estuarine work. This technique overestimates the organic matter content up to four times due to the included loss of crystal water in clay particles (Dankers & Laane, 1983).
For most estuarine work the collection will be by conventional samplers; sediment traps for the estimation of particulate fluxes will have only limited use under most estuarine conditions.

Sampling:
Most water-sampling techniques can be applied for the collection of POC. Particulate matter may settle in the sampler. Care should be taken to collect a representative sample from the sampler, by shaking shortly before collection, or by collection of the entire volume of the sampler (see S-6.2). Centrifugation gives different results from filtration , and is usually applied when large amounts of seston have to be collected, e.g. for a characterization of specific organic compounds.

Sample volume:
The collection of 1 litre of water will almost always be sufficient in estuaries. If sub-samples are to be taken from the sample bottle for filtration , avoid segregation of the sample.

Sample treatment:
The water sample is filtered over pre-treated (450 °C for 24 h) 0.45 µm glass fibre filters (GF/F type). Silver filters are also used. Filtration should be performed as soon as possible after collection, in order to avoid any decomposition of the organic matter . A controlled vacuum (about 100 kPa) should be used. After the sample has passed the filter, the salt is removed by rapidly passing through 20 ml distilled water or ammonium formate isotonic solution (roughly equivalent to the salinity of the estuarine sample). The latter method minimizes the rupture of plankton cells. The filter should be manipulated by steel forceps to minimize contamination.

Storage:
After collection rapid analysis is preferred. The filters can be stored folded in pre-treated aluminium foil, at - 20 °C, or lower in liquid nitrogen. Immediate storage in a desiccator has also been reported as a suitable method.

Analytical method(s):
Method #1. Dry combustion. The glass fibre filters are folded in crucibles and burned under oxygen in a furnace (500 - 800 °C). The evolved carbon dioxide is then analyzed in a CHN or specific carbon analyzer.
Method #2. Persulphate oxidation. The organic matter on the filter is oxidized, usually under pressure, using a solution of persulphate and phosphoric acid. The evolving CO_2 is determined using a carbon dioxide sensitive infrared detector.
Method #3. Spectrophotometric method. The organic matter is digested by "wet-ashing" of the glass fibre filter with dichromate and concentrated sulphuric acid. The decrease in extinction of the yellow dichromate solution at 440 nm is a measure for the oxidizable carbon.

Remarks:

Method(s) references:
Menzel & Vaccaro (1964); Riley (1970); Copin-Montegut & Copin-Montegut (1973); Sharp (1974); Cauwet (1981); Parsons *et al.* (1984); LeB. Williams (1985)

A-7.26

Parameter: **particulate organic nitrogen**

PON

Unit : **mg/kg**

JEEP database: **PONW, PONB**

Compartment: **seston, sediment**

Introduction:
As with POC, the particulate organic nitrogen will be a sum parameter. The sampling and sample treatment will be identical to the methods for POC. Both wet digestion and dry combustion methods have been developed, with either element specific detection or application of a CHN analyzer. The traditional method of distillation of the ammonia and titration with acid is not recommended because of the possible high blanks. The calculation of particulate organic nitrogen as the difference between total and dissolved nitrogen is also not recommended because of the low precision of the method.

Sampling:
Most water-sampling techniques can be applied for the collection of PON. Particulate matter may settle in the sampler. Care should be taken to collect a representative sample from the sampler, by shaking shortly before collection, or by collection of the entire volume of the sampler (see S-6.2). Centrifugation gives different results from filtration , and is usually used when large amounts of seston have to be collected.

Sample volume:
The collection of 1 litre of water will almost always be sufficient in estuaries. If sub-samples are to be taken from the sample bottle for filtration , avoid segregation of the sample.

Sample treatment:
The water sample is filtered over pre-treated (450 °C) 0.45 µm glass fibre filters (GF/F type). Silver filters are also used. Filtration should be performed as soon as possible after collection, in order to avoid any decomposition of the organic matter . A controlled vacuum (about 100 kPa) should be used. After the sample has passed the filter the salt is removed by rapidly passing through 20 ml of distilled water. The filter should be manipulated by steel forceps to minimize contamination.

Storage:
Rapid analysis after collection is preferred. The filters can be stored folded in pre-treated aluminium foil, at - 20 °C, or lower in liquid nitrogen.

Analytical method(s):
Method #1. Dry combustion. The glass fibre filters are folded in crucibles and burned in a furnace. A CHN analyzer is used for the detection of the nitrogen compounds evolved.

Method #2. Total Kjeldahl nitrogen. The sample is digested in aqueous sulphuric acid and the ammonia produced is determined in the neutralized digest by a colorimetric method.
Method #3. Persulphate digestion. The filter is digested with alkaline persulphate and the resulting nitrate determined as nitrite after reduction with cadmium.

Remarks:

Method(s) references:
Strickland & Parsons (1972); LeB. Williams (1985)

A-7.27

Parameter: **particulate organic phosphorus**

POP

Unit: **mg/kg**

JEEP database: **POPW, POPB**

Compartment: **seston, sediment**

Introduction:
As with POC, the particulate organic phosphorus will be a sum parameter. The sampling and sample treatment will be identical to the methods for POC. Where filtration separates dissolved from particulate matter, oxidation serves to distinguish between inorganic and organic phosphorus. Not all phosphorus is in a form that is readily available to organisms. An example of the bio-available phosphorus fraction from sediments has been estimated by Wiltshire (1991) for the Elbe estuary. Particulate inorganic phosphorus can be determined by the difference in reactive phosphorus from filtered and unfiltered samples. Analysis of particulate material after oxidation will give a measure of its total phosphorus content.
Wet digestion methods are usually applied, where the final analytical stage is based on the determination of ortho-phosphate ions (A-7.11).

Sampling:
Most water-sampling techniques can be applied for the collection of POP. Particulate matter may settle in the sampler. Care should be taken to collect a representative sample, by shaking shortly before collection, or by collection of the entire volume of the sampler (see S-6.2). Centrifugation gives different results from filtration and is usually applied when large amounts of seston have to be collected.

Sample volume:
The collection of 1 litre of water will almost always be sufficient in estuaries. If sub-samples are to be taken from the sample bottle for filtration , avoid segregation of the sample.

Sample treatment:
The water sample is filtered over pre-treated (acid leaching) 0.45 µm glass fibre filters (GF/F type). Silver filters are also used. Filtration should be performed as soon as possible after collection, in order to avoid any decomposition of the organic matter . A controlled vacuum (about 100 kPa) should be used. After the sample has passed the filter, the salt is removed by rapidly passing through 20 ml of distilled water. The filter should be manipulated by steel forceps to minimize contamination.

Storage:
Rapid analysis after collection is preferred. The filters can be stored folded in pre-treated aluminium foil, at - 20 °C, or lower in liquid nitrogen.

Analytical method(s):

Method #1. Persulphate digestion. The filter is digested with alkaline persulphate and the resulting orthophosphate determined by a spectrophotometric method (see A-7.11).

Method #2. Sulphuric acid/peroxide digestion. The filter with the residue is digested by heating with sulphuric acid and hydrogen peroxide (H_2O_2). The following determination of the ortho-phosphate can be determined either manually or by autoanalyzer method (cf. A-7.11).

Method #3. Perchloric acid digestion. The filter is treated with perchloric acid. After evaporation, the residue is heated to oxidize the organic matter and to liberate the phosphorus as inorganic phosphate which is determined by spectrophotometric analysis (A-7.11).

Remarks:

Method(s) references:

Strickland & Parsons (1972); Kattner & Brockmann (1980); Head (1985a); Yamada & Kayama (1987); Wiltshire (1991)

A-7.28

Parameter: **clay content**

< 2 µm

Unit: **%**

JEEP database: **CLAY**

Compartment: **sediment**

Introduction:
The terminology of sedimentary particle sizes is most frequently referred to the Udden-Wentworth Scale (Wentworth, 1922), which distinguish between very coarse particles (such as boulders, cobbles and pebbles), sands, silt and clay. Each boundary between fractions is double the diameter of the previous one (see A-7.29). According to this scale clay is defined as material < 3.9 µm. Generally clays are smaller than 2 µm, and are discrete particles, not aggregates (Gorsline, 1984).
The clay content of the sediment cannot be analyzed by (wet) sieving. The most frequently applied method involves the settling rates of the particles by either the "pipette" method or the Atterberg method. Differences between the methods were demonstrated to be substantial (Brennan, 1989). The principle of these analyses is that large particles in suspension in water fall faster than small ones, the fractions respectively deposition times can be calculated using the Stokes law. The procedure can be speeded up by the use of (batch) centrifugation.

Sampling:
The upper surface sediment layer gives the most recent information, although he physical and chemical information it contains will often be the net result of reworking by (bio)turbation. The top 0.5 cm is collected, taking care that the uppermost "liquid mud" layer is not washed away. Core samplers are most suitable for this purpose (see S-6.3).

Sample volume:
Depending on the diameter of the corer, between 1 - 50 cm^3 of sediment will be collected. For the determination of the clay content about 5 cm^3 is sufficient; for coarse sediments more material will be required and this may be collected by thorough mixing of several samples.

Sample treatment:
Both methods involve the dispersal of the finer particles using an anti-coagulant such as ammonia or sodiumhexametaphosphate (Calgon method).

Storage:
Untreated samples may be stored at - 20 °C.

Analytical method(s):
Method #1a. Pipette method, peroxide treatment. For this method the sample is first pre-treated with hydrogen peroxide (H_2O_2) to remove the organic coatings that glue clay particles together, and with hydrochloric acid to remove carbonates. The sediment is then brought into suspension at a preset temperature

(e.g. 20 °C). Three 20 ml pipette samples are collected from the water column at a defined depth and after a defined time period of deposition . The clay content is measured by weighing.

Method #1b. Pipette method, no peroxide treatment. For this method the sample is not pre-treated with hydrogen peroxide (H_2O_2). The further procedure is equivalent to Method #1a.

Method #2. Atterberg method. About 2 - 5 g of sediment is brought into suspension in an Atterberg tube, and left to rest at constant temperature (20 °C) untill the end of the sedimentation time of the respective fraction. After this defined sedimentation time (for the fraction < 2 µm: 23 h 22 min for a deposition length of 30 cm) the entire water column is collected and the particulate matter collected and determined by weighing. Other fractions may thus be collected afterwards (e.g. < 6.3 µm after 2 h 21 min sedimentation time, and < 20 µm after 14 min sedimentation time; 30 cm deposition path).

Method #3. Conductometric analysis (e.g. Coulter Counter). The sediment is brought into suspension, and analyzed by conductometric particle sizer (see A-7.23).

Method #4. Laser diffraction (e.g. Malvern Autosizer). The sediment is treated with peroxide to remove organic coatings, and with hydrochloric acid to remove carbonates. The sediment is brought into suspension. A laser light beam is directed towards the water sample in a stirred cell. The suspended particles in he sample scatter the light, the angle of the scattered light and its intensity are measured and interpreted in terms of size of the particle. From the size distribution spectrum the fraction < 2 µm can thus be calculated.

Remarks:

Method(s) references:

Müller (1964); Leeder (1982); Buchanan (1984); Instrument specific manuals

A-7.29

Parameter: **silt content**

< 63 µm

Unit: **%**

JEEP database: **SILT**

Compartment: **sediment**

Introduction:
Between the arbitrarily selected boundaries of clay and very fine sand (according to the Udden-Wentworth Scale) is the silt fraction. It is assumed that this fraction, together with the smaller clay fraction, can be transported in suspension under "normal" conditions. Of course larger material can be transported by strong currents or turbulence.
The true silt content is the difference between the fraction < 63 µm (silt+clay) and the fraction < 2 µm (clay). As the determination of the clay content is rather laborious, often the silt+clay fraction is determined by wet sieving.
For most estuarine purposes the fraction < 63 µm is a suitable description of the very fine sediments. For practical reasons this method will therefore have preference over the determination of the true silt content.
Coagulated particles, e.g. originating from pseudo-faeces, may interfere with the analysis. Treatment with an oxidising medium (peroxide) will break the organic bonds between the clay/silt particles.
Although, for example, laser diffraction is a suitable technique for the determination of the particle size distribution, the sieving method is considered to be more simple to perform and is therefore preferred.

Sampling:
The surface sediment layer gives the most recent information, although the physical and chemical information it contains will often be the net result of reworking by (bio)turbation. The top 0.5 cm is collected, taking care that the uppermost "liquid mud" layer is not washed away. Core samplers are most suitable for this purpose (see S-6.3).

Sample volume:
Depending on the diameter of the corer, between 1 - 50 cm^3 of sediment has to be collected. For the determination of the silt content about 5 cm^3 is sufficient; for coarse sediments more material will be required, when necessary these may be composed of separate samples which have been combined and homogenized.

Sample treatment:
Peroxide is sometimes used to decompose the organic coating that may glue small particles together, as mentioned under the methods of clay analysis.

Storage:
The untreated sample may be stored at - 20 °C.

Analytical method(s):

Method #1. Wet sieving. Depending on the estimated amount of fines, 10 - 50 cm^3 of sediment is gently washed through a 63 µm sieve using distilled water. The fraction < 63 µm is collected from the water that passes through the sieve. The coarse and fine fraction the material is dried at 95 °C. The weight is determined and the relative amount calculated [1])

Method #2. Conductometric analysis (e.g. Coulter Counter). The sediment is brought into suspension, and analyzed by conductometric particle sizer (see A-7.23), which reveals also the particle size spectrum.

Method #3. Laser diffraction (e.g. Malvern Autosizer). The sediment is brought into suspension. A laser light beam is directed towards the water sample in a stirred cell. The suspended particles in the sample scatter the light, the angle of the scattered light and its intensity are measured and interpreted in terms of size of the particle. From the size distribution spectrum the fraction < 63 µm can thus be calculated.

Remarks:

[1]) The residue on the sieve can conveniently be used for the determination of the grain size distribution of the coarser material (A-7.30).

Method(s) references:

Leeder (1982); Buchanan (1984); Instrument specific manuals

A-7.30

Parameter: **grain-size distribution**

Unit: **%**

JEEP database: **SGSZ**

Compartment: **sediment**

Introduction:

The Udden-Wentworth Scale for the classification of sediments defines a series of classes and sub-classes of sediment types.
The most important size-classes for work in estuaries are:

Name:	*size range (μm)*
clay	< 2
silt	2 - 63
very fine sand	63 - 125
fine sand	125 - 250
medium sand	250 - 500
coarse sand	500 - 1000
very coarse sand	1000 - 2000

For estuarine work in relation to the JEEP92 programme, the coarser classes belong to sediment types that are not particularly interesting. Therefore the description for this programme ends with > 2 mm.
Dry and wet sieving are both popular. The disadvantage of dry sieving is the possibility that clayish sediments when dried, tend to form lumps that consist of fine material, thus disturbing the analysis. This problem is solved, when the > 63 μm fractions are determined after the separation of the silt+clay fractions by wet sieving.

Sampling:

The surface layer of the sediment gives the most recent information, although the physical and chemical information it contains will often be the net result of reworking by (bio)turbation. The top 0.5 cm is collected. Core samplers are most suitable for this purpose, although grab samplers may be used (see S-6.3).

Sample volume:

Depending on the diameter of the corer, about 1 - 50 cm^3 of sediment will be collected. For the determination of the grain size distribution about 100 cm^3 is sufficient. Sufficient material may be collected by thorough mixing of several samples.

Sample treatment:

No special treatment is required. Ensure that the sample is prevented from drying out.

Storage:

Samples may be stored at - 20 °C until analysis.

Analytical method(s):

Method #1a. Dry sieving[1]). The material left on the 63 μm sieve is used for the determination of the < 63 μm fraction (see A-7.28) and is quantitatively transported to a petri-dish. About 100 g of sediment is needed for the analysis. After drying, the size-classes are determined by sieving over a set of nested sieves of appropriate mesh size under vibration. The sieving step should be standardized at 15 min. The separate fractions are measured by weighing and the percentages calculated.

Method #1b. Wet sieving. The (wet) sediment is passed over a set of nested sieves of appropriate mesh size under vibration and applying a gentle water flow. The separated fractions are dried, measured by weighing and the percentages calculated.

Remarks:

[1]) This dry-sieving method can only be applied when the silt+clay fractions have been removed.

Method(s) references:

Leeder (1982); Buchanan (1984); Giere *et al.* (1988)

A-7.31

Parameter: **Bacteria**

bacteria numbers

Unit: **n*10^6/ml**

JEEP database: **BANR**

Compartment: **water**

Introduction:
Total bacterial numbers in sea- and estuarine water can be estimated from direct counts under a fluorescence microscope. Other methods involve culturing techniques in specific media, which includes the most probable number (MPN) technique. These methods are rather selective for bacteria that grow faster in a defined medium. The first method is considered the simplest and most widely applied method for estimating total bacteria numbers.

Sampling:
Samples used for MPN etc. should be collected using a sterile water sampler (e.g. prewashed with 70% ethanol), or a special sampler that can be heat sterilized. To avoid the problem of a patchy distribution, e.g. due to the presence of bacteria in particulate aggregates, a number of samples (ca. 20) should be collected and these are pooled to get the final sample that is used for counting (see A-7.32).

Sample volume:
About 10 ml sample is sufficient for the counting.

Sample treatment:
About 10 ml of the estuarine water is transferred to a (sterile) screw-cap vial, and 0.5 ml of a 40% formaldehyde solution is added to preserve the sample. The preservation step may be ignored if the sample is to be analyzed immediately.

Storage:
Preserved samples should be stored in a cool dark place (10 °C) and analyzed within 2 weeks.

Analytical method(s):
Method #1. Fluorescence microscopy. Special black filters (2.5 cm ø, 0.2 μm pore size) may be obtained, or filters should be stained black. After rinsing the pipette with the sample to be analyzed, 2 ml sample is introduced in the filter holder, 0.2 ml Acridine orange or DAPI solution is added to stain the bacteria and after 2 min incubation a moderate vacuum (50 kPa) is applied for filtration . The dry filter is placed on a slide, covered with microscopy oil, and counted within 24 h; at least 10 fields should be counted with about 20 bacteria per field. To reach this, dilution of the sample may be required.

Remarks:
Formalin is a toxic substance. Please refer to the safe handling procedures for chemicals.

Method(s) references:
Vollenweider (1969); Hobbie *et al.* (1977); Parsons *et al.* (1984)

A-7.32

Parameter:	**Bacteria** **bacterial production**
Unit:	**mg C/l.h**
JEEP database:	**BAPR**
Compartment:	**water**

Introduction:
Production of heterotrophic bacteria in marine, estuarine and freshwater environments can be estimated by measuring the incorporation of radio-labelled (tritium, ^{3}H) thymidine ([methyl-^{3}H]thymidine) into DNA (tritiated thymidine incorporation, TTI).
Another method, which is far more time consuming involves the determination of the frequency of dividing cells. This method involves no radio-tracer work.

Sampling:
Most water sampling techniques can be used, but collection using a sterile water sampler (e.g. prewashed with 70% ethanol), or special sampler that can be heat sterilized is preferred. Care must be taken to collect a representative sample, especially in high turbidity zones. The pooling of a number of separate samples (20) is recommended.

Sample volume:
For most estuaries and coastal waters 50 ml samples will be sufficient for a single series of analyses.

Sample treatment:
As the turn-over of bacteria is high (reproduction rate about 20 min^{-1}), rapid action is necessary to estimate the production rate.
Of a set of six sub-samples of 5 ml each, three blanks are treated with formaldehyde to give a final concentration of 1.5 - 2 %. All sub-samples are spiked with tritiated thymidine and incubated for 30 - 45 min at *in situ* (field) temperature, in the dark. The three sub-samples that were not preserved, are spiked with formaldehyde to stop bacterial growth.

Storage:
Storage of the water sample for later analysis is not possible, immediate action is required. After incorporation of the thymidine the vials can be stored on ice until analysis. When plastic scintillation vials are used the radioactivity should be measured within 24 h after addition of the scintillation fluid.

Analytical method(s):
Method #1. TTI. After the actual incorporation step (see above), the samples are filtered over a 0.2 μm membrane filter, which has been wetted with 1 ml 5 mM unlabelled thymidine. Vacuum filtration under mild vacuum (56 kPa). After washing with trichloroacetic acid (5 % (w/v) the filters are transferred to scintillation vials, scintillation fluid is added and the radioactivity measured by

scintillation counting. From the incorporated activity the bacterial production can be calculated.
Method #2. Growth rate. This method involves the determination of the frequency of dividing cells for the determination of bacterial production.

Remarks:
As the first method involves radioactive material the proper precautions should be maintained, and the national safety regulations are to be followed.
Formalin is a toxic substance. Please refer to the safe handling procedures for chemicals.

Method(s) references:
Hagström *et al.* (1979); Fuhrman & Azam (1982)

A-7.33

Parameter: **Phytoplankton**

species abundance

Unit: **n/ml**

JEEP database: **FPSP**

Compartment: **water**

Introduction:

Phytoplankton is present in estuarine waters together with detrital and inorganic particles. The difficulty in analyzing phytoplankton is to separate living cells from dead cells, debris and other amorphous particles. Automatic methods are difficult to use and direct examination under a microscope is therefore the most likely method. From the preserved sample the micro-zooplankton should be analyzed.

As a first step, the analysis can be limited to the identification of dominant phytoplankton types and the determination of the ratio of phytoplankton cells to other particles. When the flaggelates, which are often the dominant species , are to be identified to the species level (which is not always necessary), it is essential that these fragile organisms are analyzed immediately in the non-preserved samples. This is also the case when heterotrophic and autotrophic (chlorophyll containing) flaggelates are to be distinguished.

The method described here assumes pre-concentration of the sample; this is not necessary when high densities are found.

Sampling:

Water samples are collected with any type of water sampler or pumping system, or are collected directly in the sample bottle. For optimal representativity see the sampling procedures for SPM (A-7.22).

Sample volume:

A sample volume up to one litre is collected.

Sample treatment:

No preservation is needed for immediate analysis of flaggelates. In other cases preservation of the samples is essential. Phytoplankton and planktonic protozoa for microscopic analysis can be preserved with a small amount of buffered formalin (enough to bring the sample to 1 % formalin) or with acidic Lugol's solution (made by addition of 50 g iodine and 100 ml acetic acid to a solution of 100 g KI in 1 l distilled water). Other methods of preservation involve the use of neutralized formalin or hexamethylenetetramine.

After preservation of the sample, the bottles are kept closed and in the dark at 4 °C for at least 2-3 days. After this period, the seston will be deposited on the bottom of the sample bottle. The supernatant is removed by careful suction, using a plastic tube (that is not used for other purposes as Lugol's solution is adsorbed by the plastic). Hold the tube end just below the water surface, and reduce the volume to slightly less than 100 ml. Shake the bottle to resuspend the seston, transfer quantitatively to a 100 ml cylinder and make up to 100 ml with water. Cover the cylinder and repeat the procedure to reduce the volume to 10 ml.

Storage:
Preserved material can be kept in closed brown glass containers and stored in the dark at 4 °C. If the samples are stored for a long period (months), neutralized formalin should be added to the Lugol preserved sample (1 ml of 40% formalin to 10 ml sample). For shorter storage periods some extra Lugol's solution should be added every two weeks to keep the colour dark brown.

Analytical method(s):
Method #1a. Microscopical analysis, after concentration. The sedimentation method is simple in routine use and is adequate for diatoms, most small flaggelates and some armoured dinoflaggelates, provided the estuarine water is not too high in turbidity. The use of an inverted microscope is preferred. The concentrated sample is shaken, and a sub-sample of 3 ml is pipetted into a sedimentation chamber. After a minimum of 15 min deposition (but overnight deposition is better) it is sufficient to count between 20 and 100 of the units (cells, chains or colonies) of the more abundant taxa.
Method #1b. Microscopic analysis, non-concentrated sample. The counting is equal to method 1a, the concentration step is not applied.

Remarks:
Iodine and formalin are toxic substances. Please refer to the safe handling procedures for chemicals.

Method(s) references:
Vollenweider (1969); Strickland & Parsons (1972); Sournia (1978); Throndsen (1978); Parsons *et al.* (1984); Tett (1987)

A-7.34

Parameter: **Phytoplankton primary production**

Unit: **gC/m^3.d**

JEEP database: **FPPR**

Compartment: **water**

Introduction:

The determination of primary production in waters gives a measure of the rate of growth of the phytoplankton community. Several techniques have been developed, which do not always agree well.

The performance of the measurements of primary production is based on two conceptually different approaches (Vollenweider, 1969):

a) measurements that are carried out on isolated samples (either returned to the field (*in situ)* or under controlled laboratory conditions). The latter method can be carried out under more standardized conditions, but it does not represent natural conditions (light, temperature) which may be simulated, however.

b) measurements that are carried out directly in the environment, thus in non-isolated samples, hence no sampling is involved. The advantage is that the vertical gradient, which may be very important in estuaries because of the inhibitory effect of high turbidity, is taken into account. Also the natural conditions of the measurement are advantageous, but the relation to other (sampled and analyzed) variables measured in water samples may be difficult to interpret.

The most applied techniques use discrete samples and involve the uptake of a radio-tracer (^{14}C) by the growing plankton, or the production of oxygen. As these involve batch techniques, and for the JEEP92 programme the aim is to compare different variables as close as possible from one sample, these methods are preferred over the *in situ* methods.

Sampling:

Sampling as for phytoplankton. As toxicants will hamper photosynthesis, non-metallic samplers should be used. Collection in acid-washed bottles.

Sample volume:

One litre will usually be sufficient.

Sample treatment:

Incubation should be carried out immediately after sampling in cleaned bottles. To protect the plankton from light-shock which may alter their response sub-surface samples should not be exposed to intense sunlight.

Storage:

Storage of the samples is not possible

Analytical method(s):

Method #1a. ^{14}C tracer technique. A known amount of radioactive carbonate ($^{14}CO_3^{2-}$) is added to a sea- or estuarine water sample of known carbonate content. After a specific time period (4 h) of photosynthesis by the phytoplankton under controlled conditions in an incubator, the plankton cells are filtered over a 0.45 µm membrane filter and the radioactivity from the carbon in the cells measured by (ß) scintillation counting. The uptake of radioactive carbonate, as a fraction of the total carbonate, will reflect the rate of net photosynthesis over the given period. Dark bottles are used for the calculation of the respiration.

Method #1b. ^{14}C tracer technique. As under Method #1a, but the incubation takes place *in situ,* by lowering the incubation bottles into the water column.

Method #2. Oxygen determination. The samples are incubated in dark and clear bottles for a specific time period (4 h) of photosynthesis by the phytoplankton under controlled conditions in an incubator. The evolution of the oxygen in the bottle is followed by oxygen determination (A-7.5). This can be by ion selective electrode, although the micro-Winkler titration is preferable because of its high sensitivity and the distinct ending of biological activity by the addition of chemicals (A-7.5). The dark bottle incubation will give the effect of respiration, the clear bottle the oxygen content due to the difference of respiratory consumption and primary production. From the increase in oxygen concentration the primary production is calculated.

Remarks:

^{14}C is a (weak) radio-tracer which, in most countries will require a specially equipped laboratory space . Please check on safety regulations for working with radio-tracers.

Method(s) references:

Vollenweider (1969); Parsons *et al.* (1984)

A-7.35

Parameter: **Phytoplankton biomass**

Unit: **gC/m^3**

JEEP database: **FPBM**

Compartment: **water**

Introduction:
Phytoplankton is present in estuarine waters together with detrital and inorganic particles: seston (see S-6.2; A-7.21). The difficulty in analyzing phytoplankton is to separate living cells from non-living particles. This is important for the determination of phytoplankton biomass.

Biomass of phytoplankton is usually determined using conversion factors from chlorophyll data. Although quick and easy, the method has disadvantages. The ratio chlorophyll:biomass is not constant, ranging from 20 - 100 mgC/1 mg chlorophyll. Because of variation in cell size (and form) amongst species and physiological differences, correlations between chlorophyll and cell numbers are often weak. Additionally, along the salinity gradient chlorophyll may be present in dead cells and debris, even in fecal pellets.

Other methods involve estimating biomass from cell counts and cell size measurements, and the use of Coulter counter data. The latter method has the disadvantage that living phytoplankton can not be distinguished from dead cells and inorganic particles.

All methods are based on calculation rather than on direct measurement. It seems better to store the basic data (thus chlorophyll (A-7.24), species abundance (A-7.33) or Coulter Counter (A-7.23) data) in the JEEP92 database and perform the calculations in one place only according to defined rules. Therefore the methods presented here are given an asterisk as a reminder that conversion factors are involved. Billington (1991) observed marked differences between the various methods, especially on a daily basis.

Example conversion factors from volume determinations are given in Annex VI, while Jørgensen *et al.* (1991) have summarized several conversion relations. Cell sizes are necessary for a sufficient reliable conversion. Similar procedures can be applied to bacteria and zooplankton.

Sampling:
Sampling as for the species abundance.

Sample volume:
A volume of 1 litre is sufficient for either method.

Sample treatment:
See for the different methods under the respective procedures (A-7.23, A-7.24, A-7.33). Preservation is not required.

Storage:
See respective analytical procedures (A-7.23, A-7.24, A-7.33).

Analytical method(s):
Method #1*. Conversion from chlorophyll data.
Method #2a*. Conversion from cell counts and cell measurements. The plankton cells are counted, their size is measured, and volume calculated.
Method #2b*. Conversion from Coulter Counter data. From the total particle counts the total particulate volume is calculated by multiplying the counts for each channel by the respective particle volume, assuming equivalent spherical diameters.

Remarks:

Method(s) references:
Strickland & Parsons (1972); Sournia (1978); Parsons *et al.* (1984); Tett (1987)

A-7.36

Parameter: **Zooplankton**

species abundance

Unit: n/m^3

JEEP database: ZPSP

Compartment: water

Introduction:
Members of the zooplankton vary from a few µm to several cm in size. Many phyla have members that pass at least some part of their lives as plankton in the water column. This heterogeneity of planktonic forms means that sampling design and analytical procedures must be adapted to the group(s) of species studied. In estuaries this problem is less acute than in open sea due to the generally low diversity of the zooplankton. The methods described here are applicable to the bulk of the zooplankton living in most estuaries.
From numbers and size of individuals (and of the various development stages) and the (total) dry weight the biomass can be calculated.

Sampling:
The sampling device will vary according to the type of plankton to be sampled. Water samplers or pumping systems are adequate for microplankton and mesozooplankton, while towing nets are more specifically useful for collecting large(r) species or when large numbers of organisms are required.
In many laboratories zooplankton sampling is carried out using towing nets because of their relatively low price and easy handling. However, the use of towing nets is seriously hampered in water with a high amount of suspended particulate matter. In practice, it is almost impossible to sample quantitatively turbid estuaries with towing nets with a mesh under 200 µm, due to clogging of the net. The problem is avoided by using a net of a larger mesh-size, but small zooplankton (e.g. nauplii, in case of copepod populations) are under-sampled. While 200 µm mesh collected samples are suitable for rough comparisons between estuaries, additional samples have to be collected by pumping system or bucket (100 l over 55 µm mesh size net) for demographic and production measurements. The micro-zooplankton will samples and analysed along with the phytoplankton (A-7.33)

Sample volume:
Using bucket, sampler or pumping system, 100 litres is preferred which is filtered over a 55 µm gauze. With towing nets 5 m^3 is favoured (200 µm mesh).

Sample treatment:
One litre plastic flasks are convenient for transporting living material in order to keep it cool. Net concentrated plankton should be copiously diluted with the local estuarine water.
Zooplankton should be preserved in buffered formalin (4% formaldehyde) in glass bottles, tightly closed and filled to the top. To prevent damage to the organisms, the amount of plankton should not exceed 10% of the volume of water.

Storage:
Preserved microplankton should be kept cool.
For zooplankton, preservation is improved if, after a few days of fixation, the plankton is transferred to a mixture of 9 parts estuarine water and 1 part of the following preservative: 50 ml propylene phenoxitol, 450 ml propylene glycol and 500 ml of buffered 40% formaldehyde. Formalin is best buffered with borax (excess or about 20 g/l); trimethylamine has also been used.

Analytical method(s):
Method #1. Microscopic analysis. Zooplankton samples containing much detrital and inorganic material are first treated with 1% aqueous solution of bengal rose, staining the plankton pink, which facilitates counting.
Sub-samples are taken in such a way that about 100 individuals are present in the sub-sample. The examination can be performed using a Bogorov or a Dollfus tray. All individuals of the sub-sample should be identified (including the different development statges of copepods, see A-7.37) and counted.

Remarks:
Formalin is a toxic substance. Please refer to the safe handling procedures for chemicals.

Method(s) references:
Tranter & Fraser (1968); Steedman (1976); Raymont (1983); Tett (1987)

A-7.37

Parameter: **Zooplankton**

stage distribution (copepods only)

Unit: **n/m³ (per stage)**

JEEP database: **ZPSD**

Compartment: **water**

Introduction:
For a number of selected species , i.c. copepods, it is useful to have information of the different development stages, the nauplii and copepodites. Usually 6 nauplii and 6 copepodite stages (N I - N VI and C I - C VI respectively) are distinguished, of which the C VI is the adult stage.
In routine procedures the following development stages are identified and counted:
- small nauplii (including N I - N II),
- large nauplii (including N III - N VI),
- all individual copepodite stages (C I - C IV),
- female and male (C V),
- and females and males (C VI).

Sampling, preservation and storage procedures are the same as those described for the total species abundance (A-7.36).

Sampling:
Sampling according to the description under A-7.36 for total catch.

Sample volume:
Using bucket, sampler or pumping system, 100 litres is preferred, which is filtered over a 55 µm gauze. With towing nets 5 m^3 is favoured (200 µm mesh).

Sample treatment:
As for the methods described under species distribution (A-7.36).

Storage:
As for the methods described under species distribution (A-7.36).

Analytical method(s):
Method #1. Microscopic analysis. Zooplankton samples containing much detrital and inorganic material are first treated with 1% aqueous solution of bengal rose, staining the plankton pink, to facilitate counting. Counting of sub-samples is as for total abundance.
The examination can be performed using a Bogorov or a Dollfus tray. The entire sub-sample should be searched for the different stages of the selected species , which should be reported according to the N I -VI and C I - VI nomenclature.
For the conversion to biomass size measurements of the different stages are needed.

Remarks:
Formalin is a toxic substance. Please refer to the safe handling procedures for chemicals.

Method(s) references:
Tranter & Fraser (1968); Steedman (1976); Raymont (1983); Tett (1987)

A-7.38

Parameter: **Zooplankton**

stage weights (copepods only)

Unit: **µg (per stage)**

JEEP database: **ZPSW**

Compartment: **water**

Introduction:
Of the selected species , e.g. copepodes, the life stage weights are of interest. The analysis is carried out by weighing. It will depend on the number of stages present, how many subdivisions are found, and how many can reliably be weighed. It is anticipated that different results may be obtained when samples are stored deep-frozen or preserved in glutaraldehyde (or formalin, which may result in loss of some fat). Hence separate methods are defined here.
The results should be reported as dry-weights.

Sampling:
Sampling according to the description under A-7.36 for total catchment.

Sample volume:
Using bucket, sampler or pumping system, 100 litres is preferred which is filtered over a 55 µm gauze. With towing nets 5 m^3 is favoured (200 µm mesh).

Sample treatment:
The normal formalin preservation causes unpredictable loss of individual weights and makes this treatment unsuitable for biomass estimates. Glutaraldehyde is a more effective fixative than formaldehyde because it cross-links proteins much more rapidly (Kimmerer & McKinnon, 1986). A 5% solution of glutaraldehyde in filtered seawater should be prepared in advance and maintained at or below room temperature. It should be used in a 1:1 dilution of the zooplankton sample.

Storage:
The preserved sample should be stored at or below room temperature. Dry-weight analysis should take place within a few days after fixation if possible. Zooplankton samples may be conveniently stored deep frozen if destined for chemical or biomass analysis.

Analytical method(s):
Method #1a. Glutaraldehyde (or formalin) preserved samples. Determination of dry-weight. The copepods are sorted and measured using a micrometer eyepiece fitted on stereo-microscope. They are put into in groups of similar sized individuals, transferring them in a drop of water. In practice, size classes of about 100 µm wide are used corresponding to about 10 size classes for estuarine copepods(*Eurytemora sp., Acartia sp.*) whose body lengths range from about 100 to 1000 µm. Each size class is subdivided into three batches (replicates) of at least 30 individuals (30 for the > 700 µm classes, 50 for the 400 to 700 µm classes and 100 for the < 300 µm classes).

The copepod batches are transferred to pre-weighed combustion boats by transporting them in a drop of water, after which the water is removed using a "fire thinned" glass pipette. This method also removes excess salt. The samples are dried for 24 hours at 60 °C. Until back- weighing, the samples are kept in a desiccator. Back-weighing using a microbalance with a precision of at least one µg gives the dry-weight per individual.
Method #1b. Deep frozen or fresh samples. After thawing, the procedure is identical to the method 1a.

Remarks:
Glutaraldehyde and formalin are toxic substances. Please refer to the safe handling procedures for chemicals.

Method(s) references:
Tranter & Fraser (1968); Steedman (1976); Raymont (1983); Tett (1987)

A-7.39

Parameter: **Zooplankton**

biomass

Unit: **mg dry-weight/m^3 (or mg C/m^3), (or mean stage length)**

JEEP database: **ZPBM**

Compartment: **water**

Introduction:

Zooplankton biomass estimates are crucial for secondary production assessment and should be carried out with great care and using standardized methods, allowing further comparisons between various studies and areas.

Essentially three methods are applied to estimate the zooplankton biomass: weighing (dry-weight), dry-weight estimation from length measurements and conversion from organic carbon content.

The following procedures mainly concern copepods which represent a dominant group in terms of zooplankton biomass and production.

Due to their small size, weighing copepods is a tedious job and it seems unrealistic to perform this as a routine analysis. It should be emphasized, however, that weighing gives the only true measured result (dry-weight) in contrast to the use of those calculated using conversion factors.

Being crustaceans with an exoskeleton, copepods have throughout their development, a rather standardized shape. This usually allows the establishment of log-linear regressions between the individual weight (W) and the length (L) with the function: $\log_{10}W = a \log_{10}L + b$ or $W = b * L^a$. When the parameters a and b have been determined for a population (calibration), the biomass is easily calculated from the length determination of the various development stages at each sampling date. For a mathematical treatment one is referred to Baskerville (1972), and to the summary in Annex IX.

As copepods are known to show size and shape variations according to seasons (a combination of thermic and trophic elements) it is safer to establish two regressions per year, one during spring (April) and one during autumn (October), which can be used for the summer-autumn and the winter-spring periods respectively. Copepods have then to be identified (development stages) and measured (cephalothorax length) at each (monthly) sampling interval and weighed only twice a year.

Biomass and derived production estimates are often expressed in carbon units. Despite the variations that have been found, the organic C/dry weight ratio is often assumed to be constant. Examples for a number of different zooplankton representatives are given, for example, in Parsons *et al* (1984). The 0.5 ratio given by Heinle & Flemer (1975) is often used. Such conversion procedures should only be considered as a stopgap allowing comparisons to be made with other data expressed as carbon units and biomass should preferably expressed as mg dry weight/m^3 (i.e. the actually measured values).

Conversions from volume to biomass is not recommended for estuarine zooplankton due to the usually large amount of detritus.

Biomass conversion equations have also bee collected by Jørgensen *et al* (1991). For more details see Annex VII.

Sampling:
See A-7.36

Sample volume:
Usually, abundant material will be available for the determination of biomass. This means that a sample splitter has to be used to reduce the sample size to practical but still representative proportions for determination and analysis.

Sample treatment:
The normal formalin preservation causes unpredictable loss of individual weights and makes unsuitable for biomass estimates. Glutaraldehyde is a more effective fixative than formaldehyde because it cross-links proteins much more rapidly (Kimmerer & McKinnon, 1986). The rapid reaction of glutaraldehyde causes proteins to become insoluble, which may prevent the loss of more labile organic substances.

A 5% solution of glutaraldehyde in filtered seawater should be prepared in advance and maintained at, or below, room temperature. The plankton sample, in a volume of seawater that is many times larger than the estimated settled volume of the plankton, is fixed by the addition of an equal volume of the dilute glutaraldehyde solution and gently agitated.

Storage:
The preserved sample should be stored at or below room temperature. If possible biomass analysis should take place within a few days of fixation .

Analytical method(s):
Method #1. Determination of dry-weight. After gentle rinsing with tap water the copepods are sorted under a micrometer eyepiece fitted on stereo-microscope. They are put into in groups of similar sized individuals, transferring them in a drop of water (see procedures under A-7.38). The copepod batches are transferred into pre-weighed combustion boats and dried for 24 hours at 60 °C or by freeze drying. Until weighing, the samples are kept in a desiccator. Back-weighing using a microbalance with a precision of at least one µg gives the dry-weight. For each size class the mean individual dry weight can be calculated, the summed dry-weight per m^3 is the total biomass.

Method #2*. Dry-weight calculation from body lengths. As described under Method #1 the copepods are separated in size-classes and sub-samples. In each batch, the cephalothorax lengths are accurately measured under a microscope (x100 magnification) mounted with, for example, a drawing tube, ocular micrometer or digitizing tablet.

A regression has then to be found between the mean individual dry weights (cf. A-7.38) and the mean lengths (both log transformed) of the n size classes using the least squares method (see introduction). This log-linear regression between the individual weight (W) and the length (L) is described with the function: $\log_{10}W = a \log_{10}L + b$ or $W = b * L^a$. This equation can then be used to esti-mate the development stages mean individual weights from the length measurements proceeded on each sampling occasion.

In the framework of the JEEP92 programme for this Method the actual measured data are preferred over the calculated biomass. This means that for each development stage the mean length and the mean weight (dry weight), with the numbers (n) and standard deviation (s.d.) of the data involved in the calculation of the mean have to be reported.

Method #3*. Conversion from organic carbon content. Biomass estimates are often expressed in carbon units. A usefull technique involves the direct analysis of the zooplankton of glass fibre filters (GF/C or GF/F) in a CHN analyzer (see

A-7.25). Despite the variations that have been found, the organic C:dry weight ratio is often assumed to be constant. The 0.5 ratio given by Heinle & Flemer (1975) is often used. Examples of conversion equations of different zooplankton representatives are given for example in Parsons *et al* (1984), different equations have also been collected by Jørgensen *et al* (1991). For more details see Annex VII.

Remarks:
Glutaraldehyde and formalin are toxic substances. Both should be handled carefully, and high concentrations handled only under a fume hood. Please refer to the safe handling procedures for chemicals.

Method(s) references:
Heinle & Flemer (1975); Steedman (1976); Parsons *et al.* (1984); Kimmerer & McKinnon (1986); Jørgensen *et al.* (1991)

A-7.40

Parameter: **micro-phytobenthos**

species abundance

Unit: $n*10^6/m^2$

JEEP database: **FBSP**

Compartment: **sediment**

Introduction:
In the sediment the inorganic sand and silt fractions will constitute the majority of the particles. To obtain a quantitative insight in the distribution of the various micro-phytobenthos species and their relative distributions, a separation technique has to be applied.
Several methods have been used, which unfortunately give different results. The most simple method is the use of lens tissue to harvest the mobile positive phototactic micro-phytobenthos (Eaton & Moss, 1966). Other more elaborate methods involve the use of density gradients, e.g. using a colloidal silica suspension (e.g. LUDOX, De Jonge, 1979). The collection by means of different sedimentation rates of sediment particles and microphytobenthos is not recommended, because it appears that many of these organisms are attached to the substrate.
For the identification of the species , microscopic investigation is indispensable. This method is similar to that for phytoplankton. A check list of benthic diatom species can be found in Van den Hoek *et al* (1979).

Sampling:
Samples are collected by coring (about 2.4 cm ø). The top 5 mm is collected; when deeper layers are to be examined, the layer 0.5-2 cm should be collected additionally.

Sample volume:
To eliminate natural variability, about 20 individual samples are collected which are pooled together (keeping the individual sediment layers).

Sample treatment:
Direct treatment of the sample is preferred.

Storage:
After separation of the micro-phytobenthos from the sediment, the organisms have to be preserved in formalin or in a Lugol's solution (see A-7.33). Lugol preserved samples can be kept in closed (brown) glass bottles and stored in dark at 4 °C. If the samples are to be stored for several months, formalin should be added (1 ml of 40% formalin to 10 ml sample).
As an alternative the preservation method of Van der Veer (1982) can be used.

Analytical method(s):
Method #1. Tissue technique. The sediment is spread in a petri dish, covered with lens tissue and placed in a climate room under illumination. Part of the

mobile diatoms migrate into the tissue. After one day the tissue is rinsed with seawater and the organisms are collected and counted.
Method #2. Density gradient. Differences in the specific weight of microphytobenthos species , empty diatom frustules, organic detritus and inorganic sediment are used to separate the sediment in various fractions. A step-layered gradient is prepared of different density suspensions of colloidal silica polymers (e.g. Ludox-TM). Sub-samples of suspended sediment are added in the middle of the density gradient and centrifuged at medium speed, afterwards the different fractions are harvested.

Remarks:

Method(s) references:
Eaton & Moss (1966); Cadée & Hegeman (1974); De Jonge (1979; 1992); Colijn & Dijkema (1981)

A-7.41

Parameter: **micro-phytobenthos production**

Unit: **gC/m^2.d**

JEEP database: **FBPR**

Compartment: **sediment**

Introduction:
Primary production of the benthic microflora has been measured both in the laboratory after sampling, and *in situ* using bell jars for treated field samples. For primary production measurements only the top layer of the sediment is of importance, despite the possible presence of a large part of the benthic microflora below this layer. The latter analytical method will give the total production over the sediment column, which has been collected under natural conditions. The method involving sediment sampling arbitrarily selects a sediment layer, which is incubated *in situ* or in an incubator. Differences are therefore anticipated.
As for phytoplankton, both the ^{14}C incorporation method and the oxygen production method can be applied.

Sampling:
For *in situ* determination using perspex cylinders (bell jars), no samples are collected, but the core-tube is placed in the sediment on the studied location. For incubation in bottles only the top 0.5 cm of the sediment is collected. The collection of about 20 separate samples, which are pooled to get an averaged sample, is recommended.

Sample volume:
For the bottle incubation methods 1 cm^3 is required.

Storage:
Storage of the samples is not possible, incubation should be carried out immediately after sampling in cleaned bottles.

Analytical method(s):
Method #1. ^{14}C tracer technique, *in situ*, in a bell jar. A perspex tube with a lid (7-10 cm ø) is pushed into the sediment, either leaving some water or air space . The core top part is completely filled with seawater. A known amount of radioactive carbonate ($^{14}CO_3^{2-}$) is added to an estuarine water sample of known carbonate content. After a specified time period (4 h) of photosynthesis by the phytobenthos under controlled conditions in an incubator, the suspended cells are filtered over a 0.45 µm membrane filter and the radioactivity from the carbon in the cells measured by (ß) scintillation counting. The uptake of radioactive carbonate, as a fraction of the total carbonate, will reflect the rate of photosynthesis over the given period.
Method #2a. ^{14}C tracer technique, after sampling, incubation in the field. Sediment (sub)samples are placed in cleaned glass bottles, estuarine water is added. The analytical technique as under Method #1, the incubation takes place

in situ, by placing the incubation bottles at the sediment surface, thus ensuring natural light and temperature conditions.

Method #2b. ^{14}C tracer technique, after sampling, incubation in an incubator. The analytical technique as under Method #2a, but the incubation takes place under controlled laboratory conditions by placing the bottles in an incubator.

Method #3. Oxygen determination, *in situ,* in a bell jar. The samples are incubated in either dark or clear perspex tubes inserted in the sediment for a specified time period (4 h) of photosynthesis by the phytobenthos under natural conditions. The change in concentration of the oxygen in the bottle is followed (A-7.5). This can be done by ion selective (micro)-electrode, although the micro-Winkler titration is preferable because of its high sensitivity and the distinct ending of biological activity by the addition of chemicals (A-7.5). The dark bottle incubation will give the effect of respiration, while the clear bottle will provide the oxygen content due to the difference of respiratory consumption and primary production. From the increase in oxygen concentration the primary production is calculated.

Method #4a. Oxygen production technique, after sampling, incubation in the field. Sediment (sub)samples are placed in cleaned glass bottles, and sea- or estuarine water is added. The analytical technique (oxygen determination) is as under Method #3. The incubation takes place *in situ,* by placing the incubation bottles at the sediment surface, thus ensuring natural light and temperature conditions.

Method #4b. Oxygen determination technique, after sampling, incubation in an incubator. The analytical technique as under Method #4a, but the incubation takes place under controlled laboratory conditions by placing the bottles in an incubator.

Remarks:

^{14}C is a (weak) radio-tracer which in most countries will require a specially equipped laboratory space . Please check on safety regulations for working with radio-tracers.

Method(s) references:

Vollenweider (1969); Marshall *et al.* (1973); Cadée & Hegeman (1974; 1977); Colijn & De Jonge (1984); Round & Hickman (1984)

A-7.42

Parameter: **micro-phytobenthos,**

biomass

Unit: **gC/m^2**

JEEP database: **FBBM**

Compartment: **sediment**

Introduction:
Chlorophyll *a* is not necessarily a good and conservative unit to describe the quantity of algal biomass. This is especially true for the estimation of microphytobenthos at the sediment surface, since here chlorophyll containing debris is collected (during slack tide) from the water column, which may thus cause an overestimation of the algal biomass. The determination of the microfloral biomass may be based on the organic carbon/chlorophyll *a* ratio (C/Chl-a). It appears that in time (seasonal effects) differences are found in this ratio. Fortunately, the ratio within an estuary for a given date appears to be constant (De Jonge, 1980). Differences in C/Chl-a ratios between various estuaries have not yet been recorded, but they may be expected, however.
A procedure that involves tuning of the method by using only two or three determinations of the ratio per sampling exercise along the estuary is presented here. The thus determined ratio can be used to estimate the biomass from the other stations (at that time and in that estuary) from the chlorophyll *a* determination only. This minimizes the number of organic carbon analyses, which is also not easy to perform adequately, due to the difficulties to separate microphytobenthos cells from other organic material in the sediment.

Sampling:
Samples are collected by coring (about 2.4 cm ø). The top 0.5 cm is collected; when deeper layers are to be examined; when deeper layers are to be examined, a second choice is the 0.5-2 cm layer.

Sample volume:
To eliminate natural variability, about 20 individual samples are collected and are pooled together (keeping the individual sediment layers); subsamples are taken from this homogenate for analysis.

Sample treatment:
The samples are lyophilized before analysis.

Storage:
The samples are to be stored deep-froozen at - 20 °C.

Sample volume:
Depending on the expected chlorophyll content a volume of the extraction liquid between 10 and 25 cm^3 is used for the determination of chlorophyll *a* .

Analytical method(s):

Method #1. Determination of the C/Chl-a ratio. After the sample water has been removed, the sediment is froozen and lyophilized, and the chlorophyll *a* content determined spectrophotometricaly according to Lorenzen (1967). This and other techniques have been described in A-7.24.

The organic carbon follows the procedure as described under POC (A-7.25).

The ratio (C/Chl-a) is calculated.

Once the ratio is known, the biomass for all other stations can be estimated from the chl-a determination alone.

For the description of the analytical methods one is referred to A-7.40.

Remarks:

Remember that not only vital micro-phytobenthos cells are represented by the chlorophyll *a* measurement (De Jonge, 1992).

Method(s) references:

Lorenzen (1967); De Jonge (1980; 1992); Round & Hickman (1984)

A-7.43

Parameter: **macrophytobenthos**

species abundance

Unit: **g C/m^2 (dw)(per species)**

JEEP database: **MFSP**

Compartment: **sediment**

Introduction:
For the purpose of the JEEP92 programme the species abundance is important for general classification. The macroscopic morphology which predominantly separates the different genera determines the classification. The qualitative and quantitative aspects of the species composition will be carried out in the laboratory rather than in the field. For nomenclature see for example Parke & Dixon (1976).
As numbers per m^2 give no relevant information, the species composition should be given in terms of either biomass per species or as percentage of the total biomass. The latter may be calculated from the former and therefore biomass (dry-weight) per species is preferred.

Sampling:
The macrophytobenthos is quantitatively collected from the randomly selected quadrats as defined under S-6.8. The material is kept in plastic bags.

Sample volume:
All material within each selected quadrat should be sampled for the determination of the species distribution.

Sample treatment:
The macrophytes are cleaned of adhering particulate matter and are kept moist; excess water should be avoided.

Storage:
The plastic bags should be stored in a cool place (4 °C), in the dark. The classification should be performed within 24 hours.

Analytical method(s):
Method #1. The macrophytes are to be identified at least to the level of macroscopic morphological characteristics. The numbers of each representative species and their wet-weight should be recorded, their biomass (dry-weight) determined (see A-7.44). This enables the calculation of their relative distribution.

Remarks:

Method(s) references:
Parke & Dickson (1976); Wolff (1983; 1987b); Eleftheriou & Holme (1984); Round & Hickman (1984)

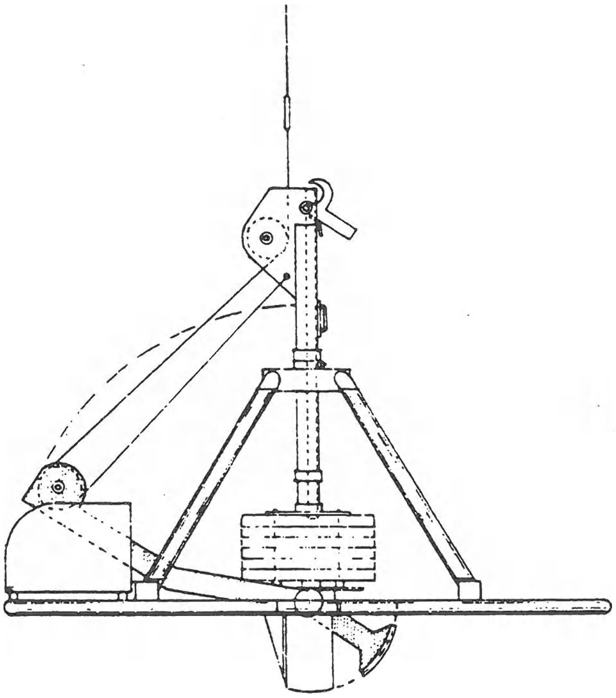


Figure 8.
The Reineck boxcorer (from: Reineck, 1963)

A-7.44

Parameter: **macrophytobenthos**

total biomass

Unit: **g/m^2 (dw)**

JEEP database: **MFBM**

Compartment: **sediment**

Introduction:
For the determination of the biomass of macrophytobenthos, the collected material is dried to determine the dry-weight. Large quantities are sometimes collected from the selected quadrats of substrate and sub-sampling may become necessary. The sub-sampling procedure should then be checked carefully to ensure collection of representative amounts are collected. This can be achieved by subsequent determination of total wet-weight of the sample (preferably per selected class), and the wet-weight of the sub-sample. The dry-weight of the latter is then determined. The total biomass should equal the sum of all different species biomass as determined for the species abundance.
The wet-weight might be measured which can be converted to dry-weight biomass by calculation. However, as this requires non-standard conversion factors the method is not recommended and hence this method has an asterisk.
It should be mentioned that also remote sensing techniques have been applied for the estimation of the biomass.

Sampling:
Because of the high variability with time of the standing stock of macrophytes (growth and erosion) Wolff (1987a,b) stresses the need for frequent sampling. The macrophytobenthos is quantitatively collected from the randomly selected squares, as defined under S-6.8. The material is sorted for the major macroscopic morphological characteristics (genera) and kept in plastic bags.

Sample volume:
The total material per sampling square is wet-weighed; sub-samples are prepared when necessary, to get about 100 g wet-weight of material for dry-weight analysis.

Sample treatment:
To avoid weighing errors from non-macrophyte material, the samples are carefully washed to remove adhering particles and organisms that may have been collected as well. After sorting into separate classes and sub-sampling when necessary, the macrophytes are kept in plastic bags.

Storage:
To prevent deterioration of the material, the samples should be stored moist (not wet) in a cool place (4 °C) in the dark. The analysis should take place within 24 h.

Analytical method(s):

Method #1. About 100 g wet material of each macrophyte class, and of each substrate square, is dried at 80 °C for 24 h. The dry-weight is averaged from the various squares, and reported as dry-weight per class of macrophytes and calculated per m^2.

Method #2. If the macrophytes are to be used for further analysis, the wet-weight is determined per class and per sampling square, after blotting with tissue paper. The results are expressed as wet-weight per class of macrophytes, and calculated per m^2.

Remarks:

Method(s) references:

Parke & Dickson (1976); Wolff (1983); Round & Hickman (1984)

A-7.45

Parameter: **meiobenthos**

species abundance

Unit: **n/m^2**

JEEP database: **MBSP**

Compartment: **sediment**

Introduction:
For the determination of the species distribution of a core sample, it is essential that a proper separation between the (inorganic) sediment and the meiofaunal organisms is quantitatively achieved. Furthermore, the number of species to be analyzed should be such that sufficient organisms are present to get a reliable estimation of their relative contributions, and that the amount of work is kept within reasonable limits. The sampling method (core diameter, core length) tries to cope with the latter aspect.
The methods of retrieval of the organisms from the sediment has extensively been treated by Pfannkuche & Thiel (1988), comparisons have been made by Uhlig *et al.* (1973).
In general the sediment is gently sieved under water to separate the animals from the abiotic particulate fraction. The following mesh sizes are proposed: 1000 μm (upper limit of meiofauna); 500 μm (mainly consisting of juvenile macrofauna, temporary meiofauna); 250 μm; 125 μm; 63 μm and 42 μm (lower limit for the retention of meiofauna).
Several methods for the quantitative extraction and concentration of meiofauna specimens have been described, none of which seems to be the ultimate technique. These rely on a) differences in the sinking rates of sediment particles, detritus and meiofauna (decantation, elutriation, flotation, filtration over a density gradient) and b) response (of living organisms) to environmental gradients (oxygen depletion, temperature, phototactic reaction). Also density gradients, e.g. using Ludox-TM have been proposed.
After quantitative retrieval of the organisms the quantity may be too large for identification of all individuals. Splitting techniques are useful in these cases.

Sampling:
The various sampling techniques of sediment for the collection of meiofauna, either in sub-tidal or inter-tidal areas, in sandy sediments or in mud, is described in section S-6.9. Standard sampling depth is 5 cm for muddy sediments, and 15 cm for sandy sediments.

Sample volume:
The sample volume is highly related to the type of sediment, and hence the number of meiofauna species to be expected. This factor determines different sampling strategies for the various substrates (S-6.9). Splitting of the sample (core) in 2 or 4 equal parts may be a possibility to reduce the sample size (check the equal splitting by weighing and counting).
Sub-sampling by splitting of the sediment extracted organisms may performed using various types of devices (Pfannkuche & Thiel, 1988).

Sample treatment:
(Sediment) samples should be stored after addition of 4% warm formalin (60 °C) in seawater solution. The elevated temperature stops nematodes rolling up, which facilitates their identification.
After extraction, identification is facilitated by staining of the entire sample with rose bengal (1 %, 48 h) or fluorescent dyes. The fauna is extracted, depending on sediment type, according to methods described in detail in Pfannkuche & Thiel (1988).

Storage:
Preserved samples can be stored until analysis.

Analytical method(s):
Method #1. Microscopic analysis, The meiofauna may be identified to major taxa and enumerated under a stereomicroscope. For more detailed taxonomic analysis organisms should be sorted into major taxa and treated according to the relevant sections in Higgins & Thiel (1988).

Remarks:
Formalin is a toxic substance. Please refer to the safe handling procedures for chemicals.

Method(s) references:
Uhlig *et al.* (1973); De Jonge & Bouwman (1977); Bouwman (1987); Higgins & Thiel (1988); Pfannkuche & Thiel (1988)

A-7.46

Parameter:	**meiobenthos**
	biomass
Unit:	**g/m^2**
JEEP database:	**MBBM**
Compartment:	**sediment**

Introduction:
Two methods that differ substantially are used for the determination of the biomass: the gravimetric and the volumetric methods.
When large numbers of meiofaunal organisms are available their biomass can be determined directly from the dry-weights.
The second method involves the "measurement" of the total volume of the various taxa. This measurement is through visual inspection of the specimens and subsequent estimation of their body volume by calculation using simple formulae for regular shaped ones (e.g. nematodes), length to volume conversions using preset conversion factors, and/or the use of clay models for irregularly shaped species or the use of slides at known equidistance for soft irregular forms. The conversion to biomass is thus derived from a calculation involving an arbitrary specific gravity (usually of 1.13) (Feller & Warwick, 1988).
While direct gravimetric measurement gives a straightforward answer, the other methods use conversion factors that may change with time or investigator. An example is the volume to dry-weight conversion factor of 1.13 (specific gravity), and a dry-weight wet-weight ratio of 0.25. They have therefore been marked with an asterisk. For the JEEP92 project the methods as given in Feller & Warwick (1988) have been chosen. Details of the conversion factors from this reference are given in Annex VIII.
It is important to have the actual measured values instead of data that are derived from unsure conversion to be stored in the database. Therefore dry-weight, ash-free dry-weight or blotted wet-weight are accepted. The first has, however, our preference.

Sampling:
The various sampling techniques of sediment for the collection of meiofauna, either in sub-tidal or inter-tidal areas, in sandy sediments or in mud, is described in section S-6.9. Standard sampling depth is 5 cm for muddy sediments, 15 cm for sandy sediments.

Sample volume:
The sample volume is highly related to the type of sediment, and hence the number of meiofauna species to be expected. This factor determines different sampling strategies for the various substrates (S-6.9). Splitting of the sample (core) in 2 or 4 equal parts may be a possibility to reduce the sample size. Check the splitting of the sediment by weighing or preferably counting of organisms.

Sample treatment:
(Sediment) samples should be stored deep frozen (-20 °C) or after addition of 4% (warm, 60 °C) formalin in seawater solution. Substantial differences in biomass have been found between preserved and non-preserved samples (Widbom, 1984) The method of retrieval of the organisms from the sediment has been extensively treated by Pfannkuche & Thiel (1988). Several methods for the quantitative extraction and concentration of meiofauna specimens has been described, none of which seems to be the ultimate technique. These rely on a) differences in the sinking rates of sediment particles, detritus and meiofauna (decantation, elutriation, flotation) and b) response (of living organisms) to environmental gradients (oxygen depletion, temperature, phototactic reaction).

Storage:
Well preserved samples, either under formalin or deep frozen can be stored for a reasonable time until analysis.

Analytical method(s):
Method #1a. Gravimetric determination of dry-weight, formalin preserved. When large numbers and/or large specimens are available, dry-weights can be determined directly on appropriately sensitive balances (sensitivity ± 0.1 µg). Prior to weighing, the organisms are rinsed by washing over a GF/C glass fibre filter and dried in an oven at 60 °C until constant weight (24 h). The should be stored in a desiccator until weighing. The biomass is given as g dry-weight per m^2.
Method #1b. Gravimetric determination of dry-weight, preserved deep frozen. The method is identical to Method #1a, except for the preservation.
Method #2a. Gravimetric determination of ash-free dry-weight, formalin preserved. As Method #1a, but after drying, the sample is ashed in an oven at 500 °C for 6 h, and expressed as ash-free dry-weight.
Method #2b. Gravimetric determination of dry-weight, preserved deep frozen. As Method #1b, but ashed in an oven and expressed as ash-free dry-weight.
Method #3a. Gravimetric determination of blotted wet-weight, formalin preserved. As Method #1a, but blotted dry using tissue paper, and expressed as blotted wet-weight.
Method #3b. Gravimetric determination of blotted wet-weight, preserved deep frozen. As Method #1b, but blotted dry using tissue paper, and expressed as blotted wet-weight.
Method #4*. Volume based conversions. Depending on the shape of the species the volume is calculated from simple equations involving length and diameter (e.g. worms) or using empirical conversion factors that take the irregular shape into account (see Annex VIII).

Remarks:
Formalin is a toxic substance. Please refer to the safe handling procedures for chemicals.

Method(s) references:
Widbom (1984); Feller & Warwick (1988); Greiser & Faubel (1988); Pfannkuche & Thiel (1988)

A-7.47

Parameter: **macrozoobenthos**

species abundance

Unit: **n/m^2**

JEEP database: **MCSP**

Compartment: **sediment**

Introduction:
Macrofauna is distinguished from the meiobenthos by arbitrary selection of mesh size of the sieve of 1 mm; sometimes 0.5 mm is preferred. The sediment sample is washed over a sieve with a gentle water stream to remove sediment and smaller organisms. Specialized techniques involve a freezing and Calgon technique (sodium hexametaphosphate for breaking up stiff clays), an elutriation technique, floatation in a liquid with higher density than water, or the use of the swimming behaviour of organisms (Hartley *et al.*, 1987).
For identification of the individual species , one is referred to the overview of the literature concerning this subject (Wolff, 1987b).

Sampling:
The use of corers is preferred to grab samplers because of the better definition of the surface area and sediment sampling depth, thus a more consistent sample size. Their use will be dependent on the sampling site (sub-or intertidal), and on the availability of a suitable ship (e.g. when using box-corers).
Sampling depth should be 25 cm, replicate samples should be collected (S-6.10).

Sample volume:
In most estuaries one core of, for example, 200 cm^2 surface area , will collect sufficient material for analysis. In sediments with high organism densities smaller cores will be sufficient. Due to heterogeneity in the distribution of the organisms in the sediment, a series of individual samples (= replicates) should be collected and analyzed separately. This will yield statistical information, e.g. about the patchiness of the macrofaunal distribution.

Sample treatment:
After extraction over a 1 mm mesh the organisms should be preserved with buffered formalin (6% formaldehyde in seawater; but if the samples contain a lot of water, 8-10% is better). Bulk staining has been proposed to facilitate sorting . A stain, like bengal rose or eosin can be added to the formalin solution.

Storage:
Formalin preserved samples can be stored until analysis. Deep freezing of fresh material is not recommended because after thawing identification of the organisms may be difficult. Fresh samples (up to a few days, stored cool at 4 °C) are sometimes easier easy to sort (moving animals) and to identify. Storage of formalin preserved samples deep froozen has been recommended as a good alternative (Salonen & Sarvale, 1985).

Analytical method(s):
Method #1. Visual inspection. The samples are placed in a white tray and checked for species which are identified. The small sized animals should be identified using a binocular microscope and possibly a compound microscope.

Remarks:
Formalin is a toxic substance. Please refer to the safe handling procedures for chemicals.

Method(s) references:
Barnet (1979); Coleman (1980); Eleftheriou & Holme (1984); Warwick (1984); Hartley *et al.* (1987)

A-7.48

Parameter: **macro-zoobenthos**

age distribution

Unit: **n/class**

JEEP database: **MCAD**

Compartment: **sediment**

Introduction:
For some important species (especially molluscs and fish) the age composition is aquired. Distinction of age can be based on either growth marks on some hard part of the organism (shells of bivalves, jaws of polychaetes, ~~otoliths or scales of~~ fish) or on size-frequency distributions. The latter method becomes problematic with the older (slower growing) individuals because of overlap between the fast growing of one year and the slow growing of the next year.

Sampling:
The same as for the total species distribution. As part of the sorting procedure the species for which the age-classes are to be determined may be kept aside for further study (sub-sampling).

Sample volume:
The same as for total species distribution.

Sample treatment:
After extraction the organisms should be preserved with buffered formalin (6% formaldehyde in seawater).

Storage:
Formalin preserved samples can be stored until analysis.

Analytical method(s):
Method #1. Growth marks. This method works provided that it can be proved that growth marks are laid down annually in e.g. the shell of a mollusc or in the jaws of a polychaete and that they can be counted and thus they organisms aged. One must be able to distinguish between growth checks produced by the slowing down of somatic growth during winter and those induced by diversion of energy into the production of gonads (seasonal effects) or caused by (accidental) adverse physical conditions e.g. prolonged low oxygen tension.
The year marks are counted and the organisms are place in the respective age classes.
Method #2. Frequency distribution. These are obtained by measuring the length (or weight) of a large number of individuals, preferably the entire sample and plotting the number of individuals within a stated size interval against size. A series of peaks can usually be distinguished.
Graphical methods may be used to separate peaks in a size/frequency distribution (Harding, 1949; Cassie, 1954) may be used to separate peaks in a size/frequency distribution. While the first two peaks generally relate to discrete age classes there is often considerable doubt as to the number of age classes in

any additional peaks. If one can estimate the number of age classes that there should be in a sample there are a number of methods (e.g. McDonald & Pitcher, 1979) by which complex size histograms can be composed to give age classes.

Remarks:
Formalin is a toxic substance. Please refer to the safe handling procedures for chemicals.

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Method(s) references:
Cassie (1954); Bhattacharya (1967); MacDonald & Pitcher (1979); Crisp (1984); Warwick (1984)

A-7.49

Parameter: **macrozoobenthos**

biomass

Unit: **g/m^2 (a.f. d.w.)**

JEEP database: **MCBM**

Compartment: **sediment**

Introduction:

Different methods are used to give a representation of the biomass estimation. They can be expressed on the basis of wet-weight, dry-weight, ash-free dry-weight or in terms of total N-content or caloric value. It will be obvious that differences between the techniques occur. Conversion factors have been evaluated for several relations between these measures which indicates that conversions are not universally applicable either in space or in time.
When the organisms contain a substantial amount of sediment in the gut (e.g. polychaetes), defecation in clean seawater for 24 h is essential to overcome errors in the biomass determination by weighing technique.
Another approach is a conversion from length (or volume) to weight. This involves either an arbitrary factor, or can be based on actual measurements from the estuary and relevant period of the year (calibration). Since macrozoobenthos is usually of sufficient size to determine the actual weight, weighing is preferred to the conversion method. Although biomass determination can be performed on (homogenised) sub-samples, the non-destructive method is preferred, as it keeps the samples available for further analyses, e.g. histo-pathology, chemical analysis of trace elements.
The use of ash-free dry-weight is the best choice under estuarine conditions. This eliminates errors caused by fluctuations in the internal salt content which varies according to estuarine conditions. As the other methods are also widely applied, they are given as well.
For animals with hard parts, such as mollusca, a length-weight conversion can be calculated. A log-linear regression is determined between the individual weight (W) and the length (L) with the function of the shape:
$\log_{10}W = a \log_{10}L + b$ or $W = b * L^a$. For a mathematical treatment of the length-weight relations, one is referred to Baskerville (1972), and to the summary in Annex IX. When the parameters a and b have been determined for a population (calibration), the biomass is easily calculated from the length determination. This weight-length relation may vary with the month of the year, and should therefore be checked. The regression will differ between estuaries and since the condition of the organisms also changes between seasons it is advisable to calibrate the method accordingly for each estuary studied.

Sampling:

As for the species distribution (A-7.47).

Sample volume:

A total of about 0.5 - 3 g of (wet) tissue, depending on the balance used, is sufficient for an (ash-free) dry-weight determination. To eliminate the variability between organisms a minimum of 20 individuals should be analyzed, either by homogenisation (and subsequent sub-sampling), or by separate analysis and calculation.

Sample treatment:
For the biomass determination the samples should preferably not be treated with formalin or any other fixing agent, as these may interfere with the analysis. Optimally fresh material should be used. As an alternative the material should be stored deep frozen (- 20 °C) until analysis, preferably after sub-sampling for species and age-class. Freezing individual animals tends to cause weight loss, however. Additionally material may be lost when tissues rupture on thawing.

Storage:
The samples should be used fresh or stored in plastic bags or containers, deep frozen (- 20 °C).
Bulk freezing of sediment samples containing the animals should be avoided at all cost.

Analytical method(s):
Method #1. Weighing (ash-free dry-weight). The sample is dried at ca 60 °C to constant weight (40-48 h), after which the sample is placed in a desiccator and allowed to cool. The sample is weighed, incinerated at about 550 °C in a muffle furnace for 2-4 h. After cooling to 80 - 90 °C, the residue is stored in a desiccator. After weighing the ash-free dry-weight is calculated by subtraction.
Method #2. Weighing (dry-weight). The sample is dried at ca 60 °C to constant weight (40-48 h), after which the sample is placed in a desiccator and allowed to cool. The sample is weighed to determine the dry-weight.
Method #3. Weighing (blotted wet-weight). The sample is blotted dry on paper tissue and weighed to determine the wet-weight. From this wet-weight the biomass can be estimated by the use of conversions. This method is relatively fast and allows the use of the organisms for other analyses. The method should be calibrated using the formula: $\log_{10}W = a \log_{10}(\text{wet-weight}) + b$, where W is the dry-weight.
Method #4*. Dry-weight calculation from body lengths. For organisms that are suitable for the method, i.e. containing hard parts, the lengths are accurately measured. For the calibration a large number of individual organisms of all sizes should be used to correlate length and weight. A regression has then to be found between the individual ash-free dry weights (cf. Method #1) and the lengths (both log transformed) of the various size classes using the least squares method (see introduction). This equation can then be used to estimate the individual dry-weights from the length measurements.
Method #5*. Dry-weight calculation from body volume. This method is important for irregular shaped organisms, where the length is not suitable for conversion purposes. As for the length - dry-weight conversion, there is no constant relationship between volume and tissue dry-weight, not even for a given species . The method should therefore be calibrated using the formula: $\log_{10}W = a \log_{10}(\text{volume}) + b$ or $W = b * (\text{volume})^a$, where W is the dry-weight.

Remarks:
Formalin is a toxic substance. Please refer to the safe handling procedures for chemicals.

Method(s) references:
Crisp (1984); Warwick (1984)

8. DATABASE

One of the necessities for introduction of information from different sources and compiled by various scientists into a database is the uniformity of the data. The use of the same units is prerequisite for uniformity, but also methods of collection (sampling, analysis) should ideally be the same. It is obvious that this can never be completely achieved.

The compilation of a data base comprising all the measurements carried out in the project is one of the major tasks of the JEEP92 project. This database will be fully computerized. In order to deal with streams of data originating from at least as many sources as there are participants in the project, a data input and editing program has to be developed and made available to the participants.

One of the aim of this manual is to guide the users for the JEEP92 project in the presentation of the proper units, and in the identification of sampling and analytical Methods. Those Methods, which are described in the sections on sampling and analysis (chapters 6 and 7) are also included in the database. Data collected are stored in the database together with coded information about the method of collection and of analysis. This ensures that if required, data collected with identical methods may be separated from those data which were gathered by techniques that do not compare well. This is not only true for physico/chemical variables like salinity, temperature, etc. of which uniformity is rather simple to perform, but also for variables that are the result of different methods, e.g. biomass calculated from size, chlorophyll or measured organic carbon content. Also scientific names of biological species (or even families) should be introduced in a uniform way. In the database the change of scientific names because new taxonomic information has become available is a simple operation.

A database has been specially designed for the collection (and interpretation) of the data from various estuaries. This computer program, the Data Entry and Editing Program (DEEP-92), is presented in a separate report (De Winter, 1992a) and is available for JEEP92 participants on diskette.

The data entry program has been especially developed for the entry of data of the JEEP92 programme, and is written using DBASE IV. It wil function on any computer platform that is able to use this database program. However, users who do not have MS-DOS systems are requested to contact the author before using it. A DBASE IV version 1.1 should be present on the computer system to run the program and to manage the database. About 1 Mb free disk space on the hard disk is required to install the program.

Initially the space requirements will be rather low, but they will grow during use of the program due to data entry.

The program is fully menu driven and controlled by function keys. It has been designed to enter data from (estuarine) surveys. Therefore it starts with the header of the sample level. From this organisation level one may go up in the hierarchy and add new stations or even estuaries. Usually data operations will occur at the same organisation level because new samples and/or observations have to be introduced. The program provides default information on locations, methods etc., based on the last entry. Abbreviations of the various variables have been introduced in the database. These abbreviations can also be found in the headings of the proceduresin the present manual.

An alphabetical list of the abbreviations is presented in Annex IV.

Annex V describes the structured scheme and list of typical information that should accompany any observation that is required for processing data into this data base.

Species lists can not be handled by the DEEP program. This is because the number of possible species is virtually unlimited and thus incompatible with the structure of an observations data base that has been based on a limited number of variables. Also the input process would become too slow.

Therefore species lists have to be converted. When they are available in a spreadsheet format (e.g. Lotus 123) or text format (ASCII) this can be handled easily by the database manager.

For the JEEP92 project, rules regarding the species names have been put forward to facilitate data entry which involve estuarine species names. The names of the genus or species and their spelling are preferably to be selected from the "Directory of the British marine fauna and flora" (Howson, 1987). This list is available both in printed format as well as on floppy disk. Some species or genera will not be available from this list. In that case the database manager will decide how to proceed.

It is realized that probably none of the participants of the project can be a specialist in the identification of all individual species of all estuarine taxa. It is strongly advised, however, to identify the organisms at least to the genera level, thus for example *Arenicola* spp.

All data entered in the program will be brought together with the data of other estuaries in the project and stored in one large data base. This database may be accessed by all the participants.

For a further description one is referred to the manual of the database (De Winter, 1992a).

9. GLOSSARY

AFDW	ash free dry weight
CHN	carbon hydrogen nitrogen
COST	Cooperation européenne dans le domaine de la recherche Scientifique et Technique
CTD	conductivity temperature depth
DNA	deoxyribo nucleic acid
DOC	dissolved organic carbon
DON	dissolved organic nitrogen
DW	dry weight
GCMS	gas chromatography mass spectrometry
HPLC	high performance liquid chromatography
IAPSO	International Association for the Physical Sciences of the Ocean
MAST	Marine Science and Technology
MPN	most probable number
nm	nano meter
PE	poly ethylene
POC	particulate organic carbon
PON	particulate organic nitrogen

POP	particulate organic phosphorus
PVC	poly vinyl chloride
SD	Secchi depth'
SED	spheric equivalent diameter
SPM	suspended particulate matter
ST	salinity - temperature
TLC	thin layer chromatography
TTI	tritiated thymidine incorporation
µm	micro meter
UNESCO	United Nations Educational, Scientific and Cultural Organisation
UV	ultra violet

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ANNEXES

Annex I

Forms for the inventory of European tidal estuaries (De Winter, 1992b)

Synopsis of the Ecology and Hydrography of European Estuaries

Name of the Estuary ....

Geographical Coordinates ....

Countries ....

Type of the Estuary [ ]

River(s) discharging ....

Human interference (like dredging): ....

....

General description ....

....

....

....

....

Additional Maps: ○ Estuary ○ Sediment ○ Catchment Area ○ Other

Maps available at ....

under number ....

Form completed by:

Institute: ....

adress: ....

....

....

....

telephone nr.: ....

fax nr.: ....

Economic Use:

Industrial Zones ....

Big cities ....

Fisheries ....

Aqua-culture ....

General Physical Characteristics:

	mean	**unit**	**minimum**		**maximum**	**also enclosed:** **time series**	**graph**
Catchment area	....	km²					
Water volume	....	* 10⁶ m³					
Residence time	....	days	....	to	....		
Number of ice days	....		....	to	....	○	○
Water temperature (seaward part)	....	°C	....	to	....	○	○
Position of turbidity maximum (from mouth)	....	km	....	to	....	○	○
Mean tidal ranges	....	m at	....				
	....	m at	....				
	....	m at	....				
	....	m at	....				

Hydrography

	Freshwater	*Oligohaline*	*Mesohaline*	*Polyhaline*	*Euhaline*	*Whole Estuary*	
Water surface at MHW:	....	....	....	....	....	....	km²
Length of section at MHW:	....	....	....	....	....	....	km
Width of section at MHW:	....	....	....	....	....	....	km
Salt marshes at MHW:	....	....	....	....	....	....	km²
0 - 5 m depth at MLW:	....	....	....	....	....	....	km²
5 -10 m depth at MLW:	....	....	....	....	....	....	km²
>10 m depth at MLW:	....	....	....	....	....	....	km²
Water volume:	....	....	....	....	....	....	10⁶m³
Residual current:	....	....	....	....	....	....	m/s

Hydrography

	Freshwater	*Oligohaline*	*Mesohaline*	*Polyhaline*	*Euhaline*	*Whole Estuary*	
areas of intertidal flats (all at MLW):							
— total surface:	....	....	....	....	....	....	**km²**
— Sandy flats (<5% clay)	....	....	....	....	....	....	**km²**
< 50% of time emerged	....	....	....	....	....	....	**km²**
≥ 50% of time emerged	....	....	....	....	....	....	**km²**
— Silty (>5% clay)	....	....	....	....	....	....	**km²**
< 50% of time emerged	....	....	....	....	....	....	**km²**
≥ 50% of time emerged	....	....	....	....	....	....	**km²**
— Beds of bivalves:	....	....	....	....	....	....	**km²**
— Beds of vegetation:	....	....	....	....	....	....	**km²**

Biology

Eco-groups:	*Freshwater*	*Oligohaline*	*Mesohaline*	*Polyhaline*	*Euhaline*	*not specified*	
Phytoplanktn Biom.:	....	....	....	....	....	....	gC/m³
Production:	....	....	....	....	....	....	gC/m³.y
remarks:							
Zooplankton Biom.:	....	....	....	....	....	....	gC/m³
Production:	....	....	....	....	....	....	gC/m³.y
remarks:							
Phytobenthos Biom.:	....	....	....	....	....	....	gC/m²
Production:	....	....	....	....	....	....	gC/m².y
remarks:							
Macrobenths Biom.:	....	....	....	....	....	....	gC/m²
Production:	....	....	....	....	....	....	gC/m².y
remarks:							
Meiobenthos Biom.:	....	....	....	....	....	....	gC/m²
Production:	....	....	....	....	....	....	gC/m².y
remarks:							
Hyperbenthos Biom.:	....	....	....	....	....	....	gC/m²
Production:	....	....	....	....	....	....	gC/m².y
remarks:							

Biological References: [1]

	species distribution/composition		energetics		
	spatial	temporal	biomass	production	consumption
phytoplankton					
zooplankton					
phytobenthos					
meiobenthos					
macrozoobenthos					
hyperbenthos					
Birdlife					
Wildlife					

[1] Please refer to numbers in the reference list you have included.

Physico-Chemical Characteristics:

	time series enclosed	graph of series enclosed [1]	reference(s)
Oxygen	○	○	
Salinity/Chlorinity	○	○	
Nitrogen	○	○	
Phosphorus	○	○	
Silicate	○	○	
DOC	○	○	
POC	○	○	
organic load in IE	○	○	
light intensity I_0	○	○	
light extinction	○	○	
temperature	○	○	
	○	○	
	○	○	

Annex II
Synopsis of Sampling Methods and Sampling (sub)codes

Physico-chemical variables water (S-6.1)

Code	Method	subcode	sample:
1a	water sampler	1	spot
1b	sample bottle	2	integrated
2	pumping system	3	profile
3	bucket	0	other
0	other		

Physico-chemical variables seston (S-6.2)

Code	Method	subcode	sample:	subcode	filter size:
1a	water sampler	1	spot	1a	0.45 µm
1b	sample bottle	2	integrated	1b	0.4 µm
2	pumping system	3	profile	2	centrifugation
3	bucket	0	other	0	other
0	other				

Physico-chemical variables sediment (S-6.3)

Code	Method	subcode	sample:
1a	handcorer	1	mixed
1b	gravity corer	2	profile
1c	piston corer	0	other
1d	box corer		
1e	vibro corer		
2	grab		
0	other		

Biota: water

Code	Method	subcode		subcode	
	bacteria (S-6.4)				
1	sample bottle				
2	pumping system				
3	(sterile) water sampler				
0	other				
	phytoplankton (S-6.5)				
1a	sample bottle				
1b	water sampler				
2	pumping system				
3	nets				
0	other				
	zooplankton (S-6.6)				
			net-size:		**volume:**
1	pump + net	1	55 or 63 µm	1	100 l
2	towing net	2	200 µm	2	5 m^3
3	high-speed sampler	0	other	0	other
4	tube + net				
5	bucket + net				
0	other				

Biota: sediment

Code Method subcode subcode

micro-phytobenthos (S-6.7)

Code	Method
1	hand corer 1 cm ø (1 cm depth)
2	box corer, subsamples
0	other

macro-phytobenthos (S-6.8)

Code	Method	subcode	**area:**
1	grid-squares	1	10 * 10 cm
0	other	2	25 * 25 cm
		0	other

meiobenthos (sub-tidal) (S-6.9)

Code	Method	subcode	**depth:**	subcode	**sieve mesh size:**
1a	box corer (2 cm Ø, mud)	1	5 cm	1	63 µm
1b	box corer (8 cm Ø, sand)	2	15 cm	2	45 µm
2a	divers (2 cm Ø, mud)	0	other	0	other
2b	divers (8 cm Ø, samd)				
3	bow wave free sampler				
4	pole, gravity, piston corers				
5	grab sampler				
0	other				

meiobenthos (inter-tidal) (S-6.9)

Code	Method	subcode	**depth:**	subcode	**sieve mesh size:**
1a	hand corer (2 cm ø, mud)	1	5 cm	1	63 µm
1b	hand corer (8 cm ø, sand)	2	15 cm	2	45 µm
0	other	0	other	0	other

macrobenthos (sub-tidal) (S-6.10)

Code	Method	subcode	**depth:**	subcode	**sieve mesh size:**
1	box corer	1	25 cm	1	1 mm
2	grab sampler	0	other	2	0.5 mm
0	other	0	other		

macrobenthos (inter-tidal) (S-6.10)

Code	Method	subcode	**depth:**	subcode	**sieve mesh size:**
1	hand corer (200 cm^2)	1	25 cm	1	1 mm
2	hand corer (other size)	0	other	2	0.5 mm
0	other			0	other

In-situ measurements: water

Code	Method	subcode	
	salinity		
1	conductivity		
2	lab. analysis		
0	other		
	temperature		
1	thermocouple		
2	thermometer		
0	other		
	pH		
1a	in-situ pH-electrode		
1b	on-board determination		
2	lab. analysis		
0	other		
	oxygen		
1	in-situ oxygen electrode		
2	lab. analysis		
0	other		
	light penetration		
1	Secchi disc		
2	irradiance meter		
0	other		
	turbidity		
			cell:
1	in-situ turbidometer	1	5 cm
2	pumping system/ on board analysis	2	10 cm
		0	other
0	other		
	fluorescence		
			cell:
1	in-situ fluorimeter	1	5 cm
2	pumping system/ on board analysis	2	1 cm
		0	other
0	other		

Annex III
Synopsis of analytical methods and methods (sub)codes

Physico-chemical variables: water

Code	Method	subcode	
	salinity (A-7.1)		
1a	conductivity		
1b	chlorinity		
0	other		
	chlorinity (A-7.2)		
1	titration		
0	other		
	temperature (A-7.3)		
1a	thermistor		
1b	resistance thermometer		
2	thermometer		
0	other		
	turbidity (A-7.4)		
			cell length:
1	Secchi depth'	1	1 cm
2	beam transmission	2	5 cm
3	nephelometer	3	10 cm
0	other	0	other
	dissolved oxygen (A-7.5)		
1a	Winkler titration		
1b	oxygen electrode		
0	other		
	pH (A-7.6)		
1	pH electrode		
0	other		

Code	Method	subcode	
	total alkalinity *(A-7.7)*		
1	pH method		
2	Gripenberg method		
3	potentiometric titration		
0	other		
	nitrate (A-7.8)		**equipment:**
1	reduction method	1	automated
0	other	2	manual
	nitrite *(A-7.9)*		**equipment:**
1	sulphanilamide method	1	automated
0	other	2	manual
	ammonia *(A-7.10)*		**equipment:**
1	oxidation method	1	automated
2	indophenol method	2	manual
0	other		
	phosphate (A-7.11)		**equipment:**
1	molybdic acid method	1	automated
0	other	2	manual
	silicate *(A-7.12)*		**equipment:**
1a	bleu colour method	1	automated
1b	yellow colour method	2	manual
0	other		
	hydrogen sulphide *(A-7.13)*		
1a	methylene blue method		
1b	Lauth's violet method		
2	manganese method		
0	other		
	sulphate *(A-7.14)*		
1	turbidimetric method		
2	gravimetric method		
3	methylthymol method		
4	sulphide method		
0	other		

Code	Method
	dissolved ***organic carbon*** *(A-7.15)*
1	high temp. catalytic oxidation (Pt, CO_2)
2	high temp. catalytic oxidation (Cu, Ni, CH_4)
3	UV oxidation
4	dry combustion
5	wet chemical oxidation
0	other
	dissolved ***organic nitrogen*** *(A-7.16)*
1	persulphate oxidation
2	micro Kjeldahl nitrogen
3	UV oxidation
0	other
	(dissolved) ***total*** **carbohydrates** *(A-7.17)*
1	tryptophan method
0	other
	(dissolved) ***mono- & oligo*** **saccharides** *(A-7.18)*
1	HPLC
2	spectrophotometric method
0	other
	(dissolved) ***total*** **amino acids** *(A-7.19)*
1	fluorimetric method
0	other
	(dissolved) proteins *(A-7.20)*
1	brilliant blue method
2	fluorimetric method
0	other
	(dissolved) lipids *(A-7.21)*
1	GCMS
0	other

Physico-chemical variables: seston

Code	Method	subcode	
	suspended particulate matter (A-7.22)		
1	filtration		
2	centrifugation		
3	calibrated turbidity		
0	other		
	particle size (per size class) (A-7.23)		
1	conductometric analysis		
2	laser diffraction		
0	other		
	pigments (A-7.24)		
			extractant:
1	inverse HPLC	1	ethanol 90%
2	spectrophotometry	2	acetone 90%
3	fluorimetric analysis	3	methanol 90%
0	other	0	other
	particulate organic carbon (A-7.25)		
1	dry combustion		
2	persulphate oxidation		
3	spectrophotometry		
0	other		
	particulate organic nitrogen (A-7.26)		
1	dry combustion method		
2	total Kjeldahl nitrogen		
3	persulphate digestion		
0	other		
	particulate organic phosphorus (A-7.27)		
1	persulphate digestion		
2	sulphuric acid/peroxide digestion		
3	perchloric acid digestion		
0	other		

Physico-chemical variables: sediment

Code	Method
	clay content (A-7.28)
1a	pipette method, peroxide
1b	pipette method, no peroxide
2	Atterberg method
3	conductometric analysis
4	laser diffraction
0	other
	silt content (A-7.29)
1	wet sieving
2	conductometric analysis
3	laser diffraction
0	other
	grain size distribution (A-7.30)
1a	dry sieving
1b	wet sieving
0	other

Biological variables: water

Code	Method
bacteria	
	total numbers (A-7.31)
1	fluorescence microscopy
0	other
	production (A-7.32)
1	thymidine method
2	growth rate
0	other
phytoplankton	
	species abundance (A-7.33)
1a	microscopy, after concentration
1b	microscopy, without concentration
0	other
	production (A-7.34)
1a	^{14}C method, incubator
1b	^{14}C method, *in situ*
2	oxygen determination method
0	other
	biomass (A-7.35)
1*	conversion from chlorophyll data
2a*	conversion from cell counts and cell measurements
2b*	conversion from coulter counter data
0	other
zooplankton	
	species abundance (A-7.36)
1	microscopic analysis
0	other
	stage distribution (key species only) (A-7.37)
1	microscopic analysis
0	other
	individual stage weights (Copepods only) (A-7.38)
1a	dry-weight, preserved
1b	dry-weight, fresh or deep froozen samples
0	other
	biomass (A-7.39)
1	dry-weight
2*	conversion from lengths
3*	conversion from organic carbon content
0	other

Biological variables: sediment

Code	Method
micro-phytobenthos	
	species abundance *(A-7.40)*
1	tissue technique
2	density gradient
0	other
	production *(A-7.41)*
1	^{14}C tracer technique, *in situ*, bell jar
2a	^{14}C tracer technique, sample, *in situ*
2b	^{14}C tracer technique, sample, incubator
3	oxygen determination, *in situ*, bell jar
4a	oxygen determination, sample, *in situ*
4b	oxygen determination, sample, incubator
0	other
	biomass *(A-7.42)*
1	C/Chl-a ratio calculation
0	other
macro-phytobenthos	
	species abundance *(A-7.43)*
1	visual inspection
0	other
	biomass *(A-7.44)*
1	dry-weight
2	wet-weight
0	other
meiobenthos	
	species abundance (major taxa) *(A-7.45)*
1	microscopic analysis
0	other
	biomass *(A-7.46)*
1a	dry weight, preserved samples
1b	dry weight, fresh/deep frozen samples
2a	ash-free dry weight, preserved samples
2b	ash-free dry weight, fresh/deep frozen samples
3a	blotted wet-weight, preserved samples
3b	blotted wet-weight, fresh/deep frozen samples
4*	volumetric based conversions
0	other

macro-zoobenthos

species abundance (A-7.47)

1	visual inspection
0	other

age distribution (selected species) (A-7.48)

1	growth marks
2	frequency distribution
0	other

biomass (A-7.49)

1	ash-free dry weight
2	dry-weight
3	blotted wet-weight
4	conversion from body lengths
5	conversion from body volume
0	other

Annex IV

Variable list sorted by abbreviation as used in the JEEP-92 database

(de Winter, 1992a)

Abbreviation	*Variable*
ALK	Total Alkalinity
BANR	Number of Bacteria
BAPR	Bacteria Production
CHLA	Chlorophyll-a
CHLB	Chlorophyll-b
CHLC	Chlorophyll-c
CHLX	Other Pigments
CL	Chlorinity
CLAY	Clay content
DLIP	Dissolved Lipids
DMOS	Dissolved Mono/OligoSaccharides
DOC	Dissolved Organic Carbon
DON	Dissolved Organic Nitrogen
DPRO	Dissolved Proteins
DTAA	Dissolved Total Amino Acids
DTCH	Dissolved Total CarboHydrates
FBBM	Phytobenthos Biomass
FBPR	Microphytobenthos Production
FBSP	Microphytobenthos #/Species
FPBM	Phytoplankton Biomass
FPPR	Phytoplankton Production
FPSP	Phytoplankton #/Species
H2S	Sulphide Concentration
ISFL	In-situ Fluorescence
ISO2	In-situ Oxygen
ISPH	In-situ pH
ISS	In-situ Salinity
ISTP	In-situ Temperature
ISTU	In-situ Turbidity
MBBM	Meiobenthos Biomass

MBSP	Meiobenthos #/Major Taxa
MCAD	Macrozoobenthos Age Distribution
MCBM	Macrozoobenthos Biomass
MCSP	Macrozoobenthos #/Species (ST)
MFBM	Macrophytobenthos Biomass AFDW
MFSP	Macrophytobenthos #/Species
NH4	Ammonia Concentration
NO2	Nitrite Concentration
NO3	Nitrate Concentration
O2	Dissolved Oxygen
PART	Particle Size (per size class)
PH	pH
PHAE	Pigments Degradation
PLIP	Particulate Lipids
PMOS	Particulate Mono/Oligosaccharides
PO4	Phosphate Concentration
POCB	POC in Sediment
POCW	POC in Seston
PONB	PON in Sediment
PONW	PON in Seston
POPB	POP in Sediment
POPW	POP in Seston
PPRO	Particulate Proteins
PTAA	Particulate Total Amino Acids
PTCH	Particulate Total Carbohydrates
S	Salinity
SECH	Light Penetration (Secchi depth')
SGSZ	Sediment Grain Size
SILT	Silt + Clay content
SIO4	Silicate Concentration
SO4	Sulphate Concentration
SPM	Suspended Particulate Matter
TEMP	Temperature
TURB	Turbidity
ZPBM	Zooplankton Biomass
ZPSD	Zooplankton Stage Distribution
ZPSL	Zooplankton Stage Lengths
ZPSP	Zooplankton #/Species
ZPSW	Zooplankton Individual Stage Weights

Annex V
Organisation of the JEEP92 database

(from: De Winter, 1992a)

ENTITY	FIELDS	EXPLANATION	REMARKS
estuary:	name	name of the estuary	
	country	country in which estuary is mainly situated	already known to the program for most estuaries
station:	reference name	short reference name (10 char.)	
	full name	a more descriptive name	
	geographical position	degrees, minutes, seconds	
	depth at MTL	water depth; may be <0 for tidal flats	
	tidal range	mean tidal range	
	% emersion time	mean percentage of time the station is not covered with water	
sample event:	station reference name	(short) name of the sampling station	
	date	sampling date	
	(starting) time	time start of sampling	all times: LOCAL TIME
	end time	time when time cycle stopped	only when different from start time in case of time cycles
	next high tide	time of the next high tide	may exceed 24h. if on the next day
observation:	sample reference	reference number	generated by the program
	parameter	parameter code	generated by the program
	value	result value	
	nr. of replicas; std. dev.	number of repeats and standard deviation	only when more than 1 replica
	time	actual sampling time	only when deferring from the sample's time
	depth	sampling depth either in the water or in the sediment	
	class	for classed observations, e.g. sediment grain size classes	optional
	method code	refer to the manual	
	responsible scientist & institute	the person to be referred to when using the data, or to make inquiries to	

Annex VI
Conversion factors for the calculation of biomass of phytoplankton

(after Strathmann, 1967)

For phytoplankton (other than diatoms):

$$\log C \equiv 0.866 \log V - 0.460$$

where C is the carbon per cell (in pg) and V is the cell volume (in μm^3).

For diatoms:

$$\log C \equiv 0.758 \log V - 0.422$$

where C is the carbon per cell (in pg) and V is the cell volume (in μm^3).

Annex VII

Conversion factors for the calculation of biomass of zooplankton

(after Parsons *et al.* 1984)

For mixed zooplankton samples containing more than 90% copepods the following relation is proposed:

$$\text{mg C} \equiv 0.49 * \text{mg (dry-weight)} - 5.19$$

ANNEX VIII
Conversion factors for the calculation of biomass of meiobenthos

(after Feller & Warwick, 1988)

- Simple approximation of the volume (V, in nl) from length (L, in µm) and width (W, in µm):

$$V = L * W^2 / 16.10^5$$

- Approximate conversion factors (C) to estimate the volume V (in nl) from the maximum width (W, in mm), total length (L, in mm) according to the formula:

$$V = L * W^2 * C$$

where C is defined:

taxon	C
Nematodes	530
Ostracods	450
Mites	399
Kinorhynchs	295
Turbellarians	550
Gastrotrichs	550
Tardigrades	614
Hydroids	385
Polychaetes	530
Oligochaetes	530
Tanaids	400
Isopods	230

- To convert volume (V, in nl) to dry-weight (in µg) multiply by specific gravity (e.g. 1.13) and dry-weight wet-weight ratio (e.g. 0.25)

Annex IX

Mathematical description of the estimation of weight from length regressions (after Baskerville, 1972)

Estimating the (mean) weight from length measurements:

1. Perform the length-weight regression according to the assumption:

$$\left.\begin{array}{llll} w = c\cdot l^{a} \Leftrightarrow & \ln(w) & = & \ln(c) + a\cdot\ln(l) \\ \text{say:} & b & = & \ln(c) \end{array}\right\} \Rightarrow \ln(w) = a\cdot\ln(l) + b$$

with the data

$$\begin{array}{cccc} l_1 & w_1 & \ln(l_1) & \ln(w_1) \\ l_2 & w_2 & \ln(l_2) & \ln(w_2) \\ \vdots & \vdots & \vdots & \vdots \\ l_n & w_n & \ln(l_n) & \ln(w_n) \end{array}$$

by calculating the following additions and coefficients of the linear regression Y = a.x + b where Y=ln(*w*) and X=ln(*l*):

$$\left.\begin{array}{l} \sum x^2 = \sum X^2 - \dfrac{(\sum X)^2}{n} \\ \sum y^2 = \sum Y^2 - \dfrac{(\sum Y)^2}{n} \\ \sum xy = \sum XY - \dfrac{\sum X \sum Y}{n} \end{array}\right] \Rightarrow \begin{array}{l} a = \dfrac{\sum xy}{\sum x^2} \\ b = \dfrac{1}{n}\cdot(\sum Y - a\cdot\sum X) \\ s_{xy}^2 = \dfrac{1}{(n-2)}\cdot(\sum y^2 - \dfrac{(\sum xy)^2}{\sum x^2}) \end{array}$$

2. To estimate the weight from lengths, use the regression coefficients to calculate the uncorrected logarithmic weight $\hat{\mu} = \ln(w)$:

$\hat{\mu} = a \bullet \ln(l) + b$

then calculate the estimated weight and its variance by taking the antilog and correcting it for the use of logarithms in the regression:

$$\hat{w} = e^{(\hat{\mu} + \frac{s_{xy}^2}{2})}$$

$$\hat{\sigma}_w^2 = e^{(2\cdot s_{xy}^2 + 2\cdot\hat{\mu})} - e^{(s_{xy}^2 + 2\cdot\hat{\mu})}$$

from these estimated weights the mean weight and its standard deviation can be calculated:

$$\left.\begin{array}{ll} \hat{w}_1 & \hat{\sigma}_{w_1}^2 \\ \hat{w}_2 & \hat{\sigma}_{w_2}^2 \\ \vdots & \vdots \\ \hat{w}_m & \hat{\sigma}_{w_m}^2 \end{array}\right] \Rightarrow \begin{array}{l} \overline{w} = \dfrac{\sum_{i=1}^{m} w_i}{m} \\ \text{s.d.}\overline{w} = \sqrt{\dfrac{\sum_{i=1}^{m} \hat{\sigma}_{w_i}^2}{m^2}} \end{array}$$

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