WATERS

EVALUATION OF METHODOLOGICAL ASPECTS ON DATA COLLECTION FOR ASSESSING ECOLOGICAL STATUS OF VEGETATION ACCORDING TO THE WFD

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WATERS is a five-year research programme that started in spring 2011. The programme's objective is to develop and improve the assessment criteria used to classify the status of Swedish coastal and inland waters in accordance with the EC Water Framework Directive (WFD). WATERS research focuses on the biological quality elements used in WFD water quality assessments: i.e. macrophytes, benthic invertebrates, phytoplankton and fish; in streams, benthic diatoms are also considered. The research programme will also refine the criteria used for integrated assessments of ecological water status.

This report is a deliverable of one of the scientific sub-projects of WATERS focusing on macrophytes in coastal waters. The aim of this report is to present and discuss the results from testing a field method that sample a fixed area with a fixed taxonomic effort and is stratified to either hard or soft substrate. We evaluate spatial, temporal and methodological variability for macrophyte indicators previously identified within the WATERS programme

WATERS is funded by the Swedish Environmental Protection Agency and coordinated by the Swedish Institute for the Marine Environment. WATERS stands for 'Waterbody Assessment Tools for Ecological Reference Conditions and Status in Sweden'. Programme details can be found at: http://www.waters.gu.se

Contents

Summary

Previous studies within the Waters project have identified a number of indicators for ecological quality of coastal vegetation (species richness, cumulative cover, community complexity and for soft substrates also a sensitivity index, MI) that could replace or complement the current indicator. Here, we present and discuss the results from testing of a field method for collecting data for these indicators. The method was chosen to overcome some of the limitations of the current transect method mainly used for vegetation surveys in Sweden and included a fixed sampling size, a defined taxonomic effort and substrate specific sampling. We also evaluated spatial, temporal and methodological variability of the indicators using the WATERS uncertainty framework, where general linear models are specified in order to capture temporal and spatial interactions and variability in sampling. Data were collected in three coastal areas on the west and east coasts of Sweden.

The results indicate that a fixed sampling size, substrate specific sampling and defined taxonomic level together reduce variation in data for the proposed hard substrate indicators compared to the current field method. As species richness increases with the size of the area sampled, a valid assessment of this indicator requires a fixed sampling size. Fixed sampling size is not as important for cover estimates as for species richness, but reduces the variation between divers.

The residual variation was lower for species richness than for cumulative cover in most of the tested models for both hard and soft substrates and lower on hard than soft substrates. Also the soft substrate MI-index based on number of species performed slightly better than the MI-index based on cover. One possible reason for the poorer performance of the cover-based indicators in the soft substrate vegetation communities is the difficulties in estimating cover for species composed of long, slim stalks without leaf canopy, which results in interpretational differences between divers.

The variation between years was only evaluated for hard substrates and was generally small compared to the within-area variation. The results indicate that sampling more sites per area every second or third year would reduce uncertainty in the status assessment more than sampling fewer sites in the same areas yearly during a six-year cycle.

Svensk sammanfattning

Tidigare studier inom WATERS har identifierat ett antal potentiella ekologiska indikatorer (artrikedom, kumulativ täckningsgrad, strukturell komplexitet och för mjukbotten även ett känslighetsindex, MI) för kustnära vegetation som kan ersätta eller komplettera den nuvarande indikatorn MSMDI. I denna rapport presenterar och diskuterar vi resultaten från test av en fältmetod för att samla in data för dessa nya indikatorer. Den testade metoden valdes för komma till rätta med några av begränsningarna i den transektmetod som främst används för vegetationsundersökningar idag. I den testade metoden ingår en given storlek på provtagningsyta, en definierad artbestämningsnivå och substratspecifik provtagning. Vi utvärderade även rumslig, temporal och metodologisk variation i indikatorerna med hjälp av Waters osäkerhetsramverk där linjära modeller specificeras för att fånga variabilitet i provtagning samt interaktioner i tid och rum. Data samlades in i tre områden på svenska väst- och ostkusten.

Resultaten visar att en given storlek på provtagningsyta, substratspecifik provtagning och definierad taxonomisk nivå tillsammans minskar variationen i data för de föreslagna indikatorerna för hårdbotten jämfört med den nuvarande fältmetoden. Eftersom artrikedomen ökar med storleken på provtagningsytan krävs en given provstorlek för att kunna göra en god bedömning av denna indikator. En given storlek på provtagningsytan är inte lika viktigt för täckningsgradsbaserade indikatorer som för artrikedom, men minskar provtagningsvariationen framförallt mellan dykare.

För både hårda och mjuka bottnar var den återstående variationen lägre för artrikedom än för kumulativ täckningsgrad och lägre på hårda än mjuka bottnar. Även MI-indexet baserat på antal arter på mjukbotten fungerade något bättre än MI-indexet baserat på täckningsgrad. En möjlig orsak till att de täckningsgradsbaserade indikatorerna fungerar sämre för mjukbottensamhällen är att det finns större utrymme för tolkningsskillnader mellan dykare när det gäller bedömningen av täckningsgrad i förhållande till skottäthet hos vegetationen. Detta återspeglar sannolikt svårigheterna i att uppskatta täckningsgraden för arter som består av långa, smala stjälkar utan bladkrona eller motsvarande, vilka utgör en större andel av arterna på mjukbotten jämfört med hårdbotten.

Variationen inom område var större än variationen mellan år. Detta antyder att provtagning av fler stationer per område t ex vartannat eller vart tredje år skulle ge en minskad osäkerhet i bedömningen över en sexårscykel jämfört med årliga provtagningar med färre stationer.

Introduction

Coastal vegetation is an important component of coastal ecosystems and proper management of coastal areas requires monitoring that can detect and follow vegetation changes over time. For instance, the EU Water Framework Directive (WFD) requires that all member states classify ecological status of coastal macrophytes. The current Swedish indicator for coastal macrophytes, the Multi Species Maximum Depth Index, MSMDI (see description in Blomqvist *et al.* 2012), is based on depth limits of selected eutrophication-sensitive perennial species. Previous studies within the WATERS project have identified a number of indicators for coastal vegetation that could replace or complement the current indicator (Blomqvist *et al.* 2014, Wikström *et al.* 2016). These include species richness, cumulative cover, community complexity and a macrophyte sensitivity index.

The choice of field methods for sampling of coastal vegetation data is essential for which indicators that can be calculated, the uncertainty of these indicators and hence which assessments that can be performed. The most common field method for monitoring macrophyte communities in Sweden is to record cover of substrate and macrophyte taxa in more or less homogenous segments of diving transects (e.g. Kautsky 1992, Blomqvist *et al.* 2012). The diver swims from deeper to shallower depths and starts a new segment if a new species appears or if the composition of species or substrate changes. Segments can thus have different lengths and span different depth intervals. This method is useful for a qualitative description of vegetation along a transect but it has limitations when it comes to generating data for calculation of the proposed new indicators (Blomqvist *et al.* 2014).

The desired taxonomic resolution for the proposed indicators can only be achieved by sampling using SCUBA technique. However, monitoring of species richness requires a fixed sampling size to be repeatable, since species richness is strongly dependent on sampling effort. Blomqvist *et al.* (2014) consequently reported a significant relationship between segment length and taxon richness in the segment. In the transect method comparisons of species richness will thus be uncertain as the sample size differs between divers, between sites and between sampling occasions on the same site.

In addition to the problem of a variable sampling effort, analyses of monitoring data collected with the transect method has indicated a large variability in the data, which limits the possibility to detect changes. One factor that may contribute to this variation is that a transect segment can and often do cover both hard and soft substrates. In the current transect method substrate in the sections is described by estimating cover of each occur-

ring substrate class (rock, boulders, stones, gravel, sand, soft sediments or other). Cover estimates and species observations are however made in relation to the area of a section regardless of substrate. As the largest differences in macrophyte community composition are between hard and soft substrates (e.g. Kautsky & van der Maarel 1990), one way to reduce variation in the vegetation data may be to stratify sampling to either stable hard substrates or soft substrates and estimate substrate-specific cover.

Another factor that may contribute to the variation is that the taxonomic resolution differs between divers and surveys (Blomqvist *et al.* 2014). This variation could be reduced by specifying regional lists of which taxa to include and to what taxonomic level they should be identified. This would increase the taxonomic coherence between different datasets allowing valid comparisons of species richness between different surveys. The Swedish coastline is characterized by a salinity gradient ranging from around 30 psu on the west coast to 0 psu in the northern Bothnian Bay. Salinity is an important factor influencing composition of the benthic macrophyte communities. Therefore, the levels of taxonomic determination need to be specific to different regions of the Swedish coast, e.g. based on the large sea basins, Skagerrak, Kattegat, South coast, Baltic Proper, Bothnian Sea and Bothnian Bay.

In the WATERS project, we have used a field method that fulfils these new demands for sampling of hard substrate macroalgae communities on both the west and east coasts and also the soft substrate communities on the east coast. The soft substrate vegetation on the more saline west coast is often dominated by a single species – the eelgrass (*Zostera marina*). The indicators suggested for this vegetation can be monitored with video-based methods, which are not further discussed in this report.

The aim of this report is to present and discuss the results from testing a field method where a fixed area is investigated with a fixed taxonomic effort and stratified to either hard or soft substrate. We evaluate spatial, temporal and methodological variability for the most promising indicators identified in Blomqvist *et al.* (2014) and Wikström *et al.* (2016) (species richness, cumulative cover, community complexity and the macrophyte sensitivity index) using the uncertainty framework developed in the WATERS project. We further report on resource requirements for the method as well as practical experiences from the field work.

Material & Analyses

Material

The field method was used within the Waters project for sampling hard substrate communities on the east and west coasts, as well as soft substrate communities on the east coast. The text below gives brief outlines of the method and our field studies in order to describe the data used in the analyses. More detailed descriptions are found in annex 1.

The field method we used includes a defined sampling size, substrate specific sampling, defined taxonomic resolutions and sampling using SCUBA-technique (annex 1). The sampling size was a rectangular area of 25 m^2 or 16 m^2 (5x5 or 4x4 m squares) that was placed on an area where either hard (rock, boulders and stones) or soft (sand, clay and mud) substrate dominated. Estimates of substrate and macrophyte cover were made on a continuous percentage scale and substrate-specific (species observations and cover in relation to either hard or soft substrate). A predetermined taxonomic resolution was used for species determinations in order to reduce variation between different divers. Annex 2 lists the taxonomic resolution used for the Baltic Proper as an example. The proposed list is based on an extensive database combined with expert knowledge on which species determinations are feasible in the field, i.e. *in situ*, and which are necessary to verify in the laboratory.

Our field studies were conducted in three coastal areas (Figure 1). Firstly, the field method was tested in two well-described pressure gradients on both the west and east coast of Sweden during 2012 and 2013 ("gradient study"). The response of vegetation indicators to the pressure indicators are reported in Wikström *et al.* (2016). Here we use the data to test temporal, spatial and spatio-temporal variability of the indicators. Hard substrate communities were sampled in five areas on the west coast and seven areas on the east coast in 2012 and again in 2013 when 8 of 10 sampling sites per area were revisited (Table 1). On each site, an area of 25 m^2 was sampled within the depth interval 2.2-5.9 m. Soft substrate communities were sampled with this method only in the seven east coast areas. Sampling was done both years but in order to reduce the variation (by reducing the number of sites without vegetation) on soft substrates, most of the sites were relocated in 2013. Therefore, only data from the last year was used in the analyses.

During the second year of the gradient study, three 4x4 m squares were sampled adjacent the 5x5 m square on two hard substrate sites per area in three of the east coast areas and

all five west coast areas (Table 1). This data was used to evaluate within-site variation in the indicators and the effect of square size on the amount of time required for the inventory.

Secondly, a smaller dataset was collected outside Västervik on the Swedish east coast (Figure 1). Here, a number of squares were surveyed by seven divers, in order to estimate the variability in sampling using subjective cover estimates according to the method. Fourteen 5x5 m squares were placed on three sites in order to sample different macrophyte communities. Five squares were sampled in a red algal community on hard substrate, where they were placed along the depth curves in the depth interval 4.9-6.4 m. A shallower *Fucus*-community was sampled by placing four squares on hard substrate between 0.9-2.8 m depths. The five squares sampled on soft substrate were placed adjacent each other in a line from 2 to 5 m depth and thus a depth gradient was sampled at this site. All seven divers individually sampled all 14 squares (Table 1).

During the studies, sampling characteristics such as dive time, time to locate new sites etc. were collected to enable an estimation of time requirements when sampling according to this method. Also, as species rich communities can be expected to require longer sampling times, the logged dive times were analysed as a function of the indicators and some other factors expected to affect sampling time in order to assess which factors that affect resource requirements.

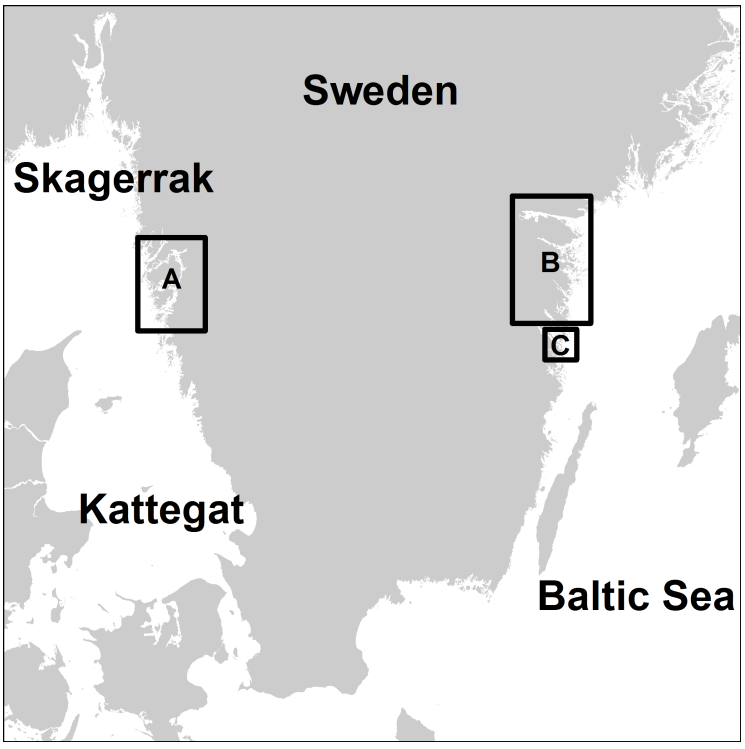

Figure 1. The location of the survey areas in the gradient study on the west (A) and east (B) coast and the Västervik study (C). More detailed maps of the study areas are presented in annex 1.

Table 1 The number of sampling sites in each area the years 2012 and 2013 on the east (A) and west (B) coasts respectively and the number of squares sampled in 2014. Large squares (5x5 m and 4x4 m) were sampled using SCUBA-technique on hard substrate sites and also on east coast soft substrate sites. The sampling period is also shown.

A. EAST COAST		**Hard substrate**				**Soft substrate**	
		2012	**2013**		**2014**	**2013**	**2014**
Area	Code	5x5 m	5x5 m	4x4 m	5x5 m	5x5 m	5x5 m
Inner Bråviken	IB	10	8			10	
Inner Slätbaken	IS	10	8	6		10	
Kaggebofjärden	KAF	10	8			10	
Outer Bråviken	OB	10	8			10	
Trännöfjärden	TF	9	8	6		10	
Lindödjupet	LD	10	8			10	
Kärrfjärden	KRF	10	8	6		10	
Västervik					7*9		7*5
Total number of samples		69	56	18	63	70	35
Sampling period (dd/mm)		20/8 - 7/9	29/7 - 5/8		10-12/6	29/7 - 5/8	10-12/6

B. WEST COAST		**Hard substrates**		
		2012	**2013**	
Area	Code	5x5 m	5x5 m	4x4 m
Byfjorden	BYF	8	8	6
Havsstenfjorden	HAV	10	8	6
Askeröfjorden	ASK	10	8	6
Hake fjord	HAK	10	8	6
Marstrandsfjorden	MAR	9	8	6
Total number of samples		47	40	30
Sampling period (dd/mm)		20/8 - 25/9	13/8 - 20/9	

Analyses

We used the WATERS uncertainty framework (Lindegarth *et al.* 2013, Bergström & Lindegarth 2016), where general linear mixed models are specified in order to capture temporal and spatial interactions and variability in sampling. The models include random (CAPITAL letters) and fixed (lowercase letters) factors and interactions.

According to the framework, all factors influencing the variance should be analysed together. However, as data was sampled in slightly different manners in the sub-studies (e.g.

different sampling area, multiple years only for hard substrates in one sub-study, and multiple divers only in one study) the data cannot be used as one dataset with all levels in the same analysis. This most likely means that the estimates of temporal and spatial variability will be too high, as it also incorporates variability between divers.

All variance estimates are given as Standard deviations. Relative uncertainty is calculated as StDev/mean.

Tested models

Hard substrates

Variability between areas and years was analysed with the model $y = \mu + area + YEAR + area*YEAR$, using data from the gradient study. Within-area variation in the data was modelled using the 4x4 m squares, where $y = \mu + area + SITE(area)$. Both the gradient study data and the 4x4 m squares were sampled at similar depths, using the same method. However, the residual variation in the gradient study data comprises the variation both within areas and sites as well as between divers, whereas the residual variation, for the within-area analysis, only contains within site and between divers variation. As only three east coast areas (out of seven) from the gradient study were sampled in the within-site analysis, the results are not fully comparable. Differences between divers (East coast only) were analysed using data from the Västervik study as $y = \mu + site + DIVER + site*DIVER$, where the additional variance due to differences between divers was tested.

Soft substrates

As soft substrate in the gradient study was sampled at different locations in 2012 and 2013, only data from 2013 was used in the analysis. Thus only area as fixed factor could be tested, $y = \mu + area$. Variance between divers (east coast only) was analysed using $y = \mu + DIVER$.

Sampling time

The effect of vegetation species richness, cumulative cover, community complexity, water transparency and siltation on the time required to sample a square (i.e. dive time) was tested with linear models, using data from the gradient study sampled in 2013.

Tested response variables

The indicators used as response variables in the tests are taken from Blomqvist *et al.* (2014) and Wikström *et al.* (2016) where we identified promising indicators for status assessment based on macrophytes. An overview of the indicators is presented in Table 2.

Table 2 Description of indicators used in this study. Further information is given in the text below the table.

Indicator	Description	Hard	Soft
Species richness	Number of taxa. Crust-forming taxa were excluded.	X	X
Cumulative cover	Sum of cover of individual taxa including epiphytes and free-living taxa. Crust-forming taxa were excluded. Can exceed 100% as species can grow in different layers.	X	X
Community complexity	Cumulative cover divided by total cover. Higher complexity indicates more layers.	X	
MI_c	Macrophyte sensitivity index, species counts, based on Hansen (2012), see Equation 1		X
MI_a	Macrophyte sensitivity index, species abundance (cover), based on Hansen (2012), see Equation 2		X

The macrophyte indices (MI) was calculated according to Hansen (2012), using the equations:

$$MI_c = \frac{N_s - N_t}{N} \times 100 \qquad \text{Equation 1}$$

$$MI_a = \frac{\sum_{i=1}^{N_s} A_i - \sum_{j=1}^{N_t} A_j}{\sum_{k=1}^{N} A_k} \times 100 \qquad \text{Equation 2}$$

where N_S is the number of sensitive species, N_t is the number of tolerant species, and N is the total number of species (including species without sensitivity classification), and A is a measure of cover. Both versions of the index produce values from -100 (all species tolerant) to +100 (all species sensitive). Classification of sensitivity to eutrophication was based on literature and is given in Wikström *et al.* (2016).

Results

Hard substrate

All tested indicators differed significantly between areas (Figure 2), see Wikström et al. (2016) for details. The variation between years and interaction between year and area were significant only for species richness on the east coast (Table 3); in two of the areas (OB and KRF), there was a clear difference between years (Figure 2). However, for several of the indicators the variation between years and/or interaction between area and year was as much as 15-20 % of the residual variation, although non-significant (Table 3). For cumulative cover and community complexity on the east coast, the variation between years was similar to or higher than that between areas between years, indicating a general difference between years across the study areas. On the west coast the variation was lower for all components, but higher for the interaction term than for year for all indicators. This implies that the difference between years is not consistent across the areas (Figure 2).

The residual variation, incorporating spatial and methodological variation, was considerably larger: 16-25 % on the west coast and 22-40 % on the east coast. It was higher for cumulative cover than species richness and community complexity and higher in the east coast compared to the west coast study area.

The analysis of within-area variation (based on 4x4-m squares) suggested that at the east coast, a large part of the spatial variation can be attributed to differences between sites within an area (Table 4). In contrast, the variation was small between sites within the west coast areas. The within-site variation was similar for all indicators and in both coastal areas (12-19 %). The residual uncertainty was also lower than for the between-area analysis, but with the difference that it contains only the variation within site, whereas the between-area analysis comprises the variation both within areas and sites.

Variation between divers in the Västervik study was relatively small (10-11%) but significant for all indicators (Table 5). For species richness and community complexity there was also a significant interaction between diver and site, showing that the effect of divers differed between the two hard substrate sites. The residual variation was similar in magnitude to the 4x4-m squares sampled in the gradient study, which supports that within-site variation was between 10-20 % in these communities.

Table 3 Variability between areas and years on hard substrate in the Gradient study data, *y = μ + area + YEAR + area*YEAR*. Significant relationships in bold (p<0.05).

Indicator	**Mean value**	**Variance estimates**			**Relative Uncertainty**		
East coast		Year	Year:Area	Residual	Year	Year:Area	Residual
Species Richness	6.168	**0.386**	**0.708**	1.684	**0.063**	**0.115**	0.273
Cumulative cover	89.048	6.234	6.117	35.741	0.070	0.069	0.401
Community Complexity	1.3192	0.041	0.000	0.284	0.031	0.000	0.215
West coast		Year	Year:Area	Residual	Year	Year:Area	Residual
Species Richness	15.61	0.082	0.558	2.808	0.005	0.036	0.180
Cumulative cover	158.82	0.000	0.000	40.203	0.000	0.000	0.253
Community Complexity	2.24	0.000	0.026	0.352	0.000	0.012	0.157

Table 4 Within-area variation (4x4 m squares) on hard substrate, *y = μ + area + SITE(area)*. Significant relationships in bold (p<0.05).

Indicator	**Mean value**	**Variance estimates**		**Relative Uncertainty**	
East coast		Site:Area	Residual	Site:Area	Residual
Species Richness	6.06	**0.94**	0.78	**0.16**	0.13
Cumulative cover	87.22	**28.65**	16.58	**0.33**	0.19
Community Complexity	1.20	**0.19**	0.14	**0.16**	0.12
West coast		Site:Area	Residual	Site:Area	Residual
Species Richness	15.53	0.47	2.46	0.03	0.16
Cumulative cover	160.60	11.84	22.63	0.07	0.14
Community Complexity	2.20	0.16	0.41	0.07	0.19

Table 5 Difference between divers (East coast only), hard substrate, *y = μ + Site + DIVER + Site*DIVER*. Significant relationships in bold (p<0.05).

Indicator	**Mean value**	**Variance estimates**			**Relative Uncertainty**		
		Diver	Diver*Site	Residual	Diver	Diver*Site	Residual
Species Richness	9.46	**0.97**	**1.31**	1.16	**0.10**	**0.14**	0.12
Cumulative cover	145.86	**14.91**	5.69	17.44	**0.10**	0.04	0.12
Community Complexity	1.55	**0.16**	**0.12**	0.19	**0.11**	**0.08**	0.12

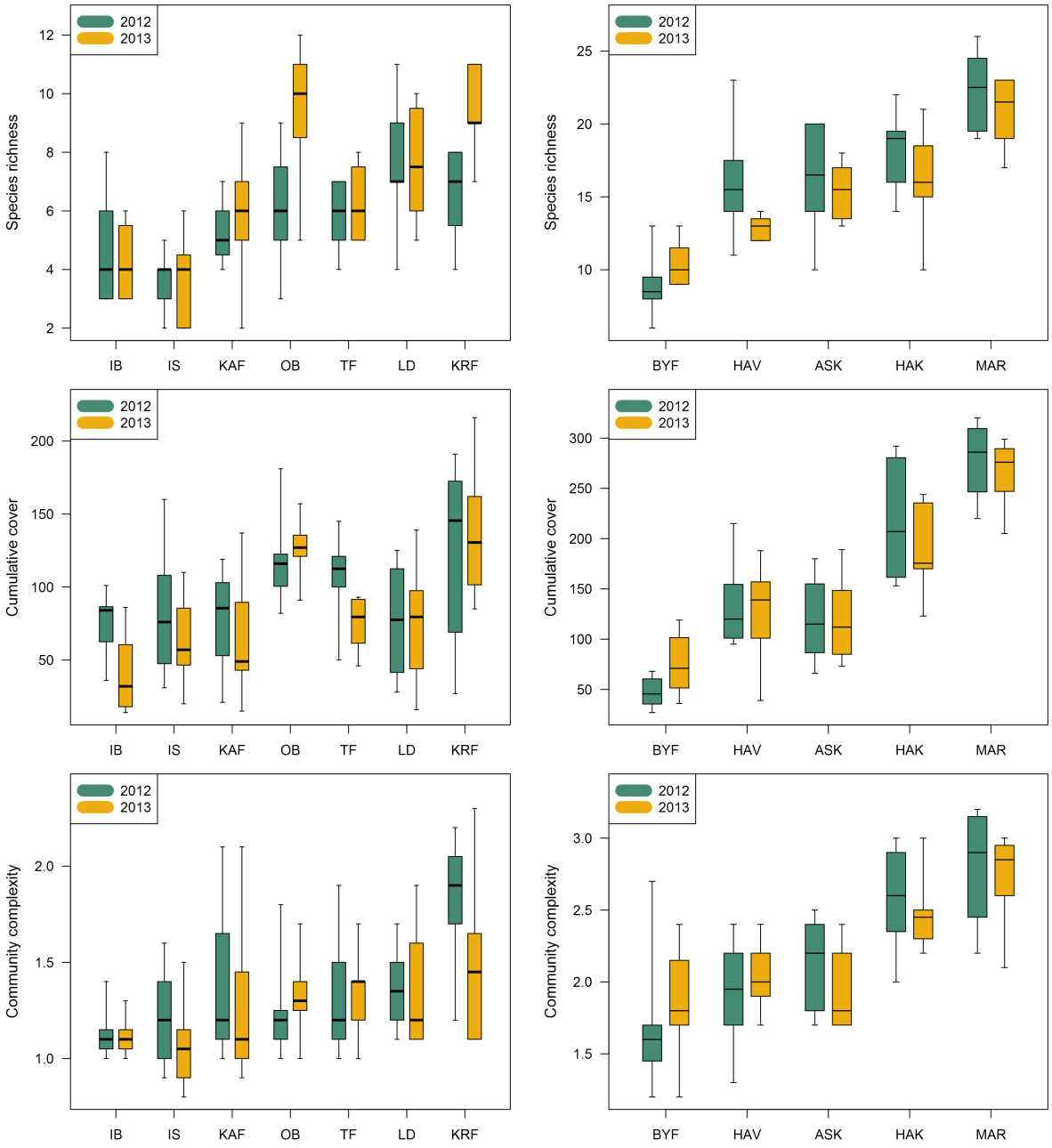

Figure 2. Hard substrate indicators, east coast areas in the left column, west coast in the right. Each box shows the first and third quartile with a horizontal line at the second quartile (median). The whiskers represent minimum and maximum values. For species richness on the east coast there was a significant interaction between area and year; the only difference that was consistent between years was a lower species richness in IS and IB compared to LD and KRF.

Soft substrate on the east coast

As only one year's sampling of soft substrate in the gradient study was used in the analysis, only area as fixed factor could be tested. The residual uncertainty was large compared to that for hard substrates (Table 6). The residual uncertainty was higher for cumulative cover than for species richness, and also higher for abundance-based MI than for count based MI. Although the uncertainty was higher overall for the Mi_c and Mi_a than for species richness and cumulative cover, this indicates lower variation in indicators based on number of species than indicators based on cover for soft substrates. The tested indicators differed significantly between areas (Figure 3), see Wikström et al.(2016) for details.

In the Västervik study, soft substrate was sampled at one site. Only for cumulative cover, the differences between divers were significant and the variance for diver was similar to the residual variance (Table 7). For the remaining indicators, the residual variance was markedly larger than the variance between divers. However, as the squares were placed along a depth gradient, higher residual variance is expected compared to the hard substrate squares which were placed within narrow depth intervals.

Table 6 Gradient study (east coast 2013), soft substrate, y = μ + area. Significant relationships in bold (p<0.05).

Indicator	**Mean value**	**Variance estimates**	**Relative Uncertainty**
		Residual	Residual
Species Richness	5.20	1.97	0.38
Cumulative cover	86.96	59.21	0.68
MI_c	-52.74	24.27	0.46
Mi_a	-47.67	45.86	0.96

Table 7 Difference between divers (east coast only), soft substrate, $y = \mu + DIVER$. Significant relationships in bold (p<0.05).

Indicator	**Mean value**	**Variance estimates**		**Relative Uncertainty**	
		Diver	Residual	Diver	Residual
Species richness	8.17	0.39	1.22	0.05	0.15
Cumulative Cover	94.00	**29.17**	26.75	**0.31**	0.28
MI_c	-9.85	2.37	13.88	0.24	1.41
MI_a	-19.25	0.00	49.02	0.00	2.55

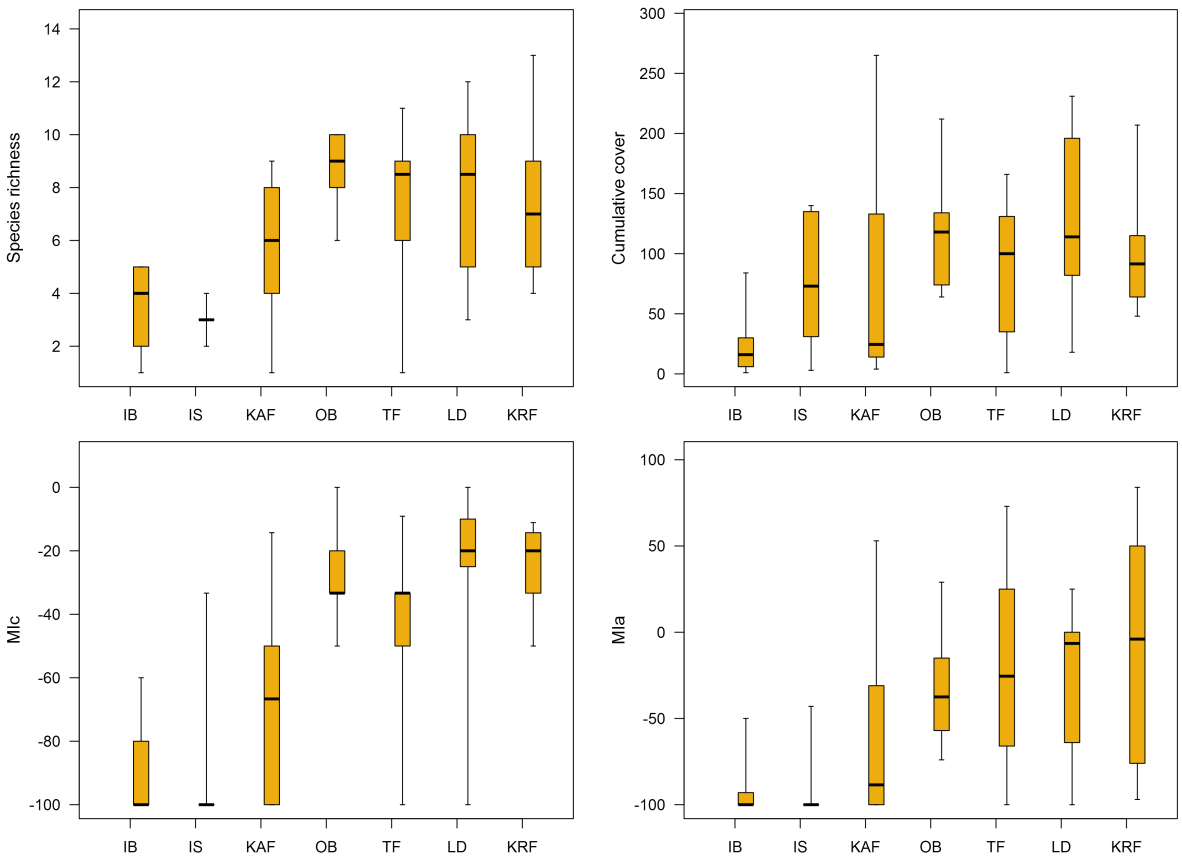

Figure 3. Soft substrate indicators. Each box shows the first and third quartile with a horizontal line at the second quartile (median). The whiskers represent minimum and maximum values.

Sampling time

Hard substrate

Time notations when sampling according to the square method showed that sampling the more diverse west coast communities required twice as long time as sampling the east coast communities. The mean dive time for the west coast sites in 2013 was 42 ± 2.1 minutes (± 95 % confidence interval) based on 40 squares (25 m^2). The corresponding time for the east coast was 19 ± 1.2 minutes based on 56 squares (25 m^2). The dive time included descent along buoy line, locating suitable area fulfilling specified criteria (annex 1), marking the square, cover estimates, depth measurements, photo documentation, removing the square markings and ascent to surface. This dive time does not include locating new sites, but is based on visiting known sites. The mean dive time, including only cover estimates and depth measurements, based on seven divers sampling 58 squares on the east coast was 13 ± 0.7 minutes.

The variation in sampling time between areas and sites was mainly due to differences in vegetation on the west coast. On the west coast, there were significant positive relationships between increasing species richness, cumulative cover, community complexity and longer dive times (Table 8). Sampling a square in the west coast area with the highest spe-

cies richness, cumulative cover and community complexity required 48 ± 1.9 minutes compared to 33 ± 4.4 minutes in the area with lowest indicator values. On the East coast the sampling time was more similar between areas and not significantly dependent on vegetation cover or composition. However, there was a weak, but significant relationship between increasing siltation and longer dive times. Water transparency (Secchi depth) did not affect the sampling time on either coast.

Table 8 Dive time for hard substrates on the east and west coasts as well as soft substrate on the east coast, vs. indicators, water transparency and siltation, sign of relationship and r2. Significant relationships in bold figures.

Dive time vs.	West coast		East coast		East coast soft
Species richness	+ **0.498**	-	0.059	+	**0.065**
Cumulative cover	+ **0.507**	-	0.020	+	0.045
Community complexity	+ **0.346**	+	0.039	+	0.049
Water transparency	+ 0.080	+	0.013	+	0.007
Siltation	- 0.030	+	**0.095**	-	0.003

Reducing the size of the sampling area from 25 m² to 16 m² only marginally affected the sampling time. The time benefit when sampling 16 instead of 25 m² was less than 5 minutes in both west and east coast vegetation communities.

Soft substrate

The dive time required for sampling soft substrate squares on the east coast was 15 ± 0.6 minutes based on 70 squares. This included all underwater activities in the method, whereas only the cover estimates and depth measurements took 13 ± 1.2 minutes (based on seven divers sampling five squares). Similar to the hard substrate squares on the east coast, the sampling time was not dependent on vegetation, water transparency or siltation (Table 8).

Practical experiences

To use a continuous scale for cover estimation instead of the 7-graded scale (1, 5, 10, 25, 50, 75 and 100 %) used in the current transect method was generally appreciated by the divers. However, it was recognized that the estimates were not truly continuous. The cover estimates for macrophytes in the method study were generally in 5 % steps, i.e. 25, 30, 35 % etc. although, in the low range, 1-15 % coverage, more detailed estimates were made and above 40 % coverage the estimates tended to be in 10 % steps. This suggest that the seven-graded scale is too coarse and that a finer graded scale should be used.

Results based on our field studies show large variation both between and within sites even though we employed stratification to reduce that variation using specific criteria including depth interval, substrate slope, proportion of substrate type in square etc. The results indicated a within-site variation between 10-20 % in hard substrate communities (Table 4

Table 5). Our sampling squares were marked by GPS-position and depth only. Revisiting was done by returning to the GPS-position and if necessarily correcting position by using sonar to find the right depth, then the diver was sent down to mark a square on the nearest surface fulfilling the specified criteria (annex 1). Thus using this approach, it was rarely the exact same surface that was revisited.

Discussion

Factors such as depth and substrate induce natural variation in benthic macrophyte communities. Therefore, the tested field method incorporated methodological changes expected to reduce this variation, with the aim to increase the chance to detect responses in the proposed indicators to changing environmental conditions, specifically nutrient induced changes.

Species richness based indicators

The residual variation was lower for species richness than for cumulative cover in most of the tested models for both hard and soft substrates. Also the soft substrate MI-index based on number of species performed slightly better than the MI-index based on cover (Table 6 and 7). Together with its previously shown ability to reflect changes in eutrophication (Blomqvist *et al.* 2014, Wikström *et al.* 2016) this supports species richness as a useful indicator for ecological status assessment.

As species richness increases with the size of the area sampled, a valid assessment of this indicator requires a fixed sampling size. The soft substrate index MI_c may be less dependent on a fixed sampling size, as it is a ratio between tolerant and sensitive species for a given area, but with increasing sampling size the chance of sampling rare (and sensitive) species increases. The use of a fixed sampling size is also incorporated in methods in use for monitoring benthic vegetation in several of our neighboring countries (see annex 3).

The potential species pool differs between hard and soft substrates as substrate is the most important factor determining the macrophyte community composition. Substrate specific sampling, with regard to both sampling units and species observations, would thus make assessments of species richness more comparable between samples. Several of our neighboring countries also use substrate specific sampling (annex 3).

Comparisons of species richness require coherent data in terms of taxonomic resolution. Incorporating a defined taxonomic resolution would make different surveys more comparable. When Blomqvist *et al.* (2014) analysed a large data set collected in multiple surveys with the transect method, differences in taxonomic resolution became evident. In order to achieve a homogenous data set that could be used to analyse the response of the proposed indicators, they found it necessary to group data to the lowest possible taxonomic level for each taxa. This however resulted in a lower taxonomic resolution than intended in many surveys. Using predefined taxonomic levels in the sampling would increase the coherence between surveys and thus increase the quality and versatility of Swedish vegetation data.

Cover based indicators

Our results indicate that the method reduces variation in cumulative cover estimates for hard substrate communities compared to estimates based on the transect method. In Blomqvist *et al.* (2014), relative uncertainty for diver in transect data was 39% (macroalgal communities in inner Baltic Proper), to be compared to 10% for the square method in the Västervik study. However, for soft substrate the reported relative uncertainty for diver in transect data was 22% (soft-bottom vegetation in inner Baltic Proper), to be compared to 31% for the square method used in our studies. The large reduction in relative uncertainty for hard substrates indicates that a fixed sampling size and substrate specific cover estimates yield data of higher quality whereas no improvement was seen for soft substrate. Substrate-specific cover estimates and fixed sampling sizes are used in other countries around the Baltic Sea. Substrate-specific cover estimates are for example recommended in HELCOM (2015), and also included in the methods used in Finland, Lithuania, Denmark and Norway. Also, estimating macroalgal and substrate cover in sampling areas with a specified size is included in the Finnish, Danish and German monitoring methods (annex 3).

Although fixed sampling size is less of a prerequisite for cover estimates than for species richness, it has the potential to reduce the variation between divers. For instance, maximum cover estimates in transects depend on how cover estimates are integrated over subjectively determined segments of varying lengths. However, in spite of this reduction in variation of cover estimates, assessment of species cover was still more variable than of species richness.

The comparison between divers in the Västervik study highlighted some differences among divers in how epiphytes are estimated, how to handle species that are loosely attached and that the cover of certain species is more difficult to estimate in a similar way by different divers, which could contribute to this variability. For example, one possible reason for the poorer performance of the cover based indicators in the soft substrate vegetation communities is interpretational differences between divers with regard to assessment of the area covered in relation to the shoot density. The results from the Västervik study show higher relative uncertainty related to diver for the indicator cumulative cover on soft substrate (0.31) compared to hard substrate (0.10) while the differences for species richness was negligible (0.05 and 0.10 on soft and hard substrate respectively). This likely reflects the difficulties in estimating cover for species composed of long, slim stalks without leaf canopy. The variation between divers could probably be reduced by a more detailed method description, including e.g. photos of different coverage of difficult species. Definitions of how to handle epiphytes and loosely attached species should also be developed together with active divers experienced in surveying benthic vegetation with this or similar methods.

General results

The indicator cumulative cover, calculated as decrease with depth, requires several samples from multiple depths and locations. When sampling a large number of units, the size

of the sampling unit needs to be considered. Different sampling units are used in our neighboring countries, e.g. rectangular sampling areas of 6 m² and 20 m² are sampled in Finland and Germany respectively and a circular area of 25 m² is sampled in the Danish method (annex 3). In our studies we mainly used a square-shaped sampling area of 25 m² but we also tested sampling in 16 m² squares. Contrary to our expectations the time gained by sampling the smaller square was small, less than 5 minutes per square on both the east and west coast, but with a large number of squares this still needs to be considered.

There are both pros and cons of smaller sampling units. For the surveying diver it is easier to overview the vegetation in a smaller square, especially in poor visibility. On the other hand, possible edge effects, e.g. over- or underestimating components close to the borders of the square, are less influential in a larger square where the border-surface ratio is smaller.

The major benefit of smaller sampling units is however that locating a suitable surface fulfilling specified criteria set to reduce natural variation (e.g. annex 1, Table 1-1) is easier, especially for hard substrates. During our surveys we had problems placing the 5*5 m squares within the specified depth interval of 3-5 m (see annex 1). This reflects the characteristics of large parts of the Swedish coastline with its complex morphology. Smaller sampling units can be placed within a narrower depth interval e.g. in the Finnish method the 6 m² frames are placed within one-meter depth intervals. Within-unit variation due to depth gradient is mainly a problem for hard substrates, where the slope of the seafloor might lead to a depth gradient within the sampling unit. For soft substrates, this would generally be less of a problem, and the sampling units should rather be as large as possible. The implications of this is that it might be necessary to use different methods on hard and soft substrate.

In our gradient study the variation between years was generally small for hard substrates in comparison to the within-area variation (soft substrates were not assessed with regard to year). The uncertainty estimates presented here could be used to calculate the number of samples and sampling frequency needed to reach a certain precision in the assessment (c.f. Bergström and Lindegarth 2016). This information is of importance for designing monitoring programs. For example, sampling more sites per area every second or third year could possibly reduce uncertainty in the status assessment more than sampling fewer sites in the same areas yearly during a six-year cycle. However, the risk with sampling every second or third year is that it takes longer to identify trends. A compromise could be to include reference areas sampled yearly in order to establish and identify trends. Such a sampling design has been used since 2007 in a regional monitoring program of macrophytes on the east coast (Länsstyrelsen Östergötland, 2011). The program includes two reference areas which are sampled yearly and nine areas sampled every third year on a rotating schedule, i.e. every year five areas are assessed and the yearly data from the reference areas are used to identify trends.

Statistical theory prescribes that sampling units are randomly positioned within the area for each survey. However, in practice this would be both time consuming and costly ending with few sampled units per area. Placing several sampling units on a transect reduces the sampling time, although a potential drawback is that it gives non-independent data. A fully randomized sampling design with stratification according to depth and substrate would also require detailed maps of these factors which are not available today. In our gradient studies we identified potential sites based on the available material such as sea charts, previous survey etc., but the time to locate suitable sites that fulfilled our criteria for this specific study was still between 10-40 minutes. In particular, suitable hard substrates were difficult to find in many areas.

Our results furthermore show that the sampling cost varies depending on vegetation composition. Surveying a sampling square on the west coast required twice as long time compared to a square on the east coast. On the west coast, there were also significant differences in sampling time between areas depending on the species richness, cover and complexity of the communities. Thus, in west coast areas the sampling cost per unit is more varied and higher compared to east coast areas.

In conclusion, fixed sampling size, substrate-specific sampling and defined taxonomic resolution, incorporated in the tested method, reduced variation in data for the proposed indicators for hard substrates, and data became more homogenous. A fixed sampling size is further required for monitoring of species richness. The large uncertainty in the cover-based indicators on soft substrate is problematic and it would be good to evaluate if the variation between divers could be reduced by a more detailed method description.

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Annex 1

Collection of data for the proposed macrophyte indicators species richness, cumulative cover, community complexity and, for soft substrate, also the MI-index, requires sampling using SCUBA-technique. In these studies, we used a square method, which includes a defined rectangular sampling area that is placed on an area where either hard or soft substrate dominates. The macrophyte cover estimates are substrate specific and on a continuous percentage scale with a predetermined taxonomic resolution.

These rectangular sampling areas, from hereon called "squares" (although they can be either quadratic or rectangular), can be placed along a transect at different depths or be positioned one square per site.

Method description

Resource requirements

Sampling with SCUBA-diving in Sweden requires a team of three persons of which at least two divers (minimum S30 professional diver certificate) and one dive leader (minimum S30 dive leader certificate). The divers need to be marine biologists with taxonomic knowledge and experience of sampling vegetation using cover estimates. Required taxonomic knowledge should be specified based on coastal area.

Locating sites

Locating suitable sites can be viewed as a twostep procedure. In step one, potential sites are identified based on previous surveys, local knowledge, sea charts, aerial photographs and depth curves. These potential sites are helpful in locating suitable sites in the field, but the final choice of site is made in the field (step two) based on a number of specific criteria listed in Table 1-1. For the soft substrate sites the criteria, "Existing vascular plants (>25% cover) on adjacent shallow substrate" is considered most important as it shows that the conditions on the site supports belt forming soft substrate vegetation including mainly vascular plants. Sonar, hand lead and/or video camera can be useful to locate suitable depth and determine substrate type.

Table 1-1Criteria for selection of vegetation sampling sites. In several cases different criteria are applied for hard and soft substrates.

Factor	Criteria	Hard	Soft
Depth	specified depth intervals, e.g. 3-5 m	X	X
Cover of substrate type in square	> 80 %	X	X
Slope	< 45 °	X	
Slope	Gentle slope (not depressions)		X
Shape of area	Square (or rectangular of equal area)	X	X
Distance between sites	> 100 m water	X	X
Exposure	Sheltered from waves, but good water circulation		X
Surroundings	Not below cliffs/rock faces		X
Surroundings	Existing vascular plants (>25% cover) on adjacent shallow substrate		X

Documenting sites

At each site one sampling square is placed. One corner of the area is marked with a surface buoy where the position (GPS) is determined. The square is described by noting the length of the sides, depth in the corners (named 1-4, corner 1 is the corner marked by the buoy and corner 2 next corner clockwise) and direction from corner 1 towards corner 3.

If sites are revisited this is done by returning to the GPS-position and, when necessary, using sonar to correct the position onsite. The surface buoy is dropped when sonar shows depths within the criteria. The divers go down to the buoy anchor and mark a sampling square on the closest surface fulfilling the criteria in Table 1-1. The buoy is moved to one corner of the square and a new position determined (GPS).

Sampling

The sampling square is marked, by the divers, with a rope or measuring tape with small weights attached. The depth is measured in the four corners of the square as well as in the middle. The compass direction (0-360 degrees) from corner 1 (marked with the buoy or closest to shore on a transect) to the centre point or corner 3 of the square is determined. Overview photographs (1-3 photos) of the vegetation should be taken in the sampling square.

A sampling square is either a hard substrate rectangular area or a soft substrate rectangular area, preferably a square, thus if the sampling size is 25 m^2 then preferably a square with 5 m sides. Hard substrate types are primarily rock and boulders, but stones, assessed as stable substrate, can also be included. Soft substrates are defined as sand and more fine-grained sediments, but not hard clay. Gravel is not included in either substrate type.

Cover estimates, projected from above, of vegetation and substrate are made within the respective substrate type (hard or soft) according to a continuous scale from 0-100%. In practice, the diver first determines how much (%) of the substrate in the area that be-

longed to either hard or soft substrate (depending on square type), and then the remaining percentage of other substrate. The diver then estimates the individual cover of respective substrates within the area's substrate type. For example, a hard substrate area was determined to consist of 90% hard substrate and 10% other substrate. The relative cover of rock and boulders within the hard substrate surface was estimated at 60 and 40% respectively. Definitions of different substrates and substrate types are shown in Table 1-2.

Table 1-2 Definition of substrates based on existing methods (e.g. Blomqvist 2009). The table also shows which substrate type each substrate belongs to.

Substrate	Definition	Substrate type
Rock	Solid rock	Hard
Boulders	> 20 cm	Hard
Stones	ca 3 cm – 20 cm	Hard/Other
Gravel	2 mm – ca 3 cm	Other
Sand	0,5 mm – 2 mm	Soft
Fine-grained sediments	< 0,5 mm	Soft

Vegetation cover, projected from above, is estimated in relation to the substrate type in question (hard or soft). Total vegetation cover includes all the macrophytes (not crust forming species) and gives an overall picture of the percentage of the bottom that is covered by vegetation (0-100%). Total vegetation cover is needed for the indicator community complexity. Cover is also estimated individually for each occurring species or taxa (macrophytes are determined to the predetermined taxonomic level specific to the region, appendix 2 for example), and should include crust forming species although these are not used in calculation of the indicators. The cumulative cover based on cover estimates of individual species is allowed to exceed 100% as the vegetation might grow in several layers.

Free living species, i.e. species that grow and thrive loose-lying on the substrate, are included in the survey (and cover estimates, including total vegetation cover) but are marked as free living. They are thus separated from dead or dying, loose algae, which have been torn loose from the substrate and drifts around before they decompose. Surface coverage of dead/dying, loose algae is only estimated as a group.

Epiphytes, plants attached to other plants, are separated from those that grow attached to the substrate. The cover is estimated individually for each epiphyte in relation to substrate type in the area. Animals can be included either using cover (mostly more or less sessile animals with surface coverage > 5%) or a quantity class (1 = single, 2 = average, 3 = very common), or the exact numbers.

The cover of silt on the substrate is estimated using a four graded scale according to the current Swedish standard method for vegetation assessment on the east coast (1 = no silt, 2 = some [if the diver touches the bottom some silt will be suspended but then quickly settle], 3 = more [the suspended silt remains in suspension for a while before it settles], 4

= heavy siltation [the suspended silt ruins the visibility for the remainder of the dive]) (Kautsky 1999).

Other parameters

Salinity and Secchi depth should be measured on the site or, regarding Secchi depth, on a nearby position with sufficient depth. Also, the prevailing wind and wave conditions on the site may be noted.

Description of studies

The gradient study

The method described above was tested in a study of well-described pressure gradients on the east and west coasts of Sweden, which covered two field seasons (2012 and 2013). The aim of the study was to test and evaluate the most promising indicators identified in the report 'Response of coastal macrophytes to pressures' by Blomqvist et al. (2014) on a homogenous data set from well-described pressure gradients on both the west and east coast. The results from the study are further reported in Wikström et al. (2016).

Five areas on the west coast and seven areas on the east coast were chosen to represent pressure gradients. The west coast areas had species rich macroalgal communities on the rocky substrates whereas the soft substrate macrophyte communities were dominated by eelgrass, *Zostera marina*. The macrophyte communities in the less saline east coast areas in the Baltic Sea were relatively species poor on hard substrate and diverse on soft substrate where the communities consisted of vascular plants with both marine and freshwater origin, Characean algae and free living macroalgae.

In 2012, 48 hard substrate sites (8-10 per area) were sampled in the five west coast areas and 140 sites, evenly distributed between area and hard and soft substrates, were sampled in the seven east coast areas (Figure 1-1). On each site, a sampling square of 25 m^2 was sampled according to the method described above, although the criteria for soft substrate sites were slightly different. The criteria were modified, based on the results and field experiences from the sampling in 2012, in order to reduce variation between soft substrate sites. The modifications were a narrower depth interval, 2-4 m, and the additional criteria: gentle slope, sheltered from waves, not below cliffs/rock faces and existing vascular plant vegetation (>25 % cover) on adjacent shallower soft substrate. The depth interval was set at 3-5 m for both hard and soft substrate sites in 2012. The actual sampled depth interval was 1.8-5.9 m due to difficulties in locating suitable sites fulfilling all criteria.

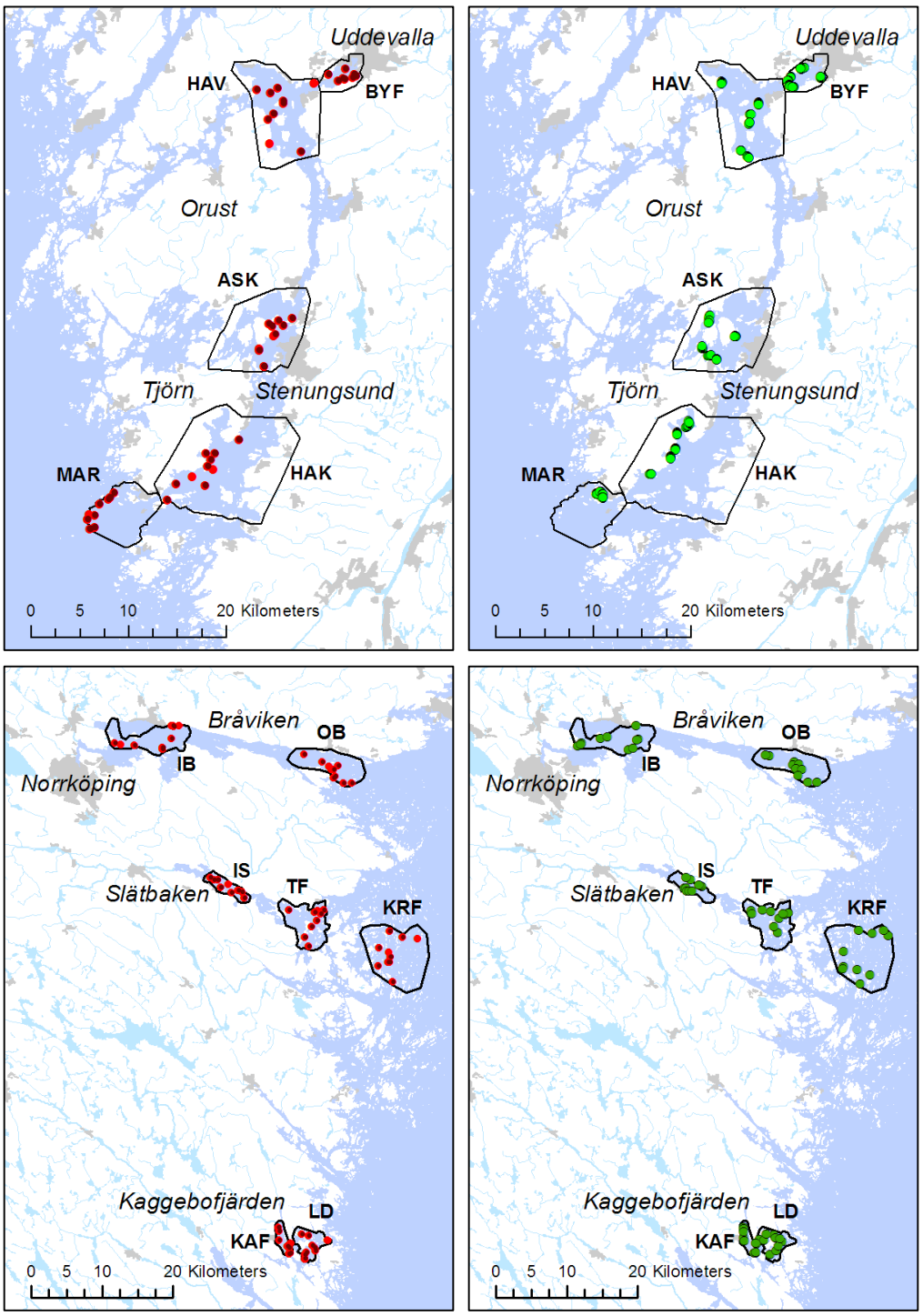


Figure 1-1 Vegetation sampling sites in the west coast areas (upper two maps) and east coast areas (lower two maps), hard substrate sites (left) and soft substrate sites (right). Lighter color and larger symbols represents sampling in 2012 and darker color and smaller symbols in top layer represents sampling in 2013.

The gradient study areas in the west coast were BYF Byfjorden, HAV Havstensfjord, ASK Askerö-/Halsefjorden, HAK Hake fjord and MAR Marstrandsfjorden. The gradient study areas on the east coast were IB Inner Bråviken, OB Outer Bråviken, IS Inner Slätbaken, TF Trännöfjärden, KRF Kärrfjärden, KAF Kaggebofjärden and LD Lindödjupet.

In 2013, 40 of the hard substrate sites (eight per area) on the west coast were revisited, as well as 56 hard substrate sites (eight per area) on the east coast. Most of the 70 soft substrate sites sampled in 2013 were new, chosen based on the new modified criteria, as only ten of the previously visited sites fulfilled the new criteria. Also, on two hard substrate sites in each of the five areas on the west coast and on two sites in three areas on the east coast, three slightly smaller squares (4x4 m) were sampled adjacent to the large 5x5 m square. This was done to assess variation in the hard substrate community composition within site and evaluate square size.

The three extra, smaller (4x4 m) squares were placed next to the standard 5x5 m square. Their positions were described relative to 5x5 m square (left, right, far left etc). The sites chosen for extra sampling had a large enough sea floor area fulfilling the criteria to fit four squares.

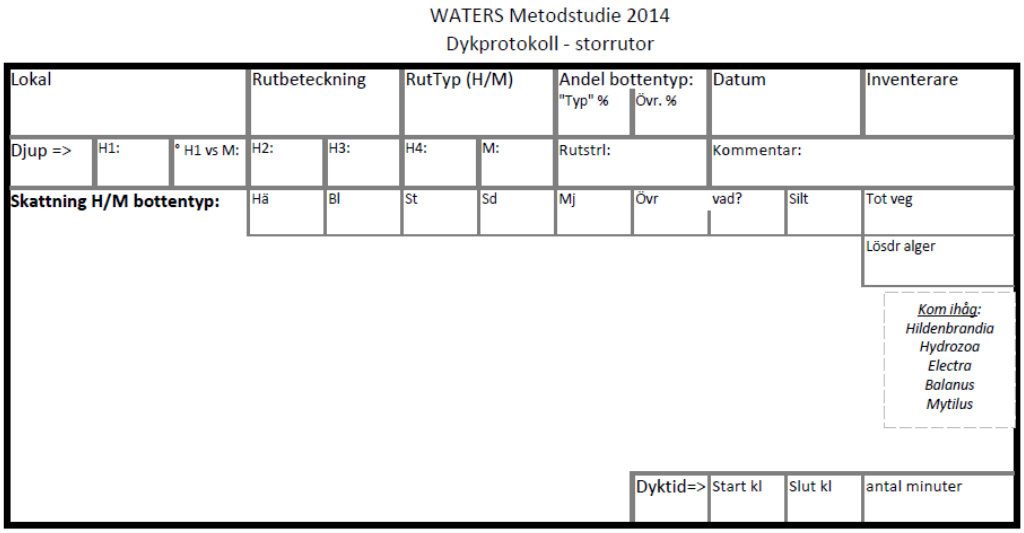
The Västervik study

The aim of the study was to determine the precision between different divers when sampling according to the method described above. In the study 14 squares (5x5 m) were sampled by seven divers during June 10-12 2014.

Seven different divers sampled all 14 squares once each. Some supporting parameters such as salinity, Secchi depth, water temperature, sea level, weather, wind direction and waves etc. were logged once per day, i.e. not by the individual divers. The positions of the sites were determined with a GPS when the sampling squares were marked.

The seven divers were marine biologists from four different organizations with experience from sampling according to the transect method, i.e. similar subjective cover estimates but using a seven-graded scale (0, 5, 10, 25, 50, 75 and 100%). Some of the divers also had experience from sampling 5x5 m sampling squares using a continuous scale, i.e. the tested method. A method description document (Blomqvist & Qvarfordt *unpubl.*), the field protocol (Figure 1-2) and a list of the desired taxonomic level for the region (appendix 2) were distributed before the study. A briefing on the method was also held prior to the field sampling.

The study was conducted on three sites in order to sample different macrophyte communities (Figure 1-3). Site A at the island Äskeskär was a gently sloping sandy bottom with a thriving macrophyte community. The five sampling squares consisted of 100 % sandy or soft substrate and were placed adjacent each other in a line from 2 to 5 m depth. The squares thus sampled a depth gradient at this site. Site B next to the island Stångklabben was a hard substrate site with a red algae community. The five squares were placed along the depth curves thus covering a narrower depth interval, 4.9-6.4 m depth. Site C, at the island St Enskär, was a shallower hard substrate community dominated by *Fucus vesiculosus*. The four squares sampled at this site (only four squares could be fitted into the area fulfilling the criteria set for this site: > 90 % hard substrate, preferably bedrock, and at least 25 % cover of *F. vesiculosus*) were placed between 0.9-2.8 m depth.


WATERS Metodstudie 2014
Dykprotokoll - storrutor

Lokal			Rutbeteckning		RutTyp (H/M)		Andel bottentyp: "Typ" %	Övr. %	Datum	Inventerare
Djup =>	H1:	° H1 vs M:	H2:	H3:	H4:	M:	Rutstrl:		Kommentar:	
Skattning H/M bottentyp:	Hä	Bl	St	Sd	Mj	Övr	vad?	Silt	Tot veg	
									Lösdr alger	

Kom ihåg:
Hildenbrandia
Hydrozoa
Electra
Balanus
Mytilus

Dyktid=>	Start kl	Slut kl	antal minuter

Figure 1-2 Field protocol used when sampling according to the square method.

All participating divers should be able to sample as many squares as possible during the few available field days. Thus, the squares were placed adjacent to each other at the sites as this set up minimized the travel time between squares. Positioning the squares close together also allowed investigation of within site variation at the hard substrate site where the squares were placed within the same depth interval.

At the soft bottom site the squares were placed in a depth gradient in order to capture different species and thus how different divers estimate their cover. This was because many of the soft bottom macrophytes consist of single, long stalks for example *Zostera marina* and *Potamogeton perfoliatus* and their cover can be difficult to determine.

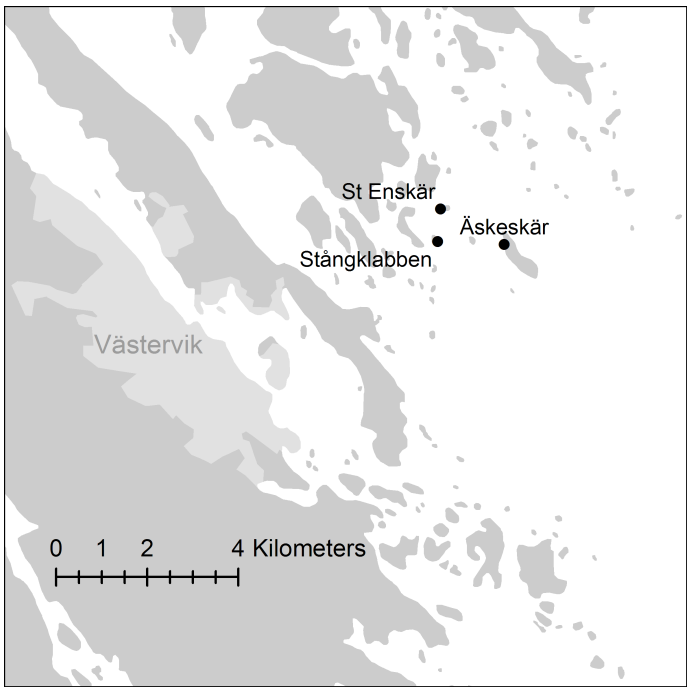


Figure 1-3 The three sites in the Västervik study.

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Annex 2

Required know-ledge	Rank	Order/Class	Name	Swedish name	Comments
Recognize *in situ*	Species	Phaeophyceae	*Battersia arctica*	ishavstofs	
	Species		*Chorda filum*	sudare	
	Species		*Dictyosiphon foeniculaceus*	smalskägg	when uncertain as species pair
	Species		*Stictyosiphon tortilis*	krulltrassel	when uncertain as species pair
	Species pair		*Dictyosiphon/Stictyosiphon*		
	Species pair		*Ectocarpus/Pylaiella*	"brunslick"	
	Species		*Elachista fucicola*	tångludd	
	Species		*Eudesme virescens*	olivslemming	
	Species		*Fucus serratus*	sågtång	should be verified near northern distribution limit
	Species		*Fucus vesiculosus*	blåstång	
	Species		*Halosiphon tomentosus*	gullsudare	
	Species		*Leathesia marina*	murkelalg	
	Species		*Scytosiphon lomentaria*	korvsnöre	
	Species	Rhodophyta	*Aglaothamnion roseum*	rosendun	
	Species		*Ceramium tenuicorne*	ullsläke	
	Species		*Ceramium virgatum*	grovsläke	verified under microscope
	Species pair		*Coccotylus/Phyllophora*	"rödblad"	
	Species		*Furcellaria lumbricalis*	kräkel	
	Species		*Hildenbrandia rubra*	havsstenhinna	
	Species		*Polysiphonia fibrillosa*	violettslick	
	Species		*Polysiphonia fucoides*	fjäderslick	
	Species		*Rhodochorton purpureum*	rödplysch	
	Species		*Rhodomela confervoides*	rödris	verified under microscope
	Species	Charophyceae	*Chara aspera*	borststräfse	
	Species		*Chara baltica*	grönsträfse	verified under microscope
	Species		*Chara canescens*	hårsträfse	verified under microscope
	Species		*Chara globularis*	skörsträfse	verified under microscope
	Species		*Chara horrida*	raggsträfse	verified under microscope
	Species		*Chara tomentosa*	rödsträfse	
	Species		*Tolypella nidifica*	havsrufse	
	Genus	Zygnematophyceae	*Spirogyra*	spiralbandsalger	verified under microscope

Required know-ledge	Rank	Order/Class	Name	Swedish name	Comments
Recognize *in situ*	Species	Chlorophyta	*Aegagropila linnaei*	getraggsalg	verified under microscope
	Species		*Chaetomorpha linum*	krullig borsttråd	
	Species		*Cladophora glomerata*	grönslick	
	Species		*Cladophora rupestris*	bergborsting	verified under microscope
	Species		*Cladophora fracta*	näckhår	verified under microscope
	Species		*Monostroma balticum*	östersjösallat	
	Genus		*Ulva sp/spp*	havssallater/tarmalger	
	Species	Magnoliophyta	*Callitriche hermaphroditica*	höstlånke	
	Species		*Ceratophyllum demersum*	hornsärv	
	Species		*Lemna trisulca*	korsandmat	
	Species		*Myriophyllum sibiricum*	knoppslinga	
	Species		*Myriophyllum spicatum*	axslinga	
	Species		*Najas marina*	havsnajas	
	Species		*Potamogeton filiformis*	trådnate	verified under microscope
	Species		*Potamogeton pectinatus*	borstnate	
	Species		*Potamogeton perfoliatus*	ålnate	
	Species		*Ranunculus circinatus*	hjulmöja	
	SubSpecies		*Ranunculus peltatus ssp. baudotii*	vitstjälksmöja	
	Species		*Ruppia cirrhosa*	skruvnating	verified under microscope
	Species		*Ruppia maritima*	hårnating	verified under microscope
	Species		*Subularia aquatica*	sylört	
	Species		*Zannichellia palustris*	hårsärv	
	Species		*Zostera marina*	bandtång	

Required know-ledge	Rank	Order/Class	Name	Swedish name	Comments
keep in mind (when observing "strange" species)	Species	Phaeophyceae	*Dictyosiphon chordaria*	gyllenskägg	verified under microscope
	Species		*Protohalopteris radicans*	stentofs	verified under microscope
	Species		*Battersia plumigera*	smal fjädertofs	verified under microscope
	Species		*Desmarestia viridis*	mjukt käringhår	verified under microscope
	Species		*Spongonema tomentosum*	repslick	verified under microscope
	Species	Rhodophyta	*Ahnfeltia plicata*	havsris	verified under microscope
	Species		*Callithamnion corymbosum*	gaffeldun	verified under microscope
	Species		*Polyides rotundus*	klyving	verified under microscope
	Species		*Polysiphonia elongata*	grovslick	verified under microscope
	Species		*Polysiphonia stricta*	rosenslick	verified under microscope
	Species	Charophyceae	*Nitella flexilis*	glansslinke	verified under microscope
	Species		*Chara connivens*	tuvsträfse	verified under microscope
	Species		*Chara virgata*	papillsträfse	verified under microscope
	Varietet		*Chara baltica var. liljebladii*	långsträfse	verified under microscope
	Species	Chlorophyta	*Acrosiphonia arcta*	liten grönkudde	verified under microscope
	Species		*Blidingia minima*	klippspringsalg	verified under microscope
	Species		*Rhizoclonium riparium*		verified under microscope
	Species		*Spongomorpha aeruginosa*	liten filtkudde	verified under microscope
	Species		*Ulothrix zonata*	skvalpalg	verified under microscope
	Species		*Urospora penicilliformis*	fransalg	verified under microscope
	Species	Magnoliophyta	*Elodea canadensis*	vattenpest	
	Species		*Myriophyllum alterniflorum*	hårslinga	
	Species		*Potamogeton crispus*	krusnate	
	Hybrid		*Potamogeton gramineus × perfolia-tus*		
	Species		*Potamogeton pusillus*	spädnate	
	Species		*Ranunculus confervoides*	hårmöja	
	Species		*Ranunculus peltatus*	sköldmöja	
	Species		*Stratiotes aloides*	vattenaloe	
	Species		*Utricularia vulgaris*	vattenbläddra	

Annex 3

Brief descriptions of the methods used for monitoring benthic vegetation for status assessment in Swedish neighboring countries.

Country/ Organisation	Method overview	Reference
Helcom Combine manual	Diving transect perpendicular to shoreline. Substrate composition in depth intervals. Vegetation coverage relative to hard or soft substrate in depth intervals. Quantitative samples to obtain species composition and biomass at given depths. Video transects to obtain coverage of conspicuous species and substrate.	Helcom 2015
Finland	1) Cover of macroalgal taxa and substrate in 2*3 m squares every depth meter along transects perpendicular to shoreline. Within each square potential growth area for macroalgae is noted (minimum 10 % of total area). Cover of each taxon relative to potential growth area is noted. 2) 10 to 30 lower growth depth limits of continuous *Fucus* belt (mature sized individuals) are noted. Method 1) and 2) can be measured independently or at the same site.	Ruuskanen 2014
Estonia	Observations along transects perpendicular to shoreline. New observation after each 1-1.5 m depth change. Cover of taxa and substrate within a radius of 2-3 m around each observation point is noted together with total cover of vegetation. Quantitative biomass samples (20*20cm frames with bags) from each community type along the transect (commonly from 5-7 depth intervals). Depth limit of vegetation is noted.	Torn *et al.* 2014
Latvia	Transects perpendicular to shoreline with observations at each depth meter. At each depth a 3 min long video is recorded by drop-down video camera. When potential maximum depth zone of phytobenthos (or *Fucus*) is reached video material is supplemented with diver observations to determine the deepest occurrence of a single plant.	M. Alberte, pers com
Lithuania	Substrate specific cover of *Furcellaria lumbricalis* along video transects. Maximum depth limit of *F. lumbricalis* is noted for each transect.	Bučas *et al.* 2007
Poland	Frame sampling (50*50 cm) for biomass determination mainly at ca 7 and 9 m depth two times yearly (June and September). Cover of phytobenthos and substrate determined from films and photographs from transects.	Saniewski 2013

Country/ Organisation	Method overview	Reference
Germany	Outer coastal waters: Zigzag video transects to determine replicates of depth limit of dense (at least 10 %) cover of Eelgrass (soft substrates) and *Fucus* spp. (hard substrates). Cover of taxa and substrate is noted within a 10 m long and 2 m wide diving transect laid out in depth intervals with the densest vegetation of eelgrass, *Fucus* or red algae biotopes respectively. Five frames are sampled within the densest vegetation of the 20 m² transect, 1 m² frames in eelgrass and 0.25 m² in *Fucus* and red algae biotopes. In each frame cover of vegetation and substrate is noted and then ¼ of the frame area is collected for biomass analysis in the laboratory. Inner coastal waters: Similar methods but diving is used instead of video for depth limits due to lower visibility. Species and cover estimates could be by raking if visibility is low (< 1m).	Fürhaupter & Meyer 2015a & 2015b
Denmark	Soft substrate: Cover and 7-10 replicated (zigzag) depth limits of eelgrass along video transect. Cover is noted in ca 2*1-1.5 m areas every 5 seconds from the video film. Stable hard substrate: Diving transects with three 25 m² circular sampling areas in each depth interval. Covers of species on stable hard substrate in sample area are noted together with total cover of vegetation.	Høgslund *et al.* 2014
Norway	Hard substrates: Diving transects 8-10 m wide from 30 m depth to surface. Covers of species on suitable substrate are noted at every second depth meter. Marked changes in community composition between fixed depths are noted separately.	Moy *et al.* 2010

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Evaluation of methodological aspects on data collection for assessing ecological status of vegetation according to the WFD

Previous studies within the Waters programme have identified a number of indicators for ecological quality of coastal vegetation that could replace or complement the current indicator. In this report we present and discuss the results from testing of a field method for collecting data for these indicators. The method was chosen to overcome some of the limitations of the current main field method for vegetation surveys in Sweden and included a fixed sampling size, a defined taxonomic effort and substrate specific sampling. We also evaluated spatial, temporal and methodological variability of the indicators using the WATERS uncertainty framework, where general linear models are specified in order to capture temporal and spatial interactions and variability in sampling.

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