Water quality — Guidance on marine biological surveys of hard-substrate communities

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ICS 13.060.10; 13.060.70

British Standards

National foreword

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This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

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Water quality - Guidance on marine biological surveys of hard-substrate communities (ISO 19493:2007)

Qualité de l'eau - Lignes directrices pour les études biologiques marines des peuplements du substrat dur (ISO 19493:2007)

Wasserbeschaffenheit - Anleitung für meeresbiologische Untersuchungen von Hartboden-Lebensgemeinschaften (ISO 19493:2007)

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Foreword

This document (EN ISO 19493:2007) has been prepared by Technical Committee CEN/TC 230 "Water analysis", the secretariat of which is held by DIN, in collaboration with Technical Committee ISO/TC 147 "Water quality".

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by December 2007, and conflicting national standards shall be withdrawn at the latest by December 2007.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

INTERNATIONAL STANDARD

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Water quality — Guidance on marine biological surveys of hard-substrate communities

Qualité de l'eau — Lignes directrices pour les études biologiques marines des peuplements du substrat dur

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 19493 was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 230, *Water analysis*, in collaboration with Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Introduction

Surveys of benthic marine algae and fauna on hard substrates represent an important part of marine environmental surveys. The species composition, both in terms of the species present and their relative abundances, is a result of the natural and anthropogenic environmental factors at the survey site. Natural factors that influence species composition include wave exposure, depth, salinity, nutrient level, type of substrate, slope, orientation, turbidity, current, temperature and grazing. Anthropogenic factors include pollution (e.g. oil, contaminants, particles), physical disturbance, elevated nutrient levels and effects from fisheries.

A number of different methods are being used to investigate flora and fauna on hard substrate according to the survey aim and the type of biotope surveyed. To allow environmental authorities and others to make use of this knowledge, it is essential that surveys are intercomparable in time and space, as well as between operators, and that the data are of a high quality. This International Standard is based on a limited selection of methods that allow precise documentation, that are replicable and which have been tested over many years. In choosing methods for this standard, semi-quantitative and quantitative techniques have been emphasized, such that species and quantities can be related to a known area of sea floor.

For the purposes of this International Standard, hard substrate is defined as bedrock, stable rocks and fixed marine constructions (e.g. pipelines and quays). The main focus is on biological surveys based on species that can be recorded in the field (i.e. that are visible to the naked eye).

The guidelines are applicable to seagrass communities and their epiflora and epifauna. They can also be used for surveys of stable substrates comprising loose pebbles/boulders, stone blocks, coarse gravel and other loose material as well as bedrock covered with loose sediment, but in general, such substrates require specially adapted techniques. Additional methods are usually required for surveys in depths greater than approximately 30 m.

For sediment sampling in marine areas, refer to ISO 5667-19. For surveys of sublittoral soft-bottom fauna, see ISO 16665.

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Water quality — Guidance on marine biological surveys of hard-substrate communities

1 Scope

This International Standard provides guidance for marine biological surveys of supralittoral, eulittoral and sublittoral hard substrate for environmental impact assessment and monitoring in coastal areas.

This International Standard comprises

— development of the sampling programme,

— survey methods,

— species identification, and

— storage of data and collected material.

This International Standard specifies the minimum requirements for environmental monitoring.

The methods are limited to surveys and semi-quantitative and quantitative recording techniques that cause little destruction of the fauna and flora. In practice, this refers to direct recording in the field and photography. Sampling by scraping off organisms, use of a suction sampler, etc. are not covered in this International Standard, but such techniques can be used as a supplement to obtain information on small-sized species or those that live hidden.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
area of influence
area influenced or expected to be influenced, based on the available information

2.2
biotope
area of uniform environmental conditions (habitat) and its characteristic assemblage of plant and/or animal species

EXAMPLE *Laminaria hyperborea* community (cuvie or tangleweed), knotted wrack community, blue mussel belt.

2.3
macroscopic organisms
algae and animals that are visible without magnification equipment ($\geqslant$ 1 mm) and which can be recorded in the field

NOTE Certain macroscopic organisms can require microscopic inspection for identification. For microscopy of collected material, the lower size limit is set to 1 mm.

2.4
hard substrate
substrate consisting of bedrock, larger rocks/stones or fixed marine constructions such as wharfs, quays and pipelines

NOTE For the purpose of this International Standard, hard substrate can also include other substrates that are not likely to be moved or turned over during a reasonable time period so that perennial species communities are likely to develop (e.g. pebbles and stones in sheltered environments).

2.5
hard substrate flora and fauna
attached algae and animals, together with relatively stationary animals living on or in close association with hard substrate

EXAMPLE Attached: kelp, seaweeds, sponges, bryozoans, corals, mussels, barnacles, ascidians. Relatively stationary: snails, sea-urchins, crabs.

2.6
supralittoral zone
zone above the eulittoral zone, which is reached by spray water

NOTE Its upper limit is normally determined by the upper limit of *Verrucaria* (black lichen belt), blue-green algae (usually *Calothrix scopulorum*) or littorinid snails.

2.7
eulittoral zone
marine intertidal zone which is submersed and emerged, either periodically due to tides or aperiodically due to irregularly occurring factors, as in the enclosed seas of the Baltic or the Mediterranean

NOTE Biologically, this zone is defined as the zone between the upper limit of barnacles and the upper limit of laminarians. In the Baltic where there is no tide, the eulittoral zone is the zone of short-lived annual algae.

2.8
sublittoral zone
zone below the eulittoral zone, which is submersed with the upper part at extreme low water levels occasionally emerging

NOTE 1 In this International Standard, the lower limit is set by the deepest occurring algae.

NOTE 2 This is also referred to as the subtidal zone.

NOTE 3 Biologically, this zone is defined as the zone between the upper limit of laminarians and the lower limit of algal vegetation (see Annex C).

2.9
level of exposure
level of wave and current exposure at a site

2.10
receiving water body
water body which receives an input of material, of either natural or anthropogenic origin

NOTE The term often appears in the context of contamination (for example effluent from municipal waste water outlets or industrial processed water). Receiving water body surveys describe the state of contamination in a given area.

2.11
sampling station
precise location where recording is carried out and any samples collected

NOTE A sampling station is defined by its geographical position (OS National Grid Reference, latitude, longitude), its depth (relative to chart datum and normalized to mean low water as given in tide tables) and any other information on physical conditions (e.g. substrate type, slope and orientation).

2.12
reference station
one or more sampling stations chosen to represent environmental conditions in a given area, i.e. free from direct anthropogenic influences

2.13
baseline survey
survey with emphasis on characterization and description of biotic and abiotic conditions in the survey area, and which forms the basis for future monitoring and/or follow-up surveys

2.14
temporal trend monitoring
surveys of the hard substrate community in response to temporal changes in chemical and/or physical conditions in the surrounding waters to document either pollution or natural variation over time

2.15
ROV
Remotely Operated Vehicle
remote-controlled underwater vehicle with video camera and often the possibility for mounting additional equipment such as sonar, manipulator arm, etc.

3 Quality and safety

3.1 Health and safety requirements

3.1.1 General

All phases of hard substrate field work and sample processing should adhere strictly to national and international health and safety regulations. The main points are listed below.

3.1.2 Laboratory safety facilities

A valid health and safety manual should be freely available in the institute or laboratory and the appropriate first aid supplies and emergency facilities (such as an eyewash station and a shower) should be installed. The laboratory and storage areas should further be equipped with point-ventilation outlets and preferably have a monitor for chemical levels in the air.

3.1.3 Requirements for diving

For surveys that require diving, this should be carried out in accordance with the appropriate rules and regulations. The diver and others participating in the diving work are required to follow the national or international regulations implemented for the prevention of accidents and health risks. Diving and communication equipment should fulfil the relevant requirements. A guide to planning and carrying out scientific diving operations is given in Reference [2]. Persons participating in diving should have the necessary certificates and official approval from the national authorities.

NOTE See also European Standards for Scientific Diving (European Scientific Diver and Advanced European Scientific Diver) http://www.soc.soton.ac.uk/OTHERS/SDSC/ESD_AESD%20Standards.pdf.

3.1.4 Field safety

Work should not be undertaken alone. Risk assessments should be addressed for the specific locations where a survey is being undertaken.

All personnel collecting and handling samples should be given training in the appropriate health and safety procedures and, where in force, have attained certification status. Refresher training should be carried out regularly. Staff should be trained in assessing risk to personnel or equipment and should follow any documented procedures.

3.1.5 Handling of chemicals

Chemicals used for fixing or preserving samples should be stored and handled with proper precautions according to health and safety regulations. Non-drip dispensers should be used for liquid chemicals.

Common chemicals used in hard substrate samples include the fixative formalin or substitutes, the preservative ethanol and biological stains.

WARNING — Formalin is particularly hazardous to health, and prolonged or intense exposure can cause long-term allergies. A number of less hazardous, but expensive, alternatives to formalin are available and should be used where possible, especially when dealing with small sample volumes.

3.2 Quality assurance and quality control

3.2.1 General

Quality assurance and quality control measures should be incorporated during all stages of marine biological surveys and sample processing programmes. These principles help to guarantee that all data produced are of a specific quality and that all parts of the work are carried out in a standardized and intercomparable manner. All procedures should therefore be clearly described and carried out openly, such that all of the laboratory's activities can be audited internally and externally at any time (see EN 14996).

NOTE The overall aim is to assure traceability and full documentation of field registration procedures, samples and equipment from beginning to end.

National and/or international accreditation should be sought if appropriate; this is required for most commercially operated laboratories. Guidance from relevant accreditation bodies should be sought in developing specific in-house quality systems, work procedures and protocols. It is recommended that the laboratories participate in intercomparative tests or learning schemes to develop expertise and maintain appropriate skills. This ensures continued standardization and reproducibility of results.

3.2.2 Scientific requirements for personnel

The surveys should be carried out by appropriately qualified personnel (marine zoologists/marine botanists). They should be able to document competence in their specialist field and regular structured training and participate in ring-testing. For surveys spanning several years, priority should be given to continuity in personnel carrying out the recordings.

4 Strategies and objectives for hard-substrate surveys

4.1 Sampling programme and plan

The sampling programme should be developed according to the individual aims of the survey, the required precision of the results, local topographical and hydrographical conditions in the survey area, information about local pollution sources, knowledge from previous surveys and any other conditions that can be of importance for the survey. For guidance on the design of sampling programme, see ISO 5667-1. The sampling programme should be determined before the survey is initiated, but appropriate adjustments may be made in the field, particularly for a pilot survey (see 4.3.2).

Surveys in partly eulittoral and sublittoral zones require different equipment and techniques. Eulittoral zone surveys are the easiest to carry out and require the least resources since surveys can normally be undertaken at low tide. Surveys in the sublittoral zone usually involve SCUBA-diving or different ROV-techniques. This International Standard describes a limited variety of different eulittoral and sublittoral survey methods. A detailed description of the recording methods is given in Annex A.

NOTE Detailed guidance on sampling programmes and methods is also given in the Marine Monitoring Handbook (http://www.jncc.gov.uk) and the Swedish EPA (http://www.naturvardsverket.se/upload/02_tillstandet_i_miljon/Miljoovervakning/undersokn_typ/hav/vegbotos.pdf).

4.2 Positioning of sampling stations

4.2.1 General

Sampling stations should be positioned according to the particular aims of the individual survey, previous surveys in the area and local topographical and hydrographical conditions. The hard-substrate community structure depends on wave exposure, type of substrate, compass direction, bottom slope, water depth and salinity. This shall be taken into account when locating the sampling and reference stations and when comparing different areas and localities.

The sampling stations should preferably be placed in areas of hard rock or other stable substrate. Areas with heterogeneous bottom conditions are difficult to record accurately and should be avoided. Particular attention should be given to station positioning in areas exposed to fresh water seeps, desiccation, ice scouring or other factors providing highly unstable conditions.

When surveys are made over time, the stratum (or strata) of the observations should be kept constant (fixed site and depth) to minimize structural variation as the temporal variation is the only one of interest. Biotopes that have little natural variation in species composition over time are best suited for temporal trend monitoring [e.g. perennial communities like the knotted wrack community (*Ascophyllum nodosum*)].

For environmental descriptions and temporal trend monitoring, a pilot survey should be carried out before sampling station positioning.

4.2.2 Strategies for locating sampling stations

Sampling stations can be located according to one or a combination of the following strategies.

— **Random sampling**. In special circumstances, sampling stations may be positioned randomly or scattered. For example, random sampling is used when no previous knowledge of the area is available as a guide to appropriate stratification, or when an unbiased value for a whole area is desired.

— **Stratified random sampling**. Based on *a priori* subdivision of the study area. The subdivisions may be delineated according to depth, substrate type, wave exposure or other factors. This ensures that all the main habitat types present on a site will be sampled and is the recommended strategy in most cases.

— **Systematic/grid sampling**. Sample stations are arranged in a regular grid-like pattern. This arrangement is appropriate for pilot surveys and for estimations of spatial pattern/extent, for instance, the zone of influence around point source discharges. The survey area should be of topographic homogeneity.

— **Gradient sampling**. Sampling stations are arranged along a known or anticipated gradient of interest. This is applicable, for instance, to trace the influence of a known pollution source.

4.2.3 Fixed sampling points

To reduce random variability when temporal changes are to be monitored, fixed sampling points can provide an effective approach.

NOTE Fixed sampling points provide a very precise measure of change and are useful for monitoring rare sessile species that are only known from specific locations. There are, however, a number of significant disadvantages to using permanent sampling points: they may be unrepresentative of the biotope/area as a whole and repeated monitoring may damage the site. In addition, it may be time-consuming to relocate fixed sampling points.

4.2.4 Reference station

For surveys carried out in affected/contaminated areas or those believed to be affected/contaminated, reference stations should be chosen outside the influenced area. The reference stations should, as far as possible, be representative of conditions unaffected by effluent sources and allow assessment of natural, temporal and spatial variation in the hard-substrate community. The reference stations should be comparable with the ordinary stations in natural conditions (similar level of wave-exposure, salinity, depth, substrate, slope and similar position in the inner, middle or outer coastal zone). The surveys at the various stations should be carried out at the same time of the year and using the same methodology.

Statistical considerations and the required precision of results dictate the number of reference stations.

4.3 Types of surveys

4.3.1 General

In receiving water bodies where there are expected effects in the surface water only and in shallow areas, the surveys should be carried out in the supralittoral and eulittoral zones (see Table 1). Examples of these are the receiving water bodies of small domestic and industrial effluent as well as aquaculture activities. In these cases, the effluent is either released directly or indirectly into the surface water.

In receiving water bodies with extensive sewage and waste-water effluents, other large-scale effluents, below fish cages and in cases where new constructions can alter the current and sedimentation conditions, there are additional requirements for sublittoral surveys (see Table 1).

Surveys in the eulittoral and sublittoral zones can be divided into three levels according to the objectives and required precision of results.

4.3.2 Pilot survey

This is an initial assessment of substrate conditions and hard-substrate flora and fauna (biotopes and characteristic species). The survey allows a coarse assessment of environmental conditions and may provide the basis for development of a more comprehensive recording and sampling programme. The survey is not suitable to record changes over time, with the exception of large-scale changes in dominant species. The requirements for sampling methodology and repeatability are usually relatively simple.

The survey is carried out by visual inspection. In the sublittoral zone, diving, a submerged video camera or a ROV may be used. There are no fixed requirements for positioning of sampling stations and the number of stations to be sampled for a pilot survey, but the stations should, as far as possible, cover the area to be described. Accurate sampling station positions, slope, tidal conditions and substrate type should be described. The wave and current exposure at the site should also be categorized and noted (see Annex E).

When pilot surveys are used as a basis to design the size and calculate statistical power for future monitoring programmes, it is desirable to have the pilot study resemble the planned monitoring programme as much as possible in terms of the spatial and temporal arrangement of samples.

4.3.3 Baseline survey

This is a detailed survey carried out to characterize the environmental conditions in the area in question. The survey may provide the basis for remedial measure or preventative action against pollution. Within the scope of this type of survey, the number of species, species composition and dominance relationships within the hard-substrate community should be recorded. The survey is carried out using semi-quantitative and/or quantitative methods, and there are specified requirements for the number of sampling stations and station positioning. The minimum requirements are given in Table 1.

In receiving water bodies for industrial and domestic effluents and aquaculture activities, the sampling stations should be positioned within the area influenced by the effluents, or which is expected to be influenced, based on the available information on the amount of effluent, current conditions and topography. The reference stations should be positioned outside the expected area of influence and should represent the natural variations in the survey area. For surveys that form the basis of remedial action, or where even slight changes in conditions can be important, more than the minimum number of stations or the use of quantitative methods may be required to increase the precision of the results. If the affected area is large or very heterogeneous, it may be necessary to increase the number of sites/stations.

In areas where possible changes in environmental conditions are to be monitored, e.g. the effects of remedial measures in polluted receiving water bodies, or in areas where activities are established that may affect the environment, a baseline survey should first be carried out with recordings over at least two consecutive years. Follow-up surveys should be carried out using the same methodology at all or a selection of the sampling stations.

4.3.4 Temporal trend monitoring

This is a survey that describes the hard-substrate community over time in order to document the natural variation and any gradual changes. A high quality of documentation and replicability is required. The surveys should be carried out using standardized methodology following an established programme. The number of sampling stations necessary to detect a trend depends on the variability within stations (temporally and spatially), effect-size and desired statistical power.

If subtle changes are to be detected in a heterogeneous area or information is needed on turnover and species dynamics, fixed sampling points can be used.

The survey should be carried out using semi-quantitative or quantitative methods. Methods should be chosen to ensure that the data can be used as a comparative basis for a baseline survey in surrounding areas. An overview of the minimum requirements for the methodology is given in Table 1.

Temporal trend monitoring can also comprise surveys of the occurrence/distribution of individual species over time. For such surveys, other methods than those described in this International Standard should be used.

Table 1 — Minimum requirements for surveys on hard-substrate according to the main aim of the survey

	Main aim	Minimum requirements for surveys in the eulittoral zone (shore) [a]	Minimum requirements for surveys in the sublittoral zone [a]
1	**Pilot survey** Used for simple rapid assessment or to give basis for designing a more detailed sampling programme.	Inspection with listing of characteristic species/biotopes.	Survey with listing of characteristic species/biotopes (diving, video, ROV, random stereo-photography).
2	**Baseline survey** Used for characterizing environmental conditions in a given area. Also maps/identifies the impact of point-source discharges.	**Areas with mean tidal range < 0,5 m:** Semi-quantitative registrations at minimum 5 assumed affected sampling stations. Number of reference stations should be balanced to stations of interest (A.1.1). **Areas with mean tidal range > 0,5 m:** Quantitative quadrat registration at minimum 5 assumed affected sampling stations. Number of reference stations should be balanced to stations of interest (A.2.1).	Semi-quantitative transect diving at minimum 2 sampling stations (A.1.2). Reference stations should be balanced to stations of interest (see 4.2.3). Registration of lower limit of algae vegetation (A.1.3).
3	**Temporal trend monitoring** Describes gradual changes in flora and fauna over time, either for detecting change in biodiversity or as applied to environmental conditions. Reference stations appropriate only if monitoring effluent impact.	**Areas with mean tidal range < 0,5 m:** Semi-quantitative registrations within fixed marked areas at minimum 5 stations (A.1.1). NOTE For temporal trend monitoring, it should be aimed for quantitative analyses also in areas with low tidal range. **Areas with mean tidal range > 0,5 m:** Quantitative quadrat registration at fixed marked areas at minimum 5 stations (A.2.1).	Semi-quantitative transect diving along marked, fixed transects at minimum 2 sampling stations (A.1.2). Registration of lower limit of algae vegetation (A.1.3). In addition, one of the following techniques should be used: – quadrat *in situ* registration (3 random replicates) (A.2.2); or – quantitative recording, e.g. using photography or video (A.2.3).
NOTE Other methods can be used for temporal trend monitoring of individual species or groups of species.			
[a] The actual number of sampling stations needed is dependent on the size of the survey area and the precision of the results. The methods (A.1 to A.2) are described in Annex A.			

5 Sampling

5.1 Defining the position of sampling stations

The position of the sampling stations for environmental descriptions and temporal trend monitoring should be defined unambiguously, such that other operators can relocate it. Positions should be defined using geographic coordinates with reference to the local geodetic system in use. Positions should be defined according to the relevant guidelines.

In addition to geographic coordinates, sampling stations may also be defined using characteristic landmarks and at least one fixed reference point or easily identifiable point immediately above the supralittoral zone. For relocation of sublittoral sampling stations, the depth and compass direction from the reference point should be given. For intertidal and shallow subtidal survey points, the distance and approximate direction from a fixed feature should be given. A tape measure can then be used to relocate the site. The level on the shore can be given in relation to zone-forming organisms, but should also be given according to chart datum.

NOTE 1 Chart datum is the height of water at the lowest possible theoretical tide, also known as the lowest astronomical tide (LAT). Calculation of the LAT only allows for gravitational effects so lower tides may occur in practice due to other factors (e.g. meterological effects, such as high-pressure systems). Tide tables give the height of the tide above chart datum. This makes it possible to calculate the depth of water at a given point and a given time.

In addition, station positioning should be documented by appropriate maps and by photography. The photography of the stations should be kept in a central image library at the institute carrying out the survey and be included in the reports where the stations are first introduced.

For temporal trend monitoring and in the case of larger oil spills, the survey area should be marked by fixed bolts in the rock. The upper left bolt should be the reference bolt and should be defined by the distance to the fixed reference point above the supralittoral zone.

NOTE 2 In practice, it can be extremely difficult to relocate bolts/holes amongst the algal vegetation. This is particularly a problem if the bolts are lost or if later follow-up surveys are carried out by new personnel.

5.2 Timing of the survey

Surveys on hard substrate are often carried out when diversity is highest and/or an impact of ephemeral algae can be expected. However, monitoring at different times of year may be necessary, depending on the scope of the survey. For temporal trend monitoring, the surveys should be carried out at the same time of year, each year. For temporal trend monitoring that already has been established, continuity should be given higher priority over the requirements for timing of the survey.

5.3 Recording of information

The following information should, as a minimum, be recorded in the field (see Annex B):

- project or contract identification code;
- person or institute responsible for the recording/sampling;
- field personnel;
- sample station identification code and geographical coordinates for each sampling station;
- date and time (start – stop);
- depth of samples (relative to chart datum and normalized to mean low water as given in tide tables);
- type of survey and methodology used (see Annex A);
- substrate types – describe the type (rock, boulder, stone, gravel, sand, etc.) and estimate the coverage; sediment deposits and other loose material on hard substrate should also be noted;
- bottom slope (for diving surveys, a bottom profile is made using slope and depth);
- type of locality (fjord – skerries – outer coast);
- station orientation;

— wave and current exposure [subjective assessment (weak – moderate – strong)]; in special circumstances, a more precise theoretical measure of wave exposure can be calculated (see Annex E);

— estimated recording conditions (% cloud cover, wind, visibility in the water, light conditions);

— horizontal limits at the site (for supralittoral/eulittoral surveys) described in words, in diagrams or by photography;

— positioning of any fixed sampling quadrats in relation to the fixed reference point;

— positioning of stations and survey areas for sublittoral surveys, including the transect route, described by giving the compass direction from a fixed reference point and by depth;

— reference to this International Standard.

Depending on the type of survey, the following useful additional parameters may be recorded:

— salinity;

— water temperature;

— secchi depth;

— nutrients;

— oxygen;

— current conditions;

— air pressure;

— tidal phase;

— ice scouring;

— drifting algae mats;

— reference to any other surveys of the area.

5.4 Sample handling

Surveys covered by this International Standard mainly use non-destructive methods which make it possible to carry out repeated surveys of the same area. Sampling should be carried out with appropriate equipment to minimize destruction and damage to the biological communities. If possible, the sampler should collect representative material from outside the marked area. Species to be identified by microscopy should be collected and kept alive or fixed for analysis in the laboratory.

Fauna should be fixed in 10 % to 20 % buffered formalin in seawater and subsequently preserved in 70 % ethanol.

Algae should be stored in 3 % to 5 % buffered formalin in seawater. They may also be stored as pressed herbarium specimens or in 70 % ethanol.

Formalin is buffered with borax ($Na_2B_4O_7 \times 10\ H_2O$) which is added until a pH $>$ 7 is obtained.

For the preservation and handling of samples, see ISO 5667-3. For more detailed information on algae collection and preservation, see the websites http://www.nmnh.si.edu/botany/projects/algae/collpres.htm and http://www.botany.uwc.ac.za/clines/colpres.htm

NOTE For studies other than morphological studies, different fixation techniques are generally required.

All biological samples and image material should be archived for future use with a minimum of the following information:

— project identification or project code;

— sampling station code;

— sampling date;

— collector/photographer;

— depth (relative to chart datum and normalized to mean low water as given in tide tables).

When photographing the survey area, the length and colour scales, together with the station code should be clearly visible.

6 Taxon identification and sample processing

6.1 Taxon identification

Flora and fauna should be determined to the most exact taxonomic level as possible (usually “species”), or to that appropriate to the aim of the survey. The identification literature shall be cited.

Nomenclature should follow authorative taxonomic lists of species.

EXAMPLE Useful online taxonomic lists include The European Register of Marine Species (http://www.marbef.org/data/erms.php), Algaebase (http://www.algaebase.org), Species 2000 (http://www.sp2000.org) and Integrated Taxonomic Information System (http://www.itis.gov).

6.2 Reference collection/herbarium

The institute/laboratory shall compile and maintain a reference collection/herbarium of all species sampled. Interlaboratory validation of the reference collection is recommended. Wherever possible, relevant taxonomic experts such as museum personnel should be asked to check specimens that are difficult to determine.

At least one individual of each species should be kept as a pressed specimen (algae), in buffered formalin in seawater (see 5.4) or in 70 % ethanol (algae, animals) in separate vials. The collection should include, as a minimum, the following information:

— species name (binomial), including author(s);

— name of collector, name of identifier and name of verifier;

— where collected, including latitude and longitude if possible, station code;

— depth, substratum type, how it was collected (SCUBA, dredge, etc.);

— date of collection, see ISO 8601;

— any nomenclatural change(s).

The collection should be updated continually as a part of all ordinary surveys and be easily available for users. Particular attention should be paid to the sealing of the vials. For particularly important specimens, the "double-vial" practice carried out by museums is recommended. The reference collection and associated species-in-situ photographs also serve as part of the quality control procedures.

NOTE If the institute/laboratory is going to be closed, arrange for the safekeeping of the reference collection/herbarium.

6.3 Data storage and reporting

All raw data should be stored electronically to enable effective data retrieval, compilation and presentation. Output from the database should be in a suitable format for further statistical treatment such as diversity calculations, dominance indices and multivariate analyses, and also linkable to background parameters.

The species lists should reflect all the species recorded at the samplings. Species not quantitatively represented in the samples should be marked as such in the taxon lists. Species recorded in the field as a group should also be treated as a group in the subsequent data treatment.

The following information should be given, as a minimum, for scientific reporting:

- complete list of species/categories recorded [species name including author(s)];
- quantity of each taxon (as degree of coverage or abundance scale) for each sample;
- table or graphs showing the number of species found at each sampling station;
- table of the most abundant species at each sampling station;
- lower limit of vegetation (for sublittoral surveys) (see A.1.3).

Annex A
(normative)

Description of methods

A.1 Semi-quantitative surveys

A.1.1 Semi-quantitative surveys in the supralittoral and eulittoral zones (shore-survey)

Each site should have a horizontal width of at least 8 m. At each site, all attached algae (benthic algae including epiphytes) and attached or slow-moving animals together with the substrate type and slope should be recorded for each separate vegetation zone within the supralittoral and eulittoral zones.

Species should be recorded in terms of presence and degree of coverage (or number of individuals in the case of large solitary animals). The degree of coverage should be estimated according to a 5-part scale (see Table A.1). The survey should be continued until the habitat is thoroughly studied and the quantities of all prominent species are recorded.

The width of the dominant vegetation zones should be measured using a tape-measure stretched along the ground from the highest to lowest levels on the shore.

Flora and fauna should be identified to species or as near to species as possible. Taxa that cannot be identified in the field should be collected for later identification. Loose/drifting algae should not be included, but it is recommended to determine the degree of coverage and to identify the most important components of the drifting mats.

For practical reasons, the quantity of certain important groups of organisms, such as isopodes (Isopoda*)* and sandhoppers (Amphipoda), are not normally recorded in this type of survey, but their presence or absence should be recorded by roughly estimating their quantity (“single”, “rare”, “many”).

Particular biological characteristics are photographed.

For temporal trend monitoring, the positioning of the survey area should be documented photographically and/or marked with fixed bolts in the rock (acid-resistant bolts with expansion shaft or nylon bolts).

Table A.1 — Scale for estimating coverage in semi-quantitative surveys

Code	Coverage (algae and small animals) %
5	75 to 100
4	50 to 75
3	25 to 50
2	5 to 25
1	< 5
0	absent

A.1.2 Transect diving

For transect diving, attached algae (benthic algae including epiphytes) and attached or slow-moving animals, together with the substrate type and slope should be recorded along a minimum 4 m wide corridor from the supralittoral zone to a maximum depth of 30 m. At each recording depth, at least 2 m^2 of substrate should be recorded. The recordings should be carried out at every second metre depth except for within the depth range of 0 m to 3 m where recordings should be carried out at each metre depth. Survey methods may include the diver being in telephone contact with an assistant on land who notes the species and quantities observed or the use of direct recordings on waterproof preprinted forms. The degree of coverage should be estimated according to a 5-part scale (see Table A.1). Depth and time should be recorded in order to correct the depth in relation to chart datum. Taxa that cannot be identified in the field should be collected for later identification.

High-quality video recording may also be used in addition to *in situ* recording and collection.

For reasons of diving physiology and recording technicalities, the recordings should start at the deepest point of the transect.

A.1.3 Lower limit of vegetation

For recording the lower limit of algae vegetation, only perennial species should be used. The degree of coverage of the actual species should be at least 5 % at the site (code 2 in Table A.1) and individuals older that 1 year must be present.

The lower limit of algae vegetation should always be included in transect diving.

A.2 Quantitative surveys

A.2.1 Quadrat survey in the eulittoral zone

For quantitative surveys in the eulittoral zone, sample quadrats of 50 cm × 50 cm should be used. The quadrats are subdivided into at least 25 smaller squares (10 cm × 10 cm), each representing 4 % of the whole quadrat. Quadrats may be subdivided to give smaller squares, thus making counting more accurate. The number of squares filled by a single species can be easily counted and will give a percentage for the whole quadrat. The quantity of species (juvenile and adults) is recorded in percent (%) coverage of the entire quadrat and the coverage should be estimated with a precision of ± 5 %. Larger solitary animals should be recorded by the number of individuals.

A minimum of four randomly distributed quadrats should be placed at each of two to three levels in the eulittoral zone (upper and middle, or upper, middle and lower). The quadrats should be placed clearly within zones rather than at zone boundaries. Where channelled wrack (*Pelvetia canaliculata*) is present, the quadrats should be placed such that they cover the belt. The knotted wrack belt (*Ascophyllum nodosum)*, or equivalent height on the shore, should also be covered by one level.

Each site should have a horizontal width of 8 m. For temporal trend monitoring, each quadrat area may be marked with fixed bolts in the rock. The position of the quadrat on the shore should be photographed and the distance to at least one fixed point should be measured. Close-up pictures should be taken of all the quadrats.

In addition to quadrate sampling, the width of each of the vegetation zones should be measured with a measuring tape stretched along the ground from the upper to lower shores [i.e. the distance between the upper and lower limits for channelled wrack (*Pelvetia canaliculata*), spiral wrack (*Fucus spiralis*), bladder wrack (*Fucus vesiculosus)*, toothed wrack (*Fucus serratus)* or other dominant and zone-forming organisms].

Flora and fauna should be determined to species or as near to species as possible. Species that cannot be identified in the field should be collected for later identification. Crustose algae may be difficult to identify to species and quantify separately, and under these circumstances species may be grouped.

In heterogeneous areas, it may be necessary to increase the number of replicate quadrats such that the survey covers as many as possible of the species present.

Recordings in the field should be carried out at low tide.

A.2.2 Quadrat survey in the sublittoral zone

For *in situ* quantitative surveys in the sublittoral zone, sample quadrats of 50 cm × 50 cm should be used. The quadrats are subdivided into at least 25 smaller squares (10 cm × 10 cm), each representing 4 % of the whole quadrat. The number of squares filled by a single species can be easily counted and will give a percentage for the whole quadrat. The quantity of species is recorded in % coverage of the entire quadrat and the coverage should be estimated with a precision of ± 5 %. Larger solitary animals should be recorded by number of individuals.

The contents of at least three parallel quadrats should be recorded at least at one fixed depth. The randomly distributed replicates should be placed within the horizontal border of a minimum 4 m wide transect. Survey methods may include the diver being in telephone contact with an assistant on land who notes the species and quantities observed or the use of direct recordings on waterproof preprinted forms.

Flora and fauna should be determined to species or as near to species as possible. Taxa that cannot be identified in the field should be collected for later identification. Crustose algae may be difficult to identify to species and quantify separately, and under these circumstances species may be grouped.

The sampling station may be positioned on a near-vertical wall to avoid large canopy-forming vegetation and sedimentation problems. For temporal trend monitoring, the quadrat areas may be marked by fixed bolts in the rock.

Due to time constraint for divers, quadrat surveys may be difficult to complete in depths below 10 m. In those cases, photography or video at fixed spaces may be an option (see Table 1, method A.2.3).

This method is not applicable to kelp forests.

A.2.3 Photography of random or fixed sampling points

A.2.3.1 General

As an alternative to *in situ* quadrat sampling, the hard-substrate communities may be recorded by photography of random or fixed sampling points. The requirements for the number of replicates and fixed depth are the same as in A.2.2.

This method is less time-consuming in the field than the *in situ* quadrat survey, but more time-consuming during processing. Using photography, the sample area should be permanently documented at the moment of photographing, and can be reviewed and re-analysed as required. The camera should be mounted on a reference frame which delimits the sampling area. The images can be processed by, for example, studying them through a magnifying glass, or they can be scanned and processed directly on computer using various imaging software. The degree of coverage, for example, can then be calculated quantitatively in relation to the sampling area. Taxon identification is more difficult than for *in situ* recordings, but can be simplified by using stereo-photography or video-photography (see below). Canopy vegetation can also complicate image processing.

A.2.3.2 Stereo-photography

Using this method, a restricted area of 50 cm × 50 cm is photographed in stereo, such that a 3D picture can be analysed. The images can be taken with two synchronized cameras mounted in parallel. The sample area should be delimited by a reference frame (0,5 m × 0,5 m × 0,1 m) which is parallel to the film plane and visible through the camera eye. The method can be used for random or fixed positioning of the sampling areas. In the case of fixed positioning, such as for temporal trend monitoring, the survey area should be marked with bolts in the rock. This method is further described in Reference [4].

Each image pair can be studied through two interconnected stereo microscopes such that a three-dimensional effect is achieved. The images are analysed qualitatively, by identifying species and abiotic factors (i.e. other features such as rock, shells and sediments). Taxa and substrate should be quantified in terms of degree of coverage or number. The percentage cover is estimated either directly or by spot-analysis (see Reference [1]).

A.2.3.3 Video-photography

Digital video may be used in conjunction with a quadrat (0,25 m^2), and the area marked with fixed bolts as appropriate. It is essential that the entire quadrat (including the frame) can be seen in view from different angles, but close-up views may also be included to aid identification.

Annex B
(informative)

Field recording form

PROJECT	LOCALITY
Project-No:	
Performing institution:	Locality:
Project manager:	
Personal in field:	Station-No:
	Station name:

Date (YY-MM-DD)	Time of start:	Time of finish:

COORDINATES

		Position from:		Datum:	
North/South		Map	☐	WGS84	☐
East/West		GPS	☐	ED50	☐
		DGPS	☐	UTM	☐
				Other	☐

METHODS/REGISTRATION

Zone		Sampling/registration		Lower limit for vegetation	
Supralittoral	☐		☐	Species	Depth
Eulittoral	☐	Semi-quant. shore survey	☐		
Sublittoral	☐	Transect diving	☐		
		Quadrat survey (eulittoral)	☐		
Photos	☐	Quadrat survey (sublittoral)	☐		
Video	☐		☐		
	☐	Stereo-photography	☐		

Positioning of quadrats in relation to fixed marks:	Substrate types: bedrock, rocks (give information on sizes), gravel, sand, etc.
	Bottom slope:
	Compass direction of the site:

Type of locality:		Degree of wave exposure		Estimated degree of wave exposure (Annex E)	
Fjord	☐	Weak	☐	Number of open sectors (of 10°, radius 7,5 km)	☐
Skerries	☐	Moderate	☐		
Outer coast	☐	Strong	☐		
Other	☐				

RECORDING CONDITIONS

Cloud cover (%):	Secchi depth (m):
Wind condition:	Wave height:

Annex C
(informative)

Biological definition of the supralittoral, eulittoral and sublittoral zones

The splash zone (= supralittoral zone) is the zone over the high water mark and is normally not submerged by water. The shore or tidal zone (= eulittoral zone) is the area between the high and low water marks, and the organisms in this zone switch between water immersion and air exposure. The marine zone (= sublittoral zone) is below the low water mark and is always submerged in water. The different zones represent very different living environments which are reflected in a predictable succession of many organisms (zonation) from the uppermost to the lowest level on the shore.

In practice, it is very time-consuming to determine the limits for mean/extreme high water and low water at a given station without reference to a nearby tidal recording device. The measured tidal level also often gives misleading information on the organisms' vertical distribution because other factors also influence their vertical distribution, such as wave exposure and geographical location. For example, it is a known phenomenon that many species are found higher up on the shore at exposed sites, compared to sheltered sites.

Therefore, a biological definition is often used and the zones are named the supralittoral, eulittoral and sublittoral zones.

The supralittoral zone is the zone between the upper limit of lichens [*Ramalina* sp., *Xanthoria parietina*, *Verrucaria maura* (black lichen belt)], blue-green algae or littorinid snails and the upper limit for barnacles (*Semibalanus balanoides*).

The eulittoral zone is defined as the zone between the upper limit for barnacles/*Pelvetia canaliculata* and the upper limit for laminarians (examples: *Laminaria* spp., *Alaria esculenta*) or *Halidrys siliquosa,* depending on the geographic area and wave exposure.

The sublittoral zone is defined as the zone between the upper limit of laminarians and the lower limit for algal vegetation. In the Baltic, the sublittoral zone is defined as the zone of perennial algae down to the lower limit of algae vegetation.

The species used as an indicator of the boundary between the eulittoral and sublittoral zones depends on the geographical location and wave exposure. It should also be emphasized that the specified limits refer to dense, multi-annual stocks. Many typical sublittoral species may be found far up in the eulittoral zone in exposed places, particularly under cover of seaweed.

Annex D
(informative)

Basis for the choice of semi-quantitative surveys in a standard

From a scientific point of view, quantitative methods are preferred over semi-quantitative surveys, amongst other reasons because a higher precision of the data is achieved. However, quantitative surveys that need to be carried out underwater in the eulittoral or sublittoral zones are extremely time-consuming and difficult to carry out due to movements of the vegetation and wave action on the sampling surface.

For areas with reduced tidal regimes, semi-quantitative methods in the eulittoral and sublittoral zone are set as the minimum requirement for environmental descriptions of receiving water bodies and for temporal trend monitoring. In areas where the surveys can be carried out at low water, quantitative analyses should be set as a minimum requirement for environmental descriptions of receiving water bodies and for temporal trend monitoring.

In addition, surveys carried out in depths greater than 9 m in the sublittoral zone are time-limited for reasons of diving physiology. This time limit becomes shorter with depth, and quantitative surveys carried out *in situ* are therefore extremely time-consuming and costly in greater depths. Therefore, quantitative surveys are a set requirement only for temporal trend monitoring.

Annex E
(informative)

Detailed method for calculating level of exposure

Wave exposure can be calculated theoretically using knowledge of wind and the position of the site.

The exposure index, E, is given by Equation (E.1):

$$E = \sum_{n=1}^{n=12} \left(V_n \times S_n\right) \tag{E.1}$$

where

V_n is the amount of wind from direction n;

S_n is the number of open sectors of 10 degrees in direction n (S_n = 0, 1, 2 or 3);

n is the given direction which makes up 1/12th of a circle of 360 degrees.

A clear plexiglas plate bearing a marked circle comprising 36 sectors is placed on the chart with the centre of the circle at the site in question. The sectors (10°) are divided into three radii: 0,5 km (R1), 7,5 km (R2) and 100 km (R3). All sectors that do not cover land within R1, R2 and R3, respectively, are recorded.

A value of E (exposure index) is calculated for each of the radii R1, R2 and R3, respectively E_L (local effect), E_F (fjord effect) and E_H (open sea effect). A compiled exposure index (EKSTAL) is calculated using Equation (E.2):

$$\mathrm{EKSTAL} = \left[E_\mathrm{L} + (10 \times E_\mathrm{F}) + (100 \times E_\mathrm{H})\right] \times 10^{-1} \tag{E.2}$$

where

E_L is the exposure index for sector radius R1 (0,5 km);

E_F is the exposure index for sector radius R2 (7,5 km);

E_H is the exposure index for sector radius R3 (100 km).

V_n is calculated from wind statistics (observations measured over a 30-year period):

$$V_n = f_\mathrm{rel} \cdot F_\mathrm{DM} \tag{E.3}$$

where

f_rel is the relative frequency of each wind direction;

F_DM is the average wind force (Beaufort) for each wind direction.

Terminology follows that given in the wind measurement tables from meteorological institutes.

NOTE The exposure index, E, is an empirical measure which has proven to be useful in many biological intertidal surveys. However, it is also possible to carry out a theoretical wave height calculation (significant wave height, H) either based on wind data and open sector length (in fjords and skerries) or based on wave statistics and sector width (in areas exposed to open ocean swell/waves).

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