HELCOM

Baltic Marine Environment Protection Commission

Recommendations and guidelines for

Benthic habitat monitoring in the Baltic Sea

Photo: Karl Florén

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Contents

1 Benthic Habitat Monitoring – the point of departure

There is a large number of survey methods used for collecting data about the benthic environment today. However, only a few methods are used in regular monitoring programmes of Baltic Sea states so far. By monitoring we mean a programme set up to collect certain information about features of interest in a systematic manner to be able to detect changes over time or perform status assessment.

Typical benthic monitoring methods that are operational today in the Baltic Sea Region and are used widely by the Contracting Parties of HELCOM are grab sampling and transect diving. However, the monitoring programmes within which these methods are performed are generally designed to describe the effect of eutrophication on the marine ecosystem. Currently, no monitoring methods are in place, which targets the **distribution or extent of the benthic habitats** in the Baltic Sea as required by the MSFD.

Methods that potentially could be used for benthic monitoring are quite numerous, for example, they could be based on various underwater video techniques, different acoustic and remote sensing methods. A useful monitoring method proposed for common use in a joint monitoring programme has to be well described, scientifically evaluated and tested. In the BALSAM project, we catalogued a large number of survey methods, including established monitoring methods, and assessed their potential usefulness to quantify benthic indicators for the MSFD as well as for the indicators listed by the HELCOM CORESET project and the LIFE MARMONI project. In MARMONI, various marine biodiversity monitoring methods and indicators were tested in the field and labs, the conclusions of which are the basis for the benthic habitat monitoring method proposal in the BALSAM project.

To monitor habitat extent, methods that fully cover selected areas, delineate habitat boundaries, or use large number of point observations may be used in order to statistically describe changes of habitat extent or size. There are several examples, including those on seagrass meadows repeatedly mapped using aqua scope, video or remote sensing, and perhaps may some of these methods qualify as monitoring methods for habitat extent. To address the perceived need for habitat quantity monitoring methods, we discussed in the BALSAM project a “drop-video” method as candidate method for surveying the size and extent of habitats, possibly in combination with a simplified version of grab sampling to improve cost-efficiency also in monitoring of the extent of soft sediment habitats.

The proposed methods may also be useful for monitoring of habitat distribution and some habitat quality aspects.

We see this “drop-video” technique in combination with traditional methods used for characterizing benthic communities (grab sampling, SCUBA diving) to be a promising, cost-effective solution for monitoring of the extent of a wide range of habitats and want to propose it herewith for standardisation and use in the Baltic Sea Region. It will be up to the HELCOM Contracting Parties to accept them for regular monitoring purposes.

2 Introduction to the publication

Work package 6 of the BALSAM Project dealt with Baltic benthic habitat monitoring methods and programmes useful for the Marine Strategy Framework Directive (MSFD). This included both assessing the usefulness of monitoring methods, which are operational today, and suggesting new methods for filling the gaps for MSFD needs.

This document describes the recommendations and guidelines for benthic habitat monitoring in the Baltic Sea, which have been developed within the BALSAM project. The work has been performed in cooperation between experts in Estonia, Latvia, Lithuania and Sweden with additional input from experts in Finland and Germany.

A large number of survey methods were catalogued within this project and gaps in existing monitoring of benthic habitats in the Baltic Sea were identified (see the online monitoring manual as well as the first BALSAM interim report).

In this report, we describe the use of drop-video and grab methods for monitoring habitat and biotope extent in shallow coastal waters of the Baltic Sea. The HELCOM Underwater Biotope and habitat classification (HELCOM HUB) is recommended for classification of benthic habitats. The HELCOM HUB system and relation to habitats listed in Habitats Directive Annex 1 are described in section 3.

Section 4 first describes area based methods for habitat and biotope monitoring and lists proposed methods for monitoring extent for HUB-classes. Method descriptions for drop-video and simplified grab sampling with a small Van-Veen grab are provided. Recommendations on identification of HUB-classes (level 5) with these methods are also provided. Section 4.4 compares cost-effectiveness of the newly proposed methods (drop-video and simplified small grab) to the conventional benthic monitoring methods (diving and grab sampling) using a large Van-Veen grab. Potential advantages of combined drop-video and grab surveys are discussed in section 4.5 and the applicability of an image recognition method for automated analysis of zoobenthos is evaluated in section 4.6.

Recommendations on habitat monitoring in the Baltic Sea based on the proposed methods are provided in section 5. Recommendations are given on aspects such as monitoring effort needed and sampling strategy based on statistical analyses performed on datasets collected in different areas.

A widely applicable and easy-to-use common data format for exchange of data is proposed in section 6.

Before launching monitoring programmes, baseline mapping surveys should be performed and background information on the diversity of existing biotopes and their distribution obtained. Since this may require specific mapping surveys and spatial modelling using experience from numerous case studies described in the literature, this is not further reflected in this report.

3 HELCOM Underwater Biotope and Habitat Classification

The HELCOM Underwater Biotope and habitat classification (HELCOM HUB) is a common system for biotope and habitat classification in the Baltic Sea. This comprehensive system is designed to be EUNIS compatible and defines 328 underwater biotopes including both common and rare Baltic biotopes.

In HELCOM HUB, a biotope is defined as the combination of a habitat and an associated community of species whereas habitat is defined as the abiotic environment which contributes to the nature of the seabed. In HELCOM HUB, Levels 1–3 can therefore be seen to describe habitats and Levels 4–6 to describe biotopes.

Many of the benthic biotopes at level 5 and 6 listed in the HELCOM HUB system are rare special cases and specially designed monitoring programmes would be needed for monitoring most of these rare biotopes. This report provides general recommendations on habitat and biotope monitoring with drop-video and grab methods. Examples based on datasets collected within the MARMONI project are also provided.

3.1 HELCOM HUB and habitats listed in the Habitats Directive Annex 1

Benthic habitats listed in Habitats Directive Annex 1 present in the Baltic Sea (table 1) are recognized as biotope complexes in HELCOM HUB. These biotope complexes consist of a number of biotopes/HUB-classes. The definitions of the biotope complexes in HELCOM HUB system follow the description for Habitats Directive Annex I Habitats in the Interpretation Manual of European Union Habitats (EUR 27, July 2007, European Commission). The descriptions of the biotope complexes and periodic reporting on the status are legally binding requirements for EU member states.

Table 1: Biotope complexes in the Habitats Directive Annex 1 present in the Baltic Sea. Table from HELCOM (2013)

Biotope Complexes (Habitats Directive Annex 1 habitats, EUR27)
1110 Sandbanks which are slightly covered by seawater all the time
1130 Estuaries
1140 Mudflats and sand flats not covered by seawater at low tide
1150 Coastal lagoons
1160 Large shallow inlets and bays
1170 Reefs
1180 Submarine structures made by leaking gas
1610 Baltic esker islands with sandy, rocky and shingle beach vegetation and sublittoral vegetation
1620 Boreal Baltic islets and small islands
1650 Boreal Baltic narrow inlets

4 Cost-effective Area Based Methods for Habitat and Biotope Monitoring

Benthic survey methods that fully cover areas in the Baltic Sea are either restricted to very shallow areas (e.g. remote sensing) or provide data on seabed substrate with very limited information on dominant benthic biota (e.g. hydroacoustics). Because of these limitations, such methods are only useful for monitoring of a small number of shallow (remote sensing), or coarser level benthic habitats and biotopes, i.e. HUB level 3 or sometimes 4 (hydroacoustics). In some cases, monitoring of distribution of certain biotopes, which correlate well with the acoustically detectable substrate types, topography or dominant species can be efficiently designed. This report suggests two methods, which may be used for cost-effective area-based monitoring of a wide range of shallow benthic habitats and biotopes. Although mainly developed for shallow coastal waters, these methods are not technically limited to shallow depths such as remote sensing methods and may therefore be used in practically all benthic biotopes in coastal areas. However for monitoring of deep off-shore areas where large ships are used, conventional methods (such as ROV - Remotely Operated Vehicle – ROV) and conventional grab sampling) can still be recommended since many of the advantages of the new cost effective methods are related to the use of small vessels with a minimum of crew (normally operated by the same persons that perform the monitoring).

4.1 Cost-effective Area Based Monitoring by Collection of Large Point Based Datasets

Cost-effective methods which allow sampling of many stations distributed over areas can be used to collect large point-based datasets useful for monitoring of extent (area) of benthic habitats and biotopes. Monitoring series of such datasets can be used in order to monitor changes in the extent of habitats and biotopes. This kind of datasets may also be used for spatial modelling in order to map the total distribution and extent of habitats and biotopes within an area, e.g. for baseline mapping.

In order to collect data from large numbers of stations in a cost-effective way, comparably fast and time-effective methods are needed. For hard and mixed substrates, drop-video is a fast and cost-effective alternative to diving. For soft substrates, a simplified grab method may be used as an alternative to conventional grab sampling. Table 2 and 3 below lists cost-effective monitoring methods for monitoring extent of HELCOM HUB-classes.

For some classes at level 5 and 6, combinations of several methods may be needed since classifications with only drop-video data will be difficult without a sampling device or combination with diving (applies mainly to classes dominated by filamentous algae). Observe that the methods given below are listed with regards to monitoring extent (area) of habitats and biotopes. Monitoring of quality aspects may require other methods.

Table 2: Recommended primary survey methods for monitoring extent of photic HELCOM HUB-classes

HUB Level 4, photic biotopes	**Recommended method for monitoring extent of biotopes**
AA.A1 Baltic photic rock and boulders characterized by macroscopic epibenthic biotic structures	Drop-video*
AA.A2 Baltic photic rock and boulders characterized by sparse macroscopic epibenthic biotic structures	Drop-video
AA.A4 Baltic photic rock and boulders characterized by no macroscopic biotic structures	Drop-video
AA.B1 Baltic photic hard clay characterized by macroscopic epibenthic biotic structures	Drop-video
AA.B2 Baltic photic hard clay characterized by sparse macroscopic epibenthic biotic structures	Drop-video
AA.B4 Baltic photic hard clay characterized by no macroscopic biotic structures	Drop-video
Baltic photic marl (marlstone rock) AA.C (level 3)	Drop-video
Baltic photic maerl beds AA.D (level 3)	Drop-video
AA.E1 Baltic photic shell gravel characterized by macroscopic epibenthic biotic structures	Drop-video
AA.E2 Baltic photic shell gravel characterized by sparse macroscopic epibenthic biotic structures	Drop-video
AA.E4 Baltic photic shell gravel characterized by no macroscopic biotic structures	Drop-video
Baltic photic ferromanganese concretion bottom AA.F (level 3)	Drop-video
Baltic photic peat bottoms AA.G (level 3)	Drop-video
AA.H1 Baltic photic muddy sediment characterized by macroscopic epibenthic biotic structures	Drop-video
AA.H3 Baltic photic muddy sediment characterized by macroscopic infaunal biotic structures	Grab
AA.H4 Baltic photic muddy sediment characterized by no macroscopic biotic structures (no infauna, no epibenthic)	Grab
AA.I1 Baltic photic coarse sediment characterized by macroscopic epibenthic biotic structures	Drop-video
AA.I2 Baltic photic coarse sediment characterized by sparse macroscopic epibenthic biotic structures	Drop-video
AA.I3 Baltic photic coarse sediment characterized by macroscopic infaunal biotic structures	Grab
AA.I4 Baltic photic coarse sediment characterized by no macroscopic biotic structures	Grab
AA.J1 Baltic photic sand characterized by macroscopic epibenthic biotic structures	Drop-video
AA.J3 Baltic photic sand characterized by macroscopic infaunal biotic structures	Grab
AA.J4 Baltic photic sand characterized by no macroscopic biotic structures	Grab
Baltic photic hard anthropogenically created substrates AA.K (level 3)	Drop-video
Baltic photic soft anthropogenically created substrates AA.L (level 3)	Drop-video
AA.M1 Baltic photic mixed substrate characterized by macroscopic epibenthic biotic structures	Drop-video*
AA.M2 Baltic photic mixed substrate characterized by sparse macroscopic epibenthic biotic structures	Drop-video
AA.M4 Baltic photic mixed substrate characterized by no macroscopic biotic structures	Drop-video

Note: *For the identification of some L5 and L6 classes (e.g. classes dominated by filamentous algae), a sampling devise or combination with diving is recommended.
The methods are suggested for monitoring extent of the level 5 and level 6 classes within the level 4 classes listed.

Table 3: Recommended primary survey methods for monitoring extent of aphotic HELCOM HUB-classes

HUB Level 4, aphotic biotopes	Recommended method for monitoring extent of biotopes
AB.A1 Baltic aphotic rock and boulder characterized by macroscopic epibenthic biotic structures	Drop-video
AB.A2 Baltic aphotic rock and boulder characterized by sparse macroscopic epibenthic biotic structures	Drop-video
AB.A4 Baltic aphotic rock and boulder characterized by no macroscopic biotic structures	Drop-video
AB.B1 Baltic aphotic hard clay characterized by macroscopic epibenthic biotic structures	Drop-video
AB.B2 Baltic aphotic hard clay characterized by sparse macroscopic epibenthic biotic structures	Drop-video
AB.B4 Baltic aphotic hard clay characterized by no macroscopic biotic structures	Drop-video
AB.C Baltic aphotic marl (marlstone rock) (level 3)	Drop-video
AB.D Baltic aphotic maerl beds (level 3)	Drop-video
AB.E1 Baltic aphotic shell gravel characterized by macroscopic epibenthic biotic structures	Drop-video
AB.E2 Baltic aphotic shell gravel characterized by sparse macroscopic epibenthic biotic structures	Drop-video
AB.E4 Baltic aphotic shell gravel characterized by no macroscopic biotic structures	Drop-video
AB.F Baltic aphotic ferromanganese concretion bottom (level 3)	Drop-video
AB.G Baltic aphotic peat bottoms (level 3)	Drop-video
AB.H1 Baltic aphotic muddy sediment characterized by macroscopic epibenthic biotic structures	Drop-video
AB.H3 Baltic aphotic muddy sediment characterized by macroscopic infaunal biotic structures	Grab
AB.H4 Baltic aphotic muddy sediment characterized by no macroscopic biotic structures	Grab
AB.I1 Baltic aphotic coarse sediment characterized by macroscopic epibenthic biotic structures	Drop-video
AB.I3 Baltic aphotic coarse sediment characterized by macroscopic infaunal biotic structures	Grab
AB.I4 Baltic aphotic coarse sediment characterized by no macroscopic biotic structures	Grab
AB.J1 Baltic aphotic sand characterized by macroscopic epibenthic biotic structures	Drop-video
AB.J3 Baltic aphotic sand characterized by macroscopic infaunal biotic structures	Grab
AB.J4 Baltic aphotic sand characterized by no macroscopic biotic structures	Grab
AB.K Baltic aphotic hard anthropogenically created substrates (level 3)	Drop-video
AB.L Baltic aphotic soft anthropogenically created substrates (level 3)	Drop-video
AB.M1 Baltic aphotic mixed substrate characterized by macroscopic epibenthic biotic structures	Grab
AB.M2 Baltic aphotic mixed substrate characterized by sparse macroscopic epibenthic biotic structures	Grab
AB.M4 Baltic aphotic mixed substrate characterized by no macroscopic biotic structures	Grab

Note: The methods are suggested for monitoring extent of the level 5 and level 6 classes belonging to the level 4 classes listed

Photo 1: Baltic photic rock and boulder biotope (author: Martin Isaeus)

4.2 Drop-video

Drop-video is a visual survey method for benthic vegetation and epifauna as well as benthic substrate. The method has the advantages of being time- and cost efficient compared to methods such as diving or ROV since limited number of staff is needed for operation, the drop-camera can be operated from a small vessel (without need for other crew than the drop-video staff), and only a few minutes are needed at each station. However, the method has a lower taxonomic resolution than methods such as diving and it may be difficult or impossible to distinguish between some species (e.g. several species of filamentous algae) with this method. A sampling device or combination with e.g. diving may be used in order to improve the taxonomic resolution in drop-video surveys.

Until now, drop-video surveys have been performed in many areas but rarely for regular monitoring. The method is performed in several different ways since there is no standard for this method in the Baltic Sea yet. A joint approach on a standard method for drop-video in shallow waters of the Baltic Sea is proposed here by the BALSAM benthic expert group.

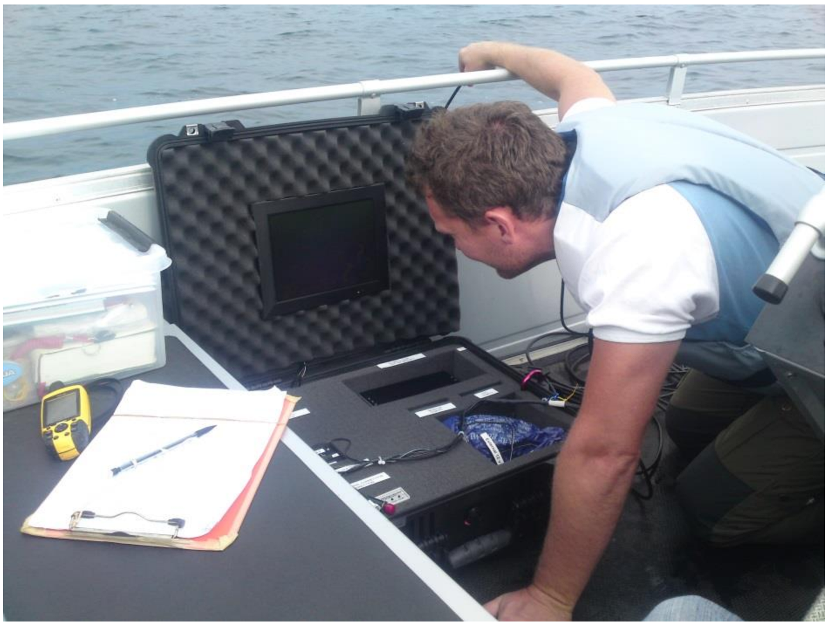

Photo 2: Performing drop video method (author: Julia Carlström)

4.2.1 Drop-video Field Procedure

Each surveyed station should have an adequate width of observation transect depending on prevailing conditions regarding visibility, bottom slope and vegetation as well as the camera's field of view. Since the field of view differs between camera systems, it is important to decide at which distance to the seafloor the desired recording width is achieved. This can easily be performed by placing a measuring tape on the bottom of a pool, shallow sandy bottom or similar and perform a test recording (or using e.g. laser ruler on the recording camera). Performing this measurement on land is not recommended since the field of view of most camera systems changes under water. It is recommended that the camera is protected with a frame or similar in order to not be damaged by contact with the bottom, boulders or similar. A well protected camera can also be used to examine the substrate by putting the camera down on the bottom. Laser pointers for distance measurement are recommended but it is also possible to lower the camera to the bottom and then lift it to the desired distance. An angle of about 30 degrees towards the bottom is recommended and, if another angle is preferred, it is recommended to stay between 20 and 45 degrees towards the bottom.

In order to achieve a good quality standard, a list of typical species that should be possible to detect with drop-video has been developed by benthic experts in the Balsam project (Annex 1). This list was further developed from a list of species, which is being produced for the drop-video method in Sweden (Swedish Agency for Marine and Water Management. In prep.). It is recommended that drop-video is only performed when the circumstances are good enough for identification of these species. For HUB-purposes, the circumstances should at least allow classification to level 5, which may differ between different HUB-classes. As a general recommendation, the vessel speed should not exceed 0.3 knots in order to achieve a good film quality (nearly 10 m per minute).

Depth and position (GPS waypoint) are noted in the protocol at the start of each transect (beginning of the video recording at the seabed). The camera is thereafter towed over the bottom until at least 5 m^2 total area has been filmed (5 m^2 is recommended sample size in Sundblad et al. 2013). Depth and position are noted in the protocol again at the end of each transect (end of video recording at the seabed).

A comparison of national and institutional drop-video methods from Sweden, Estonia, Latvia, Lithuania, Finland and Germany is available in Annex 2.

4.2.2 Video interpretation and HUB-classification of drop-video data

The recorded films are analysed in lab by personnel with good knowledge of benthic species and the surveyed habitats of the area. Substrate type and species are analysed in each film.

Presence of species is interpreted qualitatively in the entire film sequence. The cover of species may be assessed in one of the following ways: in still images extracted at frequent intervals (recommended to cover full diversity of the transect and all substrate types, with at least one image per substrate type) or assessed in the entire recording. Breakdown of the entire record into shorter intervals (e.g. 30 sec. or 1 min.) is possible in order to increase assessment accuracy if heterogeneity of a biotope (or estimated feature) is high. Only presence is noted for mobile fauna.

Drop-video surveys provide data on substrate and epibenthic species and/or groups of plants and animals that can be used for classification of drop-video stations into HUB-classes. However, some interpretations have to be performed in order to decide upon dominating epibenthic group at levels 5 and 6 in the HUB-hierarchy where biovolume is used for classification. In order to facilitate interpretations of dominating group, continuous scales are recommended as opposed to discrete classes, e.g. 3, 27 or 55 as opposed to 5, 25 or 50 (less risk of two groups having exactly the same cover). Continuous scales are also recommended from a statistic point of view (e.g. for calculations of mean or error estimates).

The HELCOM HUB report recommends measuring height of vegetation or second best to use regional height values supported by literature when determining biovolume (HELCOM 2013). There is however not yet any method for measuring height in drop-video surveys. Therefore, lists of regional height values are recommended unless a suitable and cost-effective method for height measurements is available. Other quantitative values, such as biomass, may also be used if available (e.g. in combined surveys). If nothing else is available, the cover may be used. A limitation in drop-video is that multilayer cover can’t be surveyed.

Annex 3 in HELCOM (2013) includes a list of average heights of macrophyte species along the Finnish coast.

Ground-truthing will be needed for some classes, e.g. when determining if filamentous algae are dominated by perennial or annual species. The ground-truthing can be performed with a sampling device, but also by the combination of drop-video and diving.

4.3 Benthic grab sampling

Grabs and other sampling devices are used for sampling of infauna and sediment. Several methods and sampling devices are in use in the Baltic Sea. A standard method for benthic grab sampling is described in the HELCOM COMBINE manual (HELCOM online document, last updated Feb. 2015.). This method is recommended whenever possible. However, in cases where large numbers of samples are needed or when only small vessels can be used (e.g. due to shallow water) this method may be too expensive or impossible to perform. For these cases a simplified grab method using a smaller Van Veen grab has been developed.

4.3.1 Simplified grab method using a small Van Veen grab

This grab method was first developed for mapping and spatial modelling purposes, when a large number of samples distributed over an area are needed. The purpose is to facilitate collection of large datasets at a minimum cost as well as to sample areas too shallow for large vessels. In that sense the aim is similar to drop-video which is used as a time and cost effective alternative to diving (where diving is a more exact method, which provides higher taxonomic resolution but also is more expensive and time consuming). The method compared well to the standard (large grab) method along the Swedish south coast and in the Hanö Bight but not in Øresund and Kattegat. The applicability of this method in different areas will depend on species composition and heterogeneity since both sample area and penetration depth are smaller than with the larger grab used in the standard method. The applicability of this method in the actual monitoring area should be tested before it is used in monitoring of the area.

Simplified grab method is based on the use of small Van Veen grab (sample area 0.025 m^2) instead of the standard Van Veen grab (sample area 0.1 m^2). This method may be performed from small vessels and require a minimum of crew and time. The method has been successfully performed in combination with a drop-video survey from a vessel of six m length and a crew of three people (two is the minimum).

The area of the opening of the grab sampler (width x length) should be measured and registered before sampling. The sample area may change after stations with hard objects such as stones or boulders, therefore this should be measured frequently. The sieves should be controlled in order to make sure that there are no holes and the mesh size is the same in the entire sieve. Spare grab samplers and sieves should be available during the field work.

If performed during a combined drop-video and grab survey, take a grab at the same location where the drop-video was performed, otherwise take a new waypoint. If the grab volume is less than 20 %, the grab is not regarded as a quantitative sample. The presences of species may, however, be analysed from such samples. The volume is noted in the protocol.

Successful grab samples are emptied in a box and the sampler is rinsed with water so that all sediment and animals are collected in the box.

Volumetric share of the size fractions of the sediment (classes according to EUNIS sediment fraction sizes; Davies et al. 2004) are estimated. The sum should be 100 %. If the sediment is layered, the proportions of the different layers may be noted. E.g. if a sample contains 5 cm glacial clay in the bottom with 2 cm gravel on top, the following is written:

Row 1: granules and pebbles 29 %, clay and silt 71 % OR

Row 1: granules and pebbles 100 %, upper layer, 2 cm.

Row 2: Clay 100 %, lower layer, 5 cm.

Table 4: Sediment classes

Class	Grain size
Gravel (granules, pebbles, cobbles)	> 2 mm
Sand and coarse sand	0.25 – 2 mm
Fine sand	63 – 250 µm
Clay/Mud and silt	< 2 µm – 63 µm

Note: *For details see Wentworth (1922)

A sieve with a 1 mm mesh size is the standard in surveys of macrofauna (Leonardsson, 2004; HELCOM MONAS 2003), but 0.5 mm is also frequently used in the central and northern Baltic Sea. Sieving should be done with care as a potentially large source of error is loss of animals due to rough sieving of the sediment. This is of especially large importance for animals without shells, such as polychaete worms.

Animals found should be identified to species as far as possible in field and counted. In cases where exact number of individuals is difficult to decide due to extremely high abundances (range of thousands in one sample), an estimate may be used for that species. Additionally, estimated biomass proportions by taxa in the HUB-system split rules should be noted. E.g. for classification to level 5, an estimation of the dominating group is performed between bivalves, polychaetes, crustaceans or insect larvae. This is an important step when samples aren't saved for biomass analyses in lab.

Many species are fragile and may have been broken into pieces during the handling. It is important that only pieces that are easy to identify to species (normally the head) are counted for each broken individual in order not to over-estimate the number of individuals.

The number of individuals for each species is noted in the protocol. In order to get individuals/m^2 for each species, the number of individuals should be divided by the sample area of the grab sampler used. Sampling in areas shallower than 70 m should be performed during daytime since some benthic species are semi-pelagic during night time.

Photo 3: Benthic grab sampling (author: Karl Florén)

4.3.2 HUB-classification of grab data

Classes dominated by infauna are split at level 5 and 6 by biomass. If biomasses have been analysed in lab, this data should be used. Otherwise field estimates of dominating biomass should be used. A single grab sample should generally not be considered a biotope and classed solely (except from very homogenous environments). Therefore it is recommended to perform more than one grab at each station, which should be HUB-classed using grab data. The combination of grab and drop-video may also be useful to describe the biotope.

4.4 Cost-effectiveness of drop-video and simplified grab method compared to conventional monitoring methods

In this section, we compare the cost per sampled station between the new methods for habitat and biotope extent proposed in this report and conventional benthic monitoring methods currently performed in the Baltic Sea. It should, however, be mentioned that the conventional monitoring methods used in these comparisons are not indented for monitoring of habitat or biotope extent. But since currently no monitoring of the extent of biotopes or habitats is performed (except special cases such as seagrass meadows), these methods were selected for comparison since they are well described and currently performed in benthic monitoring (although for other purposes). The conventional methods collect data about the same benthic groups and species as the proposed habitat and biotope monitoring methods, but they are designed to collect detailed data with high taxonomic resolution from a small number of stations whereas the newly proposed biotope and habitat monitoring methods are designed to collect less detailed data with lower taxonomic resolution from a much larger number of stations. The proposed habitat and biotope monitoring methods increase cost-efficiency since they minimize time and personnel needed per station and since only small vessels are needed (no other crew than the people performing the monitoring is needed).

The cost of different monitoring methods for benthic species and habitats depends on a number of factors and differs between campaigns. Geographic spread of the stations (travel distances), number of stations and hour rates of personnel are factors greatly affecting the cost per sampled station. The costs presented here are based on examples from Sweden.

4.4.1 Drop-video compared to diving

The Swedish Institute for the Marine Environment (Svensson et al, 2011) compared costs of a number of different methods including drop-video and diving transects. A total of 61 diving transects from three different areas in the Baltic Sea were used for cost analysis and the average cost for one transect (station) was EUR 969. The average cost for one drop-video station was estimated to EUR 41. Stations from a monitoring campaign in Östergötland County were used for calculations.

The difference in costs between the two methods is largely due to amount of time required for the different methods. Based on field experience from the MARMONI (2012) project (Wijkmark et al. 2014), approximately 3 stations can be sampled in one day using a conventional transect based diving method for phytobenthos surveys. Approximately 30 stations can be sampled in a day using the drop-video method. This figure was also estimated from the field campaign in the Koster Hvaler area (2012) within the "Hav möter Land" project (Sundblad et al, 2013). Furthermore, diving always requires a minimum of three persons (by Swedish law) whereas the drop-video method requires only two persons.

The drop-video method used in MARMONI and in the monitoring campaign in Östergötland sampled an area of approximately 25 m^2. Within the ongoing research programme WATERS, a new sampling method is evaluated where a 25 m^2 square is sampled by diving. Preliminary results from the project indicate that with this method approximately 9 stations could be sampled per day. Compared to conventional dive transects this method would roughly halve the sampling cost per station.

4.4.2 Drop-video compared to diving

Costs of conventional grab sampling were also analysed by Svensson et al (2011). Data consisted of 150 grabs from the Swedish monitoring programme of soft bottom macrofauna collected in the Bothnian Bay between 1995 and 1997. Costs of simplified grab sampling are based on field experience from the MARMONI project where 460 grabs were sampled. Table 5 summarizes the result.

Table 5: Costs per day (EUR)

	Conventional grab sampling	Simplified grab sampling (Small van-veen grab)
Boat	5000	350
Personnel for sampling	500	1000
Lab analysis	2600	0
Total cost per day	8100	1350
Grabs per day	13	30
Cost per grab (EUR)	623	45

Note: The personnel cost for the simplified grab sampling is higher since the personnel in this method also are the boat crew while crew costs are included in the boat costs for conventional sampling (a large vessel is used). A considerably shorter sieving time for simplified method (smaller grab and therefore less sediment in each grab) facilitates collection of more grabs per day with this method. Times and costs for the conventional method are taken from Svensson et al. (2011)

Costs for personnel and lab analysis are based on an hour rate of 50 EUR for both methods. The rate has a great impact on the costs and differs between campaigns. Conventional grab sampling requires a large vessel including a crew. This cost item is by far the most expensive for the method.

4.5 Combined drop-video and grab surveys

Surveys combining drop-video and grab from the same vessel have several advantages, one of the most obvious being cost- and time effectiveness. For the purpose of mapping or monitoring HELCOM HUB classes in an area, this approach facilitates the use of both methods at same stations, which will often be an advantage in HUB classification. Whenever a sandy or soft sediment substrate without vegetation is encountered with drop-video, the grab is used for sampling of infauna. Some grab samplers may also be used for sampling of vegetation when needed (e.g. at stations dominated by filamentous macroalgae), which may improve the quality of drop-video interpretations.

A combined drop-video and grab survey was performed in the Swedish study area "The Hanö Bight" within the MARMONI project. This survey was performed from a small vessel (6 m length) with a crew of three people (a minimum of two is needed). Grab samples were taken with a small Van-Veen grab (sample size 0.025 m^2) and sieved, sorted and counted in field. The data from this survey was categorized into HUB-classes and used in the analyses in the section *Recommendations based on analyses from MARMONI-data below*.

Another way of increasing taxonomic resolution in the surveys is to combine the surveys with diving. Surveys combining diving, video and grab are described in Martin et al. (2013).

4.6 Testing of a scanning method for macrofauna abundance and bio-volumes in order to increase accuracy and cost-efficiency

This section contains a summary of testing image recognition software for automatic identification, counting and other measurements of benthic macrofauna. The testing is described in detail in Annex 4 "Note on Automated Analysis of Zoobenthos using ZooImage Software".

A method for automated recognition and measurements of scanned macrofauna samples was tested. The image recognition software ZooImage (originally developed for zooplankton) was used in the testing. Since manual sorting and counting in lab is a time consuming task an automated approach was tested. Grab samples were scanned in Petri dishes in a flatbed scanner and image analysis was used for automated identification and counting of macrofauna.

It was clear that the image recognition method can't replace manual sorting of the sample due to the large amounts of gravel and/or detritus in the samples which fully or partly cover specimens in scanned images. When tested on already sorted samples, the method is technically functional and has a high classifying capacity when enough specimens are used in the training of the classifier. However, the accuracy never exceeded the accuracy in manual sorting.

ZooImage provides a range of measurements and characteristics for each specimen. The process is fast and automated and may therefore potentially be used as an alternative to manual analyses in lab such as biovolume and biomass analyses. For such uses, functions for conversion of certain measurements (e.g. length) to biovolume or biomasses are needed. Due to soft bodies or variable body shapes within most zoobenthic invertebrate species, this is currently only possible for very few zoobenthic species commonly found in the Baltic Sea. With further developments of software, such measurements may be possible for more species and thereby saving time by decreasing the need for time-consuming manual lab work. For the purpose of HUB-classification by dominating biomass or biovolume, the most time efficient way is, however, estimates performed directly in field (sorting directly in the sieve when using the simplified method described in this manual) or alternatively an estimate made during sorting of the sample in lab (if the conventional grab method is used).

5 Recommendations for Benthic Habitat Monitoring in the Baltic Sea

5.1 Monitoring period and frequency

The minimum monitoring frequency of habitat and biotope extent is once every sixth years (once every MSFD monitoring period). Field surveys of phytobenthos should be performed during the vegetation season while lab work such as video interpretation may be performed later at another time of the year. Since monitoring extent of benthic habitats and biotopes can be a quite extensive task too, short or limited time windows for monitoring can't be provided.

5.2 Monitoring areas

Since a dense sampling of the entire Baltic Sea with drop-video and grab is impossible, a number of monitoring areas should be selected in different parts of the Baltic Sea including dominating habitats and biotopes. Before areas are selected and sampling is designed, baseline mappings should be performed. A recommendation is to choose two monitoring areas in each HELCOM sub-basin (HELCOM Monitoring and Assessment Strategy, Attachment 4). Diversity and distribution of habitats and biotopes within the monitoring areas need to be mapped within the baseline mapping before monitoring starts.

Monitoring methods and required number of stations will vary between areas depending on the biotopes and habitats present as well as physical factors such as depth, exposure, seabed substrate, heterogeneity etc. Recommendations for sampling within monitoring areas are given in the section "Recommendations based on analyses from existing datasets" below.

5.3 Recommendations based on analyses from existing datasets from study areas

This section contains recommendations and results based on statistical analyses of drop-video and grab data. The datasets were collected in study areas in Estonia, Latvia, Lithuania and Sweden within other projects such as MARMONI. Recommendations are given based on these results.

Drop-video datasets (in some areas also combined with grab) from areas in Sweden, Estonia, Latvia, Lithuania and Germany were classed into HUB-classes. The benthic environments as well as purposes and sampling designs of analysed datasets vary between areas. The Swedish dataset was collected in a rather heterogeneous environment and was sampled for mapping and modelling purposes and therefore designed to include the variety of biotopes on all kinds of substrates in the area. Drop-video was substituted with grab sampling at the stations with soft sediments. As a result of this, a wide range of benthic biotopes were found in the Swedish dataset. In Latvia, most of the sampling was performed on mixed substrates. In Lithuania, most of the sampling was performed on rock and boulders.

In order to illustrate how sampling affects the number of stations needed for monitoring of HUB biotopes at level 4, 5 and 6, examples from AA.A (Baltic photic rock and boulders) are provided from the Swedish dataset and Lithuanian datasets and examples from AA.M (Baltic photic mixed substrate) are presented from the Latvian dataset. From Sweden, an example from AA.J (Baltic photic sand) is also presented, since both drop-video and grab was used in the Swedish area. Detailed results from all analysed datasets are provided in Annex 3.

Sweden

Field sampling was performed in 2011 and 2012 in the Hanö Bight study area within the MARMONI project. The sampling was performed in a stratified random way in order to include the depth and exposure ranges and the combinations of these in the area. In depths between 0 and 41 meters, all substrate types were sampled and the stratification was weighted to prioritize shallow bottoms. All stations were surveyed with drop-video. Grab samples (using a small Van Veen grab) for infauna were taken at all stations where this was possible (sand, fine sediments and mixed substrates when possible).

From this dataset, 876 stations were classified into HUB-classes as far as possible (mostly to level 6, but sometimes also to coarser levels when no finer level was available or when enough information for level 6 was not available). In total, 61 HUB-classes at finest level were found in the data. Almost half of these (27 biotopes) were found in only 1 - 3 stations (0.1 – 0.3 % of the stations) and another 13 biotopes were found in only 4 – 9 stations (0.5 – 1 % of the stations).

In order to detect a 20 % change of any of the 27 biotopes that occur in only 0.1 – 0.3% of the data with a statistical power of 80 %, between ca 100,000 and 300,000 stations would be needed. Monitoring therefore need to be restricted to certain habitats in order to decrease the needed sampling effort. If sampling would be performed within e.g. AA.A1 Baltic photic rock and boulders, in total seven level 6 biotopes were encountered. Four of these were more or less common in this dataset and two were rare. The "rare" biotopes occurred in only two stations each and were either dominated by annual algae or only sparsely inhabited by epibenthos. The common biotopes were dominated by *Fucus spp.*, perennial non-filamentous corticated red algae, perennial filamentous algae or by *Mytilidae* with perennial filamentous algae being most common (found in 42 % of the stations) and *Fucus spp.* being the least common (found in 10 % of the stations).

Distinguishing between filamentous algae species is often difficult in drop-video and extra measures should therefore be considered in stations dominated with filamentous algae in order to support the interpretations. In the surveys in the Hanö Bight, this was solved by the collection of algal samples during the drop-video survey. Dives were also performed in the area before the surveys. During the diving, the field staff learned about local conditions and macroalgae community. Filamentous red algae dominate many bottoms in the Hanö Bight. *Polysiphonia fucoides* and *Rhodomela confervoides* were most often the dominating species among filamentous red algae during this survey and both these were considered perennial here. Most stations dominated by filamentous red algae were therefore classed as "dominated by perennial filamentous algae" in the HUB-classification.

Table 6: Example from "AA.A Baltic photic rock and boulders" from a combined drop-video and grab survey in the Hanö Bight, Sweden

	HUB-class	Presences of HUB class in drop-video data	N drop-video stations (n)	N stations needed to detect a difference of 20% with 80% power	N stations needed to detect a difference of 50% with 80% power
AA.A If all habitats are sampled, HUB finest level (L5-L6)	AA.A1C1 Baltic photic rock and boulders dominated by *Fucus spp.*	23	876	13116	1753
	AA.A1C2 Baltic photic rock and boulders dominated by perennial non-filamentous corticated red algae	54	876	5402	724
	AA.A1C5 Baltic photic rock and boulders dominated by perennial filamentous algae	92	876	3038	409
	AA.A1E1 Baltic photic rock and boulders dominated by *Mytilidae*	48	876	6118	820
	AA.A1S Baltic photic rock and boulders characterized by annual algae	2	876	154205	20565
	AA.A2T Baltic photic rock and boulders characterized by sparse epibenthic macrocommunity	2	876	154205	20565
AA.A if only AA.A is sampled, HUB finest level (L5-L6)	AA.A1C1 Baltic photic rock and boulders dominated by *Fucus spp.*	23	221	3069	413
	AA.A1C2 Baltic photic rock and boulders dominated by perennial non-filamentous corticated red algae	54	221	1123	154
	AA.A1C5 Baltic photic rock and boulders dominated by perennial filamentous algae	92	221	527	74
	AA.A1E1 Baltic photic rock and boulders dominated by *Mytilidae*	48	221	1303	178

	HUB-class	Presences of HUB class in drop-video data	N drop-video stations (n)	N stations needed to detect a difference of 20% with 80% power	N stations needed to detect a difference of 50% with 80% power
AA.A if only AA.A is sampled, HUB L5	AA.A1S Baltic photic rock and boulders characterized by annual algae	2	221	38663	5159
	AA.A2T Baltic photic rock and boulders characterized by sparse epibenthic macrocommunity	2	221	38663	5159
	AA.A1C Baltic photic rock and boulders characterized by perennial algae	169	221	140	23
	AA.A1E Baltic photic rock and boulders characterized by epibenthic bivalves	48	221	1303	178
	AA.A1S Baltic photic rock and boulders characterized by annual algae	2	221	38663	5159
	AA.A2T Baltic photic rock and boulders characterized by sparse epibenthic macrocommunity	2	221	38663	5159
AA.A if only AA.A is sam-pled, HUB L4	AA.A1 Baltic photic rock and boulders characterized by macroscopic epiben-thic biotic structures	221	221	32	8
	AA.A2 Baltic photic rock and boulders characterized by sparse macroscopic epibenthic biotic structures	2	221	38663	5159

Table 7: Example from "AA.J Baltic photic sand" from a combined drop-video and grab survey in the Hanö Bight

	HUB-class	Presences of HUB class in drop-video data	N drop-video/grab stations (n)	N stations needed to detect a difference of 20% with 80% power	N stations needed to detect a difference of 50% with 80% power
AA.J if only AA.J is sampled, HUB finest level (L5-L6)	AA.J1B1 Baltic photic sand dom. by pondweed (*Potamogeton perfoliatus* and/or *Stuckenia pectinata*)	38	255	2047	277
	AA.J1B2 Baltic photic sand dom. by *Zannichellia spp.* and/or *Ruppia spp.* and/or *Zostera noltii*	9	255	9675	1294
	AA.J1B7 Baltic photic sand dom. by common eelgrass (*Zostera marina*)	30	255	2678	361
	AA.J1E1 Baltic photic sand dom. by *Mytilidae*	2	255	44661	5959
	AA.J1Q1 Baltic photic sand dom. by stable aggregations of unattached *Fucus spp.* (typical form)	5	255	17672	2360
	AA.J1S Baltic photic sand charact. by annual algae	3	255	29667	3960
	AA.J1V Baltic photic sand charact. by mixed epibenthic macrocommunity	9	255	9675	1294
	AA.J3L Baltic photic sand charact. by infaunal bivalves	27	255	3011	406
	AA.J3L1 Baltic photic sand dom. by Baltic tellin (*Macoma balthica*)	51	255	1443	196
	AA.J3M5 Baltic photic sand dom. by multiple infaunal polychaete species: *Pygospio elegans, Marenzelleria spp., Hediste diversicolor*)	25	255	3278	441
	AA.J3N Baltic photic sand charact. by infaunal crustaceans	27	255	3011	406
	AA.J3P Baltic photic sand charact. by infaunal insect larvae	1	255	89643	11956

	HUB-class	Presences of HUB class in drop-video data	N drop-video/grab stations (n)	N stations needed to detect a difference of 20% with 80% power	N stations needed to detect a difference of 50% with 80% power
	AA.J4U Baltic photic sand charachterized by no macrocommunity	28	255	2892	390
AA.J if only AA.J is sampled, HUB L5	AA.J1B Baltic photic sand charact. by submerged rooted plants	77	255	847	117
	AA.J1E Baltic photic sand charact. by epibenthic bivalves	2	255	44661	5959
	AA.J1Q Baltic photic sand charact. by stable aggregations of unattached perennial vegetation	5	255	17672	2360
	AA.J1S Baltic photic sand charact. by annual algae	3	255	29667	3960
	AA.J1V Baltic photic sand charact. by mixed epibenthic macrocommunity	9	255	9675	1294
	AA.J3L Baltic photic sand charact. by infaunal bivalves	78	255	832	115
	AA.J3M Baltic photic sand charact. by infaunal polychaetes	25	255	3278	441
	AA.J3N Baltic photic sand charact. by infaunal crustaceans	27	255	3011	406
	AA.J3P Baltic photic sand charact. by infaunal insect larvae	1	255	89643	11956
	AA.J4U Baltic photic sand charachterized by no macrocommunity	28	255	2892	390
AA.J if only AA.J is sampled, HUB L4	AA.J1 Baltic photic sand charact. by macroscopic epibenthic biotic structures	96	255	616	86
	AA.J3 Baltic photic sand charact. by macroscopic infaunal biotic structures	131	255	366	53
	AA.J4 Baltic photic sand charact. by no macroscopic biotic structures	28	255	2892	390

Latvia

Drop-video surveys were performed during the period from April to May 2012 and from August to September 2013 in the frame of MARMONI project. Video record data on the type of substrate and coverage of biological organisms were collected from 215 stations. The sampling was performed in a regular way in order to evenly cover the whole assessment territory. The depth interval of stations was from 6 to 24 m.

To verify drop-video data, 17 hard bottom stations were selected according to depth, substrate type, biodiversity and significant coverage of macro-benthic species where SCUBA divers collected samples for further analysis. The data were obtained on detailed macroalgal species composition and wet biomass as well as macrobenthic invertebrate species composition, abundance and wet biomass. Results for all analysed habitats in Latvia are available in Annex 3.

Table 8: Example from a drop-video survey in Irbe Strait, Latvia

	HUB-class	Presences of HUB class in drop-video data	Total number of drop-video stations (n)	Number of stations needed to detect a difference of 20% with 80% power	Number of stations needed to detect a difference of 50% with 80% power
AA.M if all habitats are sampled, HUB L5	AA.M1E Baltic photic mixed substrate characterized by epibenthic bivalves	121	204	274	41
	AA.M2T Baltic photic mixed substrate characterized by sparse epibenthic macrocommunity	32	204	1928	261
	AA.M4U Baltic photic mixed substrate characterized by no macrocommunity	4	204	17672	2360
	AA.M1C Baltic photic mixed substrate characterized by perennial algae	10	204	6876	921
	AA.M1S Baltic photic mixed substrate characterized by annual algae	2	204	35665	4759
AA.M, if only AA.M is sampled, HUB L5	AA.M1E Baltic photic mixed substrate characterized by epibenthic bivalves	121	169	172	27
	AA.M2T Baltic photic mixed substrate characterized by sparse epibenthic macrocommunity	32	169	1542	210
	AA.M4U Baltic photic mixed substrate characterized by no macrocommunity	4	169	14585	1949
	AA.M1C Baltic photic mixed substrate characterized by perennial algae	10	169	5641	756
	AA.M1S Baltic photic mixed substrate characterized by annual algae	2	169	29491	3936

Lithuania

Drop-video surveys were performed during the period from April to August in 2006 and 2007 in the frame of the LIFE BALTIC MPA project. Video record data on the type of substrate and species coverage were collected in depths from 2 to 20 m. The sampling was stratified according to the depth and substrate. Duration of video transects was set to ca. 3 minutes and resulted in approximately 40 m distance covered by drop-video in each site. Results for all analysed habitats in Lithuania are available in Annex 3.

Table 9: Examples from a drop-video survey in Karklė - Šventoji area, Lithuania

	HUB-class	Presences of HUB class in drop-video data	Total number of drop-video stations (n)	Number of stations needed to detect a difference of 20% with 80% power	Number of stations needed to detect a difference of 50% with 80% power
AA.A if all habitats are sampled, HUB L5	AA.A1C Baltic photic rock and boulders characterized by perennial algae	49	287	1745	237
	AA.A1E Baltic photic rock and boulders characterized by epibenthic bivalves	79	287	961	132
	AA.A1I Baltic photic rock and boulders characterized by epibenthic crustacea	24	287	3898	524
	AA.A1S Baltic photic rock and boulders characterized by annual algae	12	287	8117	1086
AA.A if only AA.A is sampled, HUB L5	AA.A1C Baltic photic rock and boulders characterized by perennial algae	49	164	860	119
	AA.A1E Baltic photic rock and boulders characterized by epibenthic bivalves	79	164	411	59
	AA.A1I Baltic photic rock and boulders characterized by epibenthic crustacea	24	164	2090	283
	AA.A1S Baltic photic rock and boulders characterized by annual algae	12	164	4501	604

Estonia

The analysed area is the surroundings of a peninsula in the western part of the Hiiumaa Island. The region is dominated by active hydrodynamic processes. The peninsula is a natural barrier for currents, which cause upwelling of cold and more saline water to the coastal area of the peninsula. The analysed area was 110 km^2. Data was collected in June of 2011 by means of grid sampling. Results for all analysed habitats in Estonia are available in Annex 3.

Table 10: Examples from a drop-video survey in Kõpu, Estonia

	HUB-class	Presences of HUB class in drop-video data	Total number of drop-video stations (n)	Number of stations needed to detect a difference of 20% with 80% power	Number of stations needed to detect a difference of 50% with 80% power
AA.A if all habitats are sampled, HUB L5	AA.A1C Baltic photic rock and boulders characterized by perennial algae	92	496	1581	215
	AA.A1E Baltic photic rock and boulders characterized by epibenthic bivalves	7	496	24677	3294
	AA.A1S Baltic photic rock and boulders characterized by annual algae	36	496	4540	609
AA.A if only AA.A sampled, HUB L5	AA.A1C Baltic photic rock and boulders characterized by perennial algae	92	135	197	30
	AA.A1E Baltic photic rock and boulders characterized by epibenthic bivalves	7	135	6483	868
	AA.A1S Baltic photic rock and boulders characterized by annual algae	36	135	1002	138

5.4 Discussion and overall recommendations

A good assessment of habitat and biotope extent is a work intensive task also when cost-effective monitoring methods are used. Rather extensive sampling is needed in order to detect areal changes of biotopes and habitats with point datasets. At present, no truly area covering methods provide enough information for monitoring of benthic HUB-classes at level 5. The cost effective point based methods drop-video and simplified grab sampling with small van Veen-grab are therefore recommended for monitoring habitat and biotope extent in shallow coastal areas. In offshore areas were large vessels are used, conventional methods such as ROV and conventional grab sampling will often still be the best option, since many of the advantages of the cost-effective methods presented here depend on the use of small vessels with no other crew than the people performing the monitoring. Deep offshore bottoms are usually more homogenous than bottoms in shallow coastal areas, wherefore offshore areas may be sampled less densely than coastal areas.

In most areas, only very few biotopes would be possible to monitor with a random sampling design, which includes the variety of biotopes in the area (such as the datasets in the examples presented by this report). Monitoring of the extent of benthic HUB-biotopes should therefore be restricted to certain selected environments, which would reduce the required sampling effort significantly, and only extent of dominating biotopes may be assessed in a broad scale assessment, which will be sufficient for MSFD reporting needs. Monitoring of rare biotopes will only be possible with specific monitoring programmes for these biotopes, since extremely large monitoring efforts would be needed in order to reach enough stations within rare biotopes if a broad non biotope specific sampling is performed. In order to design monitoring programmes, baseline mappings are needed.

In general, the following set of **recommendations** has been developed for sampling benthic habitat and biotope monitoring:

- ❖ An initial baseline biotope mapping of the monitoring area and its benthic biotopes is performed (the baseline mapping procedure is not described within BALSAM). Benthic HUB Level 5 biotopes and biotope complexes (habitats) of Habitat Directive Annex 1 should be mapped.
- ❖ Monitoring of biotope area should be performed at HELCOM HUB Level 5 classes (or finer).
- ❖ To keep monitoring efforts at a reasonable level, only extent of dominating biotopes is recommended in a broad scale assessment.
- ❖ Monitoring of rare biotopes may be performed too, but only if specific sampling is performed. An initial mapping of these biotopes is therefore required.

6 Common Data Format for Interchange of Data

A common data format for interchange of data should be widely compatible with different software. It should also include a minimum amount of information and be easy to understand as well as to import and export with different applications.

CSV (Comma-Separated Values) is recommended since it is a widely compatible format, which is simple and supported by almost all spreadsheets and database management systems (e.g. it can easily be opened in MS Excel and imported in most database systems).

A minimum set of information in a standard format is also required for the interchange of data. Table 11 lists mandatory information, which applies to all benthic methods. Taxonomic information should follow "World Register of Marine Species" (http://www.marinespecies.org/). The way other information (such as species or seabed substrate information) is registered differs between methods (e.g. cover or number of specimens of a species or group).

Table 11: Mandatory information for all benthic methods

Information	Specification	Example
Lat*	WGS84 decimal degrees	58.2409
Long*	WGS84 decimal degrees	19.3918
Date	Date/time	2014-08-12
Method	Free text	Drop-video
Sample area or volume	Sample area or volume, depending on method performed	5 m^2
Depth	Depth in meters	16.5
Reference	Text. Reference to Survey or survey leader or responsible person	Name Surname
Institute	Name of institute or company	Institution Name

Note: * Coordinates may be single coordinates (point samples such as grab), start and end (e.g. video transects) or other depending on method performed

7 The Way Forward

As stated in our manual, monitoring of biotope and/or habitat distribution and extent is largely missing in the Baltic Sea today (a few special examples such as eelgrass meadows are monitored in some areas). Good methodological approach as well as regular monitoring activities are needed. It is important that methodology and actual monitoring activities do not only focus on one or a few special cases such as eelgrass (*Zostera marina*) meadows but is more widely applicable on a wide range of benthic biotopes.

The BALSAM benthic expert group proposed and described methods, which are useful for a wide range of biotopes in different areas in the Baltic Sea. These methods are both detailed enough to distinguish between different biotopes (at least to HUB level 5) and not restricted to only shallow depths such as aerial or satellite remote sensing methods, and therefore much more widely applicable. Common indicator/indicators for distribution and extent need to be agreed on and thereafter a monitoring strategy for these indicators, including methods described in BALSAM (probably in combination with some conventional methods), should be developed. Baseline mappings will be needed for the areas selected for monitoring.

How?

The way forward to reach common monitoring of benthic habitats has been started by setting up a new HELCOM inter-sessional expert network on benthic habitat monitoring in November 2014. The BALSAM benthic expert group is the core group and HELCOM contracting parties have been/are invited to nominate experts to it. Estonia has agreed to lead this expert network, BALSAM expert Georg Martin from Estonian Marine Institute is acting as chairman. The group had its first working meeting in Jūrmala, Latvia, on January 28, 2015 and developed a roadmap to achieve harmonization of methods and monitoring strategies for benthic habitats in the Baltic Sea area.

The Baltic benthic habitat experts have already participated actively at the European Commission’s 1st Marine Biogeographic Seminar in St. Malo in May 2015 and at the LIFE Marine project Platform in Madrid in March 2015. In both events, the need for better marine habitat definitions, survey methods and monitoring strategies has been concluded and encouraged, the lack of suitable and detailed marine habitat definitions in the Habitats Directive has been pointed out as major concern and initiative among European benthic habitat experts has started to foster development.

The work will be continued and attached to projects and events, whenever possible.

8 List of annexes

Annex 1. List of species for identification with drop-video

Annex 2. Drop-video method comparison

Annex 3. Power calculation examples from different areas in the Baltic Sea

Annex 4. Note on Automated Analysis of Zoobenthos using ZooImage Software

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Baltic Sea Pilot Project: Testing new concepts for integrated environmental monitoring of the Baltic Sea (Project acronym - **BALSAM**).

Project website: http://helcom.fi/helcom-at-work/projects/balsam/

Project lead: HELCOM (Baltic Marine Environment Protection Commission - Helsinki Commission) Secretariat
Katajanokanlaituri 6 B, FI-00160 Helsinki, Finland
http://www.helcom.fi

Annex 1. List of species for identification with drop-video

	Species/group	**Proposed common list**	**Sweden**	**Lithuania**	**Latvia**	**Estonia**	**Germany**	**Comment**
Macroalgae	*Aegagropila linnaei*							
	Ascophyllum nodosum	x	x					
	Battersia arctica					x		
	Brownalgae filamentous (as a group)	x	x				x	
	Ceramium tenuicorne					x		
	Ceramium virgatum							
	Chara aspera							
	Chara baltica							
	Chara canescens							
	Chara globularis							
	Chara sp	x	x			x		
	Chara tomentosa	x	x					
	Chondrus crispus							
	Chorda filum	x	x			x		
	Cladophora glomerata					x		
	Cladophora rupestris							
	Coccotylus truncatus/Phyllophora pseudoceranoides (species pair)	x		x				Not included in Swedish list since it is often covered by filamentous species, especially in the Swedish west coast. Possible to identify on the Swedish east coast.
	Dictyosiphon foeniculaceus					x		

Species/group	Proposed common list	Sweden	Lithuania	Latvia	Estonia	Germany	Comment
Dictyosiphon/Stichtyosiphon	x	x					
Ectocarpus siliculosus							
Enteromorpha ahlneriana							
Enteromorpha intestinalis							
Fucus spp.	x	x				x	
Fucus radicans							
Fucus serratus	x	x					
Fucus spiralis							
Fucus vesiculosus	x	x			x		
Furcellaria lumbricalis	x		x		x		Not included in Swedish list since it is often covered by filamentous species, especially in the Swedish west coast. Possible to identify on the Swedish east coast.
Gayralia oxysperma							
Halidrys siliquosa	x	x					
Halosiphon tomentosus							
Laminaria sp	x	x				x	
Laminaria digitata							
Laminaria hyperborea							
Lamprothamnium papulosum							
Limosella aquatica							
Monostroma balticum							
Nitellopsis obtusa							
Phyllophora pseudoceranoïdes							
Polyides rotundus							

	Species/group	**Proposed common list**	**Sweden**	**Lithuania**	**Latvia**	**Estonia**	**Germany**	**Comment**
	Polysiphonia fibrillosa							
	Polysiphonia fucoides					x		
	Pylaiella littoralis/Ectocarpus siliculosus (species pair)	x	x			x		Only included as a species pair. Not possible to distinguish between *Pylaiella* and *Ectocarpus* in drop-video.
	Red algae filamentous (as a group)	x	x				x	
	Red algae non-filamentous (as a group)	x	x				x	
	Rhodomela confervoides							
	Saccharina latissima	x	x					
	Stictyosiphon tortilis							
	Tolypella nidifica	x	x			x		
Vascular plants	*Ceratophyllum demersum*	x	x					
	Isoëtes lacustris							
	Lemna trisulca	x	x					
	Myriophyllum alterniflorum							
	Myriophyllum sibiricum							
	Myriophyllum spicatum					x		
	Myriophyllum sp	x	x					
	Myriophyllum verticillatum							
	Najas marina	x	x			x		
	Potamogeton filiformis							
	Potamogeton friesii							
	Potamogeton obtusifolius							

	Species/group	Proposed common list	Sweden	Lithuania	Latvia	Estonia	Germany	Comment
	Potamogeton pectinatus					x		
	Potamogeton perfoliatus	x	x			x		
	Potamogeton pusillus							
	Potamogeton vaginatus							
	Ranunculus circinatus	x	x					
	Ranunculus confervoides							
	Ranunculus peltatus ssp. baudotii	x	x			x		
	Ruppia cirrhosa							
	Ruppia maritima					x		
	Zannichellia palustris	x	x			x		
	Zostera marina	x	x			x		
	Zostera noltii							
Animals	*Mytilus edulis*	x		x				Not included in Swedish list since it is often covered by filamentous species, especially in the Swedish west coast. Possible to identify on the Swedish east coast.
	Electra crustulenta	x		x				
	Cordylophora caspia	x	x	x				
	Amphibalanus improvisus	x		x		x		
	Theodoxus fluviatilis					x		
	Ulva intestinalis					x		
	Hydrozoa					x		
	Mytilus trossulus					x		

Note: * Coordinates may be single coordinates (point samples such as grab), start and end (e.g. video transects) or other depending on method performed

Annex 2. Drop-video method comparison

	Questions	Sweden	Germany	Lithuania	Latvia	Finland	Estonia
GENERAL AND TECHNICAL QUESTIONS							
1.	Is the method standard fixed? If the method standard is fixed, which level? (institutional, national, other level)	Fixed (but under development) National level (in manual for visual methods for monitoring of marine habitats/biotopes and typical species)	No	No	Institutional	Method is fixed within national VELMU participants including Finnish Environment Institute, Finnish Parks & Wildlife, Centre for Economic Development, Transport and the Environment, Geological Survey of Finland	Institutional
2.	Recorded area or film length: fixed or not? If fixed: what area (or interval)?	Fixed. 5 m^2	No, minimum 5 minutes, up to 4 hours at heterogeneous substrates	Yes (30 min)	3 minutes	Not fixed, but at least one minute of good quality film is required	Not fixed, usually 1 min
3.	Height over bottom: fixed or not? If fixed: what height (or interval)?	Not fixed but 0.5 m above bottom is recommended	No, but approx. 1.5m above seafloor	Fixed on soft bottoms (sledge) - 40 cm, not fixed in hard bottoms	Not fixed (depends on visibility)	Not fixed but different bottom habitats and species need to be distinguished and analysed properly	Not fixed, approximately 1 m above the seafloor. For the identification of the species might be dropped lower
4.	Recorded width: fixed or not? If fixed: what width (or interval)?	Not fixed but 1 m is recommended	No	Fixed on soft bottoms (sledge) - 60 cm, not fixed in hard bottoms	Not fixed	No fixed, but approximately 20 square meter is filmed	Not fixed
5.	Angle of camera: fixed or not? If fixed: what angle (or interval)?	20-45 degrees towards bottom is recommended	No, but approx. 35°	70-80 degrees	Yes, 40 degrees	Not fixed, depends on the camera type, but approximately 45° angle	Not fixed, approx. 35°
6.	Scale (e.g. laser beams) used? (yes/no)	Recommended but not demanded	Yes, two laser	Yes	No	In some drop-videos in use, for example Finnish Parks & Wildlife's	No

	Questions	Sweden	Germany	Lithuania	Latvia	Finland	Estonia
						HD-drop video systems has a laser beam	
7.	Speed of vessel fixed? If fixed: what speed (or interval)?	Recommended maximum speed 0.3 knots	Not fixed, mainly wind drift, max. 1kn	Less than 0.4 knots	Not fixed (boat is on anchor)	Not fixed, but the quality of the video needs to be good and analysed	Not fixed
8.	Weather limitations? What limitations?	Recording should not be performed in weather conditions in which the quality demands can't be met (as specified by the species list)	Sea state 3	Less than 3 bofort	No limitations fixed in method	No fixed limitations, but the quality of the filmed video needs to be good so wind and wave conditions need to be no more than moderate	No limitations
9.	Resolution requirements? (e.g. HD)	HD	HD (1920x1080, 30fps), Stills with 4048 × 3040 px		No resolution requirements fixed	HD is preferred	Not fixed, 720 tvl used
10.	Lights? (yes/no, how much, when)	Yes. To be used whenever needed.	Yes, three LED lights, depends on turbidity	4 bulbs x 50 W	No	Yes, extra light is used when needed	Yes, 2 bulbs are used
11.	Sledge used? (yes/no)	No	Partly	Yes (soft bottom)	No	There are no fixed drop video systems so equipment in use vary between different authors. Finnish Parks & Wildlife's newer HD drop video systems are factory-made with sledges, older ones are self-made and models vary greatly	No
12.	Frame used? (yes/no?)	Protective frame recommended	Partly	Yes (hard bottom)	No	See the previous answer	Protective frame
13.	Field protocol used?	Yes	Digital, Video Annotation Software VIDEOMON	No	Yes	Yes. Before actual filming, date, name of the boat, field crew, used devices, environment conditions like wind	Yes

	Questions	Sweden	Germany	Lithuania	Latvia	Finland	Estonia
						speed, direction, weather, temperature of the water, Secchi depth and salinity are written down. When filming, the start and end points (coordinates) of the film are taken with separate GPS device if the drop video system does not record the coordinates. Also depths in the beginning and in the end of the film are written down. As well as the length of the film	
14.	Text overlay? Yes/No? If overlay: what is included?	Not demanded	Yes: coordinates, depth, date, time, transect name	Yes, GPS coordinates, depth, time, station number	No	Drop video equipment vary between different authors, for example Finnish Parks & Wildlife's newer HD-video systems depth, temperature, salinity, record time and coordinates are included	No
15.	Coordinates: When are coordinates taken? Beginning of transect/end/middle/beginning and end/other?	Beginning and end of transect	During full transect, DGPS from research vessel via NMEA	Beginning and end of the transect, continuous recording on text overlay	Beginning	In older video systems coordinates are always taken in the beginning and in the end of the film but newer HD drop video systems with NMEA cable takes coordinates through the whole film	Beginning, if the drift is high then beginning and end

	Questions	Sweden	Germany	Lithuania	Latvia	Finland	Estonia
LAB PROCEDURE (OR INTERPRETATION PROCEDURE IN FIELD)							
16.	Interpretation in field or lab?	Lab	Both	Lab	In lab	Actual interpretation in the lab, some general observation also in the field	Actual interpretation in the lab, some general observation also in the field
17.	Biotopes defined in field/in lab/later based on data/other?	Later	Later in the lab. based on video footage and other data (grain size...)	Depending on purpose: new areas and new biotopes - based on analysis, typical areas and typical (published) biotopes - by expert judgment	In lab	?	Lab
19.	Entire video or sections/still images analysed? (entire, sections, still, combination, explain)	Recommendation: Entire video-transect ($5m^2$) is analysed for presence of species. Cover of species is analysed in still images extracted at ten stops (during paused video). The film is divided into 10 sections of same length and one stop is randomly selected within each section. Each extracted still image is analysed in ten points. Each time a species touches a point in a still image it is given 1 % cover. If a species touches ten points in all ten still images it is given 100 % cover which is the	Entire video	Early development phase - still images, last 5 years - video records	Entire video	If the quality of the film is good, the interpretation starts from the beginning. If the habitat is not changing within 30 seconds, only one interpretation is done. If habitats vary, the interpretation is done by very single habitat so one video gets many interpretations. If the biotope is clearly same in the film at least 10 seconds, it is interpret as a one habitat	Entire video

	Questions	Sweden	Germany	Lithuania	Latvia	Finland	Estonia
		maximum cover for a species in this method					
20.	If sections/still images: length/number/?	10 still images (for cover of species)	-	Video sections - 30 sec.	-	Sections, length depends on quality of the video and the habitat	-
21.	Substrate classes: what classes?	Bedrock Boulders (>200 mm) Stones (20-200 mm) Gravel (2-20 mm) Sand (0.06-2 mm) Fine sediments (silt/clay, <0.06 mm)	Mud, fine sand, medium/coarse sand, gravel, stones, boulders, shell gravel, peat, till	Mud/clay/sand/Pebble/Cobble/Boulders	Mud/clay/sand/Pebble/Cobble/Boulders	Rock, Boulder > 3000 mm, Boulder 1200-3000 mm, Boulder 600-1200 mm, Large cobbles 100-600 mm, Small cobbles 60-100 mm, Gravel 2,0-60 mm, Sand 0,06-2 mm, Silt 0,002-0,06 mm, Clay < 0,002 mm, Hard clay, Mud < 0,002 mm, Concretions / Iron manganese nodules, Sandstone, Artificial substrate / Manmade structures, Peat, Tree trunks/branches	Mud <0,063 mm, clay <0,063 mm, fine sand <0,25 mm, sand 0,25-0,5 mm, coarse sand 0,5-2 mm , gravel 2-20 mm , small stones 20-200 mm , boulders >200 mm , slab-stone plate, hard clay
22.	Substrate classes: how are they determined?	Video interpretation	Optically, but validation by ground-truthing (grab and lab analysis)	Visually and capacity to resuspend	Visually	Substrates are fixed within national VELMU participants	Visually
23.	How is substrate cover interpreted? Fixed classes? Free estimates?	Percent cover	Fixed classes	+/- 10%	Fixed, 10 %	Free estimates	Percent cover
24.	How is species cover interpreted? Fixed classes? Free estimates?	See question 19 above	Fixed classes	+/- 10%	Fixed, 10 %	Free estimates	Percent cover
25.	Species requirements? E.g. is there a list if species that should be detected? If there is a list: please attach it!	Yes. See those who is suitability for identification by HD video	Yes	Dominant, clearly visually identifiable	No	See the attached Excel for interpretations. Algae and macrophytes are hard to determine	Yes

	Questions	Sweden	Germany	Lithuania	Latvia	Finland	Estonia
		(Potential typical species video identification, Sweden 2007-2013.docx). Scientific species names included in list. Colour codes: Green - Can be identified with camera Yellow - Can be identified to genus level with camera Red - Can't be identified with camera due to species specific characters or depending on the way the species grows (e.g. hidden under other species). Also attached the list of all reported typical species for the Habitats Directive 2013 (Reported typical species video Habitats Directive, Sweden 2013)				in genus level, so upper levels are preferred like "filamentous red algae". Only large and unique species can determine to genus level like *Furcellaria lumbricalis* and *Potamogeton perfoliatus*. All sessile animals (bivalvia, *Balanus improvisus, Electra crustulenta, polyps*), invertebrates like *Saduria entomon* and *Mycidae* and Fishes are detected	
26.	During the same survey: How many persons are allowed to work with interpretations? Are they intercalibrated?	Number of video interpreters is not restricted. Intercalibration of video interpreters should be performed.	2, partly	5, no official intercalibration	Not defined	All interpreters are intercalibrated. During intercalibration, interpretations are performed by 1-3 persons, but usually there is one person doing the actual interpretation.	Number of video interpreters is not restricted. Intercalibration of video interpreters should be performed

Questions	Sweden	Germany	Lithuania	Latvia	Finland	Estonia
					Finnish Parks & Wild-life's interpretations has done approximately 30-40 different persons within VELMU project but all are trained first	

Annex 3. Power calculation examples from different areas in the Baltic Sea

Table 1: Analysis of number of drop-video stations needed to detect 20% or 50% differences in biotope amount. Example from "AA.A Baltic photic rock and boulders" from a combined drop-video and grab survey in the Hanö Bight, Sweden

	HUB-class	Presences of HUB class in drop-video data	N drop-video stations (n)	N stations needed to detect a difference of 20% with 80% power	N stations needed to detect a difference of 50% with 80% power
AA.A If all habitats are sampled, HUB finest level (L5-L6)	AA.A1C1 Baltic photic rock and boulders dominated by *Fucus spp.*	23	876	13116	1753
	AA.A1C2 Baltic photic rock and boulders dominated by perennial non-filamentous corticated red algae	54	876	5402	724
	AA.A1C5 Baltic photic rock and boulders dominated by perennial filamentous algae	92	876	3038	409
	AA.A1E1 Baltic photic rock and boulders dominated by *Mytilidae*	48	876	6118	820
	AA.A1S Baltic photic rock and boulders characterized by annual algae	2	876	154205	20565
	AA.A2T Baltic photic rock and boulders characterized by sparse epibenthic macrocommunity	2	876	154205	20565

	HUB-class	Presences of HUB class in drop-video data	N drop-video stations (n)	N stations needed to detect a difference of 20% with 80% power	N stations needed to detect a difference of 50% with 80% power
AA.A if only AA.A is sampled, HUB finest level (L5-L6)	AA.A1C1 Baltic photic rock and boulders dominated by *Fucus spp.*	23	221	3069	413
	AA.A1C2 Baltic photic rock and boulders dominated by perennial non-filamentous corticated red algae	54	221	1123	154
	AA.A1C5 Baltic photic rock and boulders dominated by perennial filamentous algae	92	221	527	74
	AA.A1E1 Baltic photic rock and boulders dominated by *Mytilidae*	48	221	1303	178
	AA.A1S Baltic photic rock and boulders characterized by annual algae	2	221	38663	5159
	AA.A2T Baltic photic rock and boulders characterized by sparse epibenthic macrocommunity	2	221	38663	5159
AA.A if only AA.A is sampled, HUB L5	AA.A1C Baltic photic rock and boulders characterized by perennial algae	169	221	140	23
	AA.A1E Baltic photic rock and boulders characterized by epibenthic bivalves	48	221	1303	178
	AA.A1S Baltic photic rock and boulders characterized by annual algae	2	221	38663	5159
	AA.A2T Baltic photic rock and boulders characterized by sparse epibenthic macrocommunity	2	221	38663	5159
AA.A if only AA.A is sampled, HUB L4	AA.A1 Baltic photic rock and boulders characterized by macroscopic epibenthic biotic structures	221	221	32	8
	AA.A2 Baltic photic rock and boulders characterized by sparse macroscopic epibenthic biotic structures	2	221	38663	5159

Table 2: Analysis of number of drop-video or benthic grab stations needed to detect 20% or 50% differences in biotope amount. Example from "AA.J Baltic photic sand" from a combined drop-video and grab survey in the Hanö Bight

	HUB-class	Presences of HUB class in drop-video data	N drop-video/grab stations (n)	N stations needed to detect a difference of 20% with 80% power	N stations needed to detect a difference of 50% with 80% power
AA.J if only AA.J is sampled, HUB finest level (L5-L6)	AA.J1B1 Baltic photic sand dom. by pondweed (*Potamogeton perfoliatus* and/or *Stuckenia pectinata*)	38	255	2047	277
	AA.J1B2 Baltic photic sand dom. by *Zannichellia spp.* and/or *Ruppia spp.* and/or *Zostera noltii*	9	255	9675	1294
	AA.J1B7 Baltic photic sand dom. by common eelgrass (*Zostera marina*)	30	255	2678	361
	AA.J1E1 Baltic photic sand dom. by *Mytilidae*	2	255	44661	5959
	AA.J1Q1 Baltic photic sand dom. by stable aggregations of unattached *Fucus spp.* (typical form)	5	255	17672	2360
	AA.J1S Baltic photic sand charact. by annual algae	3	255	29667	3960
	AA.J1V Baltic photic sand charact. by mixed epibenthic macrocommunity	9	255	9675	1294
	AA.J3L Baltic photic sand charact. by infaunal bivalves	27	255	3011	406
	AA.J3L1 Baltic photic sand dom. by Baltic tellin (*Macoma balthica*)	51	255	1443	196
	AA.J3M5 Baltic photic sand dom. by multiple infaunal polychaete species: *Pygospio elegans, Marenzelleria spp., Hediste diversicolor*)	25	255	3278	441
	AA.J3N Baltic photic sand charact. by infaunal crustaceans	27	255	3011	406
	AA.J3P Baltic photic sand charact. by infaunal insect larvae	1	255	89643	11956
	AA.J4U Baltic photic sand charachterized by no macrocommunity	28	255	2892	390

	HUB-class	Presences of HUB class in drop-video data	N drop-video/grab stations (n)	N stations needed to detect a difference of 20% with 80% power	N stations needed to detect a difference of 50% with 80% power
AA.J if only AA.J is sampled, HUB L5	AA.J1B Baltic photic sand charact. by submerged rooted plants	77	255	847	117
	AA.J1E Baltic photic sand charact. by epibenthic bivalves	2	255	44661	5959
	AA.J1Q Baltic photic sand charact. by stable aggregations of unattached perennial vegetation	5	255	17672	2360
	AA.J1S Baltic photic sand charact. by annual algae	3	255	29667	3960
	AA.J1V Baltic photic sand charact. by mixed epibenthic macrocommunity	9	255	9675	1294
	AA.J3L Baltic photic sand charact. by infaunal bivalves	78	255	832	115
	AA.J3M Baltic photic sand charact. by infaunal polychaetes	25	255	3278	441
	AA.J3N Baltic photic sand charact. by infaunal crustaceans	27	255	3011	406
	AA.J3P Baltic photic sand charact. by infaunal insect larvae	1	255	89643	11956
	AA.J4U Baltic photic sand charachterized by no macrocommunity	28	255	2892	390
AA.J if only AA.J is sampled, HUB L4	AA.J1 Baltic photic sand charact. by macroscopic epibenthic biotic structures	96	255	616	86
	AA.J3 Baltic photic sand charact. by macroscopic infaunal biotic structures	131	255	366	53
	AA.J4 Baltic photic sand charact. by no macroscopic biotic structures	28	255	2892	390

Table 3: Analysis of number of drop-video stations needed to detect 20% or 50% differences in biotope amount. Example from a drop-video survey in Irbe Strait, Latvia

	HUB-class	Presences of HUB class in drop-video data	Total number of drop-video stations (n)	Number of stations needed to detect a difference of 20% with 80% power	Number of stations needed to detect a difference of 50% with 80% power
AA.M if all habitats are sampled, HUB finest level (L5-L6)	AA.M1E1 Baltic photic mixed substrate dominated by *Mytilidae*	19	204	3467	466
	AA.M1E Baltic photic mixed substrate characterized by epibenthic bivalves	102	204	385	55
	AA.M2T Baltic photic mixed substrate characterized by sparse epibenthic macrocommunity	32	204	1928	261
	AA.M4U Baltic photic mixed substrate characterized by no macrocommunity	4	204	17672	2360
	AA.M1C Baltic photic mixed substrate characterized by perennial algae	10	204	6876	921
	AA.M1S Baltic photic mixed substrate characterized by annual algae	2	204	35665	4759
AA.M, if only AA.M is sampled, HUB finest level (L5-L6)	AA.M1E1 Baltic photic mixed substrate dominated by *Mytilidae*	19	169	2817	380
	AA.M1E Baltic photic mixed substrate characterized by epibenthic bivalves	102	169	264	39
	AA.M2T Baltic photic mixed substrate characterized by sparse epibenthic macrocommunity	32	169	1542	210
	AA.M4U Baltic photic mixed substrate characterized by no macrocommunity	4	169	14585	1949
	AA.M1C Baltic photic mixed substrate characterized by perennial algae	10	169	5641	756
	AA.M1S Baltic photic mixed substrate characterized by annual algae	2	169	29491	3936

	HUB-class	Presences of HUB class in drop-video data	Total number of drop-video stations (n)	Number of stations needed to detect a difference of 20% with 80% power	Number of stations needed to detect a difference of 50% with 80% power
AA.M, if only AA.M is sampled, HUB L5	AA.M1E Baltic photic mixed substrate characterized by epibenthic bivalves	121	169	172	27
	AA.M2T Baltic photic mixed substrate characterized by sparse epibenthic macrocommunity	32	169	1542	210
	AA.M4U Baltic photic mixed substrate characterized by no macrocommunity	4	169	14585	1949
	AA.M1C Baltic photic mixed substrate characterized by perennial algae	10	169	5641	756
	AA.M1S Baltic photic mixed substrate characterized by annual algae	2	169	29491	3936
AA.M, if only AA.M is sampled, HUB L4	AA.M1 Baltic photic mixed substrate characterized by macroscopic epibenthic biotic structures	133	169	127	21
	AA.M2 Baltic photic mixed substrate characterized by sparse macroscopic epibenthic biotic structures	32	169	1542	210
	AA.M4 Baltic photic mixed substrate characterized by no macroscopic biotic structures	4	169	14585	1949

Table 4: Analysis of number of drop-video stations needed to detect 20% or 50% differences in biotope amount. Examples from a drop-video survey in Kõpu, Estonia

	HUB-class	Presences of HUB class in drop-video data	Total number of drop-video stations (n)	Number of stations needed to detect a difference of 20% with 80% power	Number of stations needed to detect a difference of 50% with 80% power
All habitats sampled	AA.A1C Baltic photic rock and boulders characterized by perennial algae	92	496	1581	215
	AA.A1E Baltic photic rock and boulders characterized by epibenthic bivalves	7	496	24677	3294
	AA.A1S Baltic photic rock and boulders characterized by annual algae	36	496	4540	609
	AA.J1B Baltic photic sand characterized by submerged rooted plants	1	496	174668	23293
	AA.J1V Baltic photic sand characterized by mixed epibenthic macrocommunity	32	496	5147	690
	AA.J4U Baltic photic sand characterised by no macrocommunity	91	496	1602	218
	AA.M1B Baltic photic mixed substrate characterized by submerged rooted plants	1	496	174668	23293
	AA.M1C Baltic photic mixed substrate characterized by perennial algae	112	496	1241	170
	AA.M1E Baltic photic mixed substrate characterized by epibenthic bivalves	57	496	2749	371
	AA.M1S Baltic photic mixed substrate characterized by annual algae	27	496	6160	825
	AA.M2T Baltic photic mixed substrate characterized by sparse epibenthic macrocommunity	24	496	6970	933
	AA.M4U Baltic photic mixed substrate characterized by no macrocommunity	2	496	87173	11627
	AB.J1V Baltic aphotic sand characterized by mixed epibenthic macroscopic community	3	496	58009	7739

	HUB-class	Presences of HUB class in drop-video data	Total number of drop-video stations (n)	Number of stations needed to detect a difference of 20% with 80% power	Number of stations needed to detect a difference of 50% with 80% power
	AB.J4U Baltic aphotic sand characterized by no macrocommunity	2	496	87173	11627
	AB.M1E Baltic aphotic mixed substrate characterized by epibenthic bivalves	7	496	24677	3294
	AB.M2T Baltic aphotic mixed substrate characterized by sparse epibenthic macrocommunity	1	496	174668	23293
	AB.M4U Baltic aphotic mixed substrate characterized by no macrocommunity	1	496	174668	23293
AA.A if only AA.A sampled	AA.A1C Baltic photic rock and boulders characterized by perennial algae	92	135	197	30
	AA.A1E Baltic photic rock and boulders characterized by epibenthic bivalves	7	135	6483	868
	AA.A1S Baltic photic rock and boulders characterized by annual algae	36	135	1002	138

Table 5: Analysis of number of drop-video stations needed to detect 20% or 50% differences in biotope amount. Examples from a drop-video survey in Karklė - Šventoji area, Lithuania

	HUB-class	Presences of HUB class in drop-video data	Total number of drop-video stations (n)	Number of stations needed to detect a difference of 20% with 80% power	Number of stations needed to detect a difference of 50% with 80% power
AA.A if all habitats are sampled, HUB L6	AA.A1C2 Baltic photic rock and boulders dominated by perennial non-filamentous corticated red algae	46	287	1880	255
	AA.A1C5 Baltic photic rock and boulders dominated by perennial filamentous algae	3	287	33430	4461
	A.A1E1 Baltic photic rock and boulders dominated by *Mytilidae*	79	287	961	132
	AA.A1I1 Baltic photic rock and boulders dominated by barnacles (*Balanidae*)	24	287	3898	524
	AA.A1S Baltic photic rock and boulders characterized by annual algae	12	287	8117	1086
AA.A if only AA.A is sampled, HUB L6	AA.A1C2 Baltic photic rock and boulders dominated by perennial non-filamentous corticated red algae	46	164	937	129
	AA.A1C5 Baltic photic rock and boulders dominated by perennial filamentous algae	3	164	18965	2533
	A.A1E1 Baltic photic rock and boulders dominated by *Mytilidae*	79	164	411	59
	AA.A1I1 Baltic photic rock and boulders dominated by barnacles (*Balanidae*)	24	164	2090	283
	AA.A1S Baltic photic rock and boulders characterized by annual algae	12	164	4501	604
AA.A if only AA.A is sampled, HUB L5	AA.A1C Baltic photic rock and boulders characterized by perennial algae	49	164	860	119
	AA.A1E Baltic photic rock and boulders characterized by epibenthic bivalves	79	164	411	59
	AA.A1I Baltic photic rock and boulders characterized by epibenthic crustacea	24	164	2090	283

HUB-class	Presences of HUB class in drop-video data	Total number of drop-video stations (n)	Number of stations needed to detect a difference of 20% with 80% power	Number of stations needed to detect a difference of 50% with 80% power
AA.A1S Baltic photic rock and boulders characterized by annual algae	12	164	4501	604

Table 6: Analysis of number of drop-video stations needed to detect 20% or 50% differences in biotope amount. Example from a drop-video survey in Germany

	HUB-class	Presences of HUB class in drop-video data	Total number of drop-video stations (n)	Number of stations needed to detect a difference of 20% with 80% power	Number of stations needed to detect a difference of 50% with 80% power
All habitats sampled, HUB L5 (whenever possible, otherwise finest level possible)	AA.B1E Baltic photic hard clay characterized by epibenthic bivalves	2	228	39898	5324
	AA.H Baltic aphotic muddy sediment	7	228	11170	1493
	AA.H1E Baltic photic muddy sediment characterized by epibenthic bivalves	1	228	80117	10686
	AA.H1Q Baltic photic muddy sediment characterized by stable aggregations of unattached perennial vegetation	1	228	80117	10686
	AA.H2T Baltic aphotic muddy sediment characterized by sparse macroscopic epibenthic biotic structures	1	228	80117	10686
	AA.I1C Baltic photic coarse sediment characterized by perennial algae	10	228	7723	1034
	AA.I1E Baltic photic coarse sediment characterized by epibenthic bivalves	2	228	39898	5324
	AA.J Baltic photic sand	22	228	3335	449
	AA.J1E Baltic photic sand characterized by epibenthic bivalves	19	228	3913	526
	AA.J1Q Baltic photic sand characterized by stable aggregations of unattached perennial vegetation	4	228	19789	2643
	AA.J1S Baltic photic sand characterized by annual algae	14	228	5425	727
	AA.J2T Baltic aphotic muddy sediment characterized by sparse macroscopic epibenthic biotic structures	6	228	13085	1749
	AA.M1C Baltic photic mixed substrate characterized by perennial algae	24	228	3031	408

HUB-class	Presences of HUB class in drop-video data	Total number of drop-video stations (n)	Number of stations needed to detect a difference of 20% with 80% power	Number of stations needed to detect a difference of 50% with 80% power
AA.M1E Baltic photic mixed substrate characterized by epibenthic bivalves	8	228	9734	1302
AA.M1F Baltic photic mixed substrate characterized by epibenthic chordates	3	228	26492	3536
AA.M1G Baltic photic mixed substrate characterized by epibenthic cnidarians	2	228	39898	5324
AA.M1J Baltic photic mixed substrate characterized by epibenthic sponges (Porifera)	1	228	80117	10686
AA.M1S Baltic photic mixed substrate characterized by annual algae	1	228	80117	10686
AB.A1G Baltic aphotic rock and boulders characterized by epibenthic cnidarians	2	228	39898	5324
AB.B2T Baltic aphotic hard clay characterized by sparse epibenthic macrocommunity	2	228	39898	5324
AB.H Baltic aphotic muddy sediment	19	228	3913	526
AB.H1G Baltic aphotic muddy sediment characterized by epibenthic cnidarians	3	228	26492	3536
AB.H2T Baltic aphotic muddy sediment characterized by sparse epibenthic macrocommunity	3	228	26492	3536
AB.I1E Baltic aphotic coarse sediment characterized by epibenthic bivalves	1	228	80117	10686
AB.J Baltic aphotic sand	10	228	7723	1034
AB.J1E Baltic aphotic sand characterized by epibenthic bivalves	15	228	5042	676
AB.J1V Baltic aphotic sand characterized by mixed epibenthic macroscopic community	2	228	39898	5324
AB.J2T Baltic aphotic sand characterized by sparse epibenthic macrocommunity	7	228	11170	1493

HUB-class	Presences of HUB class in drop-video data	Total number of drop-video stations (n)	Number of stations needed to detect a difference of 20% with 80% power	Number of stations needed to detect a difference of 50% with 80% power
AB.M Baltic aphotic mixed substrate	1	228	80117	10686
AB.M1E Baltic aphotic mixed substrate characterized by epibenthic bivalves	12	228	6382	855
AB.M1G Baltic aphotic mixed substrate characterized by epibenthic cnidarians	18	228	4148	557
AB.M1J Baltic aphotic mixed substrate characterized by epibenthic sponges (*Porifera*)	3	228	26492	3536
AB.M1V Baltic aphotic mixed substrate characterized by mixed epibenthic macrocommunity	1	228	80117	10686
AB.M2T Baltic aphotic mixed substrate characterized by sparse epibenthic macrocommunity	1	228	80117	10686

Annex 4. Note on automated analyses of zoobenthos using zooimage software – can image analyses increase cost-efficiency or accuracy in analyses of zoobenthos samples?

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Introduction

Software for automatic image analysis of organisms such as plankton already exists and has been shown to function for automatic recognition at least at coarser taxonomic levels (e.g. Bell and Hopcroft 2008). Analysis of benthic macrofauna (abundances, weight, length etc.) is a task, which is still performed manually. This manual work is a slow time consuming process (Nygård et al. 2014), which may be speeded up by automation of certain analysis steps. Biases due to differences between experts may also be reduced or eliminated by the use of automated analysis methods.

Typically time consuming manual analysis steps

- **Sorting of sample.** The samples are typically sieved in field. Specimens and other objects larger than the sieve mesh size (detritus, gravel, stones, shells etc.) are collected and brought to lab for sorting. During the sorting in lab, specimens are manually separated from other objects and counted. In many cases (in the Baltic Sea), most specimens can be identified to species or genus level immediately during the sorting without the need for extensive microscoping. This sorting is often a time-consuming task.
- **Identification of specimens.** Identification of some specimens (certain groups, damaged specimens etc.) will not be possible immediately during the sorting of the sample. Identification of these specimens will require more work, typically including microscoping. Time required for this task will vary depending on factors such as taxa found and taxonomic resolution required in the survey.
- **Weighing, measuring etc.** Additional analyses such as measuring or weighing specimens are often performed. Weights can be performed per taxonomic group or per specimen. Manual measurements are slow and time consuming tasks (Nygård et al. 2014).

The sorting step is a manual step which is always performed. In this report, it is evaluated if manual sorting and counting may be replaced with an automated image analysis of scanned samples using the software ZooImage and if the automated analysis process is faster and/or more precise than manual sorting and counting.

Methods

The ZooImage software (Grosjean and Denis 2007) was utilized in image analysis of scanned zoobenthos samples collected with Van-Veen grab in Kalmarsund (southern Baltic Sea) in 2014.

Samples were sieved and collected in plastic jars containing the sieved samples preserved in ethanol. Each sample contained the sieved zoobenthos specimens as well as detritus and gravel too large to pass through the sieve but too small to be removed manually during sieving.

In lab, each sample was placed in water in plastic Petri-dishes and scanned using a Canon Canoscan 9900F at the transparency setting at 800 dpi in colour mode. Images were saved in jpeg at the highest quality setting.

Images were imported using the Canoscan driver and GIMP (GNU Image Manipulation Programme) 2.8 and thereafter processed in the ZooImage software. ZooImage processing and analyses are performed in a stepwise process in which software such as R (R2.4.1) and Image J (1.38r) are utilized in different steps. In short images are first imported and metadata is attached. Thereafter the particles in the samples are automatically cut into individual images (vignettes) and a number of characters (size, shape etc.) of each particle are analysed and saved. Acquired data can thereafter be utilized as a training set in order to train a classifier or be analysed using an existing classifier. Several classifiers are available in ZooImage. In this case randomForest was used.

Results

The ZooImage software (Grosjean and Denis 2007) was utilized in image analysis of scanned zoobenthos samples collected with Van-Veen grab in Kalmarsund (southern Baltic Sea) in 2014.

Substitution of manual sorting by automated identification of specimens in raw sample (including other objects)

Two raw samples were analysed. The samples contained 0.5 and 0.4 l of gravel and other objects, respectively. Gravel and detritus made the scanning of zoobenthos impossible since the specimens were covered by gravel and detritus and therefore not seen in the scanned images. Also when the samples were divided into 20 sub-samples, the gravel and detritus content was too high (Figure 1). No specimens could be separated by the software among the gravel and detritus since most specimens were entirely or partly covered by gravel and detritus.

Figure 1: Example from scanned raw unsorted sample (sub-sample 1 of 20)

Identification of specimens from sorted sample

The samples above were sorted manually and specimens were separated from gravel and detritus and scanned. A randomForest classifier was created in

ZooImage using specimens from these samples plus extra specimens from five other samples in order to increase the number of specimens, especially for less abundant species.

In order to test the classifying capacity of ZooImage this classifier was used to classify the scanned specimens (Table 1). Species that were abundant in the training set were very well classified (e.g. *Marenzelleria sp.* of which 100 % of the specimens were correctly classified) while the rarest species in the training dataset were usually wrongly classified. The samples also contained a few specimens of *Saduria entomon* but this species could not be included in the analysis since there were too few specimens for training of the classifier.

Table 1: Classification results from randomForest classifier in ZooImage

		Classified in ZooImage by randomForest classifier							
		Asellus	***Bylgides***	**detritus**	***Halicryptus***	***Macoma***	***Marenzelleria***	***Mytilus***	**letters**
Manually identified	***Asellus***	1	0	0	0	0	5	0	0
	Bylgides	0	5	0	0	0	1	0	0
	detritus	0	0	10	0	0	1	0	3
	Halicryptus	0	0	0	0	2	1	1	0
	Macoma	0	0	0	0	50	0	2	0
	Marenzelleria	0	0	0	0	0	73	0	0
	Mytilus	0	0	0	0	4	2	28	0
	Letters	0	0	1	0	0	0	0	15

Note: Detritus are parts of specimens or other detritus remaining after sorting. “Letters” are small transparent letters printed in the bottom of the Petri dishes by the manufacturer (these are also identified as objects in ZooImage). This table includes both samples together.

Automatic measurements of specimens for calculation biovolume and biomass

ZooImage provides a large number of values characterising each scanned specimen retrieved from automatically created miniature images (vignettes) in ImageJ. These measurements can be used for automated analyses of biovolume, biomass, length and other parameters. In order to use such measurements for biomass and/or biovolume values such as a length or similar should be related to biovolume or biomass, e.g. from the literature. Secondly, the parameter used in this relation (e.g. the length of a certain body part of a species) needs to be related to a parameter retrieved during the image analysis. This is performed by manual measurements of the vignettes in ImageJ.

The ZooImage software was however designed for zooplankton species and not for zoobenthos. Several groups (e.g. most worms) are soft and lay curved, bent or coiled in any position in the scanned samples. They may also be retracted or protracted, which significantly affects the length. Many crustaceans (e.g. amphipods) are also usually more or less bent. This makes establishment of such relations much harder for zoobenthos than for many zooplankton species (e.g. copepods have comparably hard and distinct prosomes that can be measured).

Automated biomass and biovolume measurements of zoobenthos using currently available software should therefore be restricted to groups with hard bodies or hard and distinct body parts that appear clearly and may be measured in scanned images. The applicability of such measurements for species included in this test is listed in Table 2. These recommendations are applicable also for other species with similar characteristics (e.g. not only *Marenzelleria*, but also other long and slender worms will be impossible or difficult to measure with this technique whereas almost all bivalves will be suitable).

Table 2: Suitability for automated biomass or biovolume calculations in ImageJ for species scanned during this test

Species	Suitability for automated biomass or biovolume calculations: 3 = good, 2 = potential, 1 = not suitable	Reason/comment
Asellus aquaticus	2	May be bent. Dorsal or ventral side may be seen in the image.
Bylgides sarsi	1	Often bent. Fragile and often damaged.
Halicryptus spinulosus	1	Soft body in varying shapes.
Macoma balthica	3	Hard and distinct shell. Open shells are possible problems.
Marenzelleria sp.	1	Soft body and normally coiled.
Mytilus edulis	3	Hard and distinct shell. Open shells are possible problems.
Saduria entomon	2	May be bent. Dorsal or ventral side may be seen in the image.

Note: With future software development, more groups may be suitable for automated biomass or biovolume calculations.

Due to their shape and hard shells, bivalves are well suited for use in automated analyses. Nygård et al. successfully performed automated length measurements of the bivalve *Macoma balthica* in ImageJ (used for image analysis in ZooImage) within the MARMONI project. In addition to this testing, they also developed the software ACSA (Aquatic Crustacean Scan Analyzer), which may potentially also be used for size measurements of amphipods and polychaete worms. Such measurements can be related to biomasses and biovolumes using the approach described above. ImageJ utilized in the ZooImage software only provides the longest perimeter of the measured specimens, which makes it less useful for other groups than bivalves.

Discussion and conclusions

The aim with this testing was primarily to identify a method for increased cost-efficiency and/or accuracy in analyses of macrofauna abundance and bio-volumes by the substitution of manual laboratory work with automated image analysis steps. The tested software is technically functional, but with most zoobenthos species and present grab sampling technique this automated method still provides very few advantages over manual laboratory analyses.

Automated abundance analyses don't increase time efficiency because manual sorting is needed anyway since large amounts of detritus and/or gravel in most samples make scanning of raw samples impossible. The classifying capacity for zoobenthos was therefore tested with already sorted samples. The powerful randomForest algorithm (Breiman 2001) was used as a classifier. Common species in the training data were very well classified while rare species were usually wrongly classified. This result was expected since randomForest is known to classify common classes in the training data better than rare (e.g. Yao et al. 2013). A large and balanced (all classes of equal size) training dataset is therefore recommended. Training of a good classifier will demand both time and effort, but it only needs to be performed once if all species in the area are included in the training dataset.

For automatic analyses of biovolumes and biomasses, good measurements of lengths or similar are needed. The tested software (ImageJ with the ZooPhytoImage plugin) could not provide useful measures for biomass or biovolume for any zoobenthos species other than bivalves. With further development, different software may provide this function for more groups in the future. For example, the new software ACSA may be adjusted to function for groups such as polychaetes and amphipods (Nygård et. al 2014).

For the purpose of determining dominating group by biovolume for classification of grab-data into HUB-classes (HELCOM Underwater Biotope and habitat classification, HELCOM 2013), visual judgments during sorting of samples will often be sufficient and much faster than manual or automated analyses.

For specific purposes when biovolume, biomass or length distribution data are needed, image-analysis software may provide cost-efficient alternatives to conventional manual laboratory analyses. At present, such automated analyses are only possible for very few zoobenthos species but further technical development may increase the functionality. Some manual work will most likely be needed in the process anyway, such as manually separating specimens before scanning or in the scanned images in order to avoid errors due to analyses of adjacent specimens as a single large specimen.

In general, automated methods may potentially increase accuracy since biases due to different knowledge levels of experts are avoided. In this case, further development of the technique is needed in order to reach and exceed accuracy from manual analyses.

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