



# OPERATIONALIZATION OF MACROALGAE MONITORING FOR WFD CUMULATIVE COVER INDICATOR

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Scientific Report from DCE - Danish Centre for Environment and Energy

No. 708

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# Data sheet

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Abstract:	The potential for complementing diver observations of macroalgae cumulative cover with ROV surveys at deeper locations was investigated. When the macroalgae community is one-layered, there is no systematic deviation between diver and ROV observations. Including ROV observations at deeper locations will improve the macroalgae indicator precision and allow for estimating the indicator for more water bodies. It is recommended to incorporate ROV surveys for deeper locations in the national monitoring program and streamlining data flows for indicator estimation.
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## Preface

This project was initiated by the Danish Agency for Green Transition and Aquatic Environment (Styrelsen for Grøn Arealomlægning og Vandmiljø, SGAV) and carried out by Aarhus University/DCE. The objective was to evaluate the macroalgae indicator cumulative cover for status assessment across the 42 water bodies under the Water Framework Directive (WFD) using monitoring data for the period 2019-2024 and to develop a python code routine that can be incorporated into the data processing system at SGAV. A second objective was to investigate the potential of using Remotely Operated Vehicle (ROV) for assessing cumulative cover at deeper locations, including the effect on the macroalgae indicator cumulative cover. In case this is feasible, a revised technical guidance for macroalgae monitoring will be drafted.

This project was carried out and the report written entirely by the Danish Centre for Environment and Energy (DCE) at Aarhus University (AU). The report has been reviewed by SGAV and revised after their comments.

## Sammenfatning

I henhold til Vandrammedirektivet skal den danske metode til vurdering af økologisk tilstand inkludere indikatorer for makroalger. Der er udviklet en indikator for kumulativ dækning af makroalger, men manglen på dybere observationer i mange danske vandområder medfører, at indikatoren ikke kan bestemmes for alle vandområder. Den nuværende overvågning af makroalger på dybere forekomster er omkostningsfuld og logistisk udfordrende på grund af reduceret dykkertid med stigende dybde. Alternativt kan makroalgedækket på dybere steder vurderes ved hjælp af optagelser fra ROV-undersøgelser, der efterbehandles gennem visuel inspektion. Denne rapport viser, at observationer af den samlede makroalgedækning med ROV er variable men sammenlignelige med dykkerobservationer af kumulativ dækning. Det vurderes, at den væsentligste årsag til forskel mellem dykker og ROV observationer formodes at skyldes forskelle i hvilket område, der er undersøgt. Det anbefales dog at foretage yderligere undersøgelser for at fastlægge denne antagelse. Rapporten viser også, at dybere ROV-observationer kan forbedre præcisionen af makroalgeindikatoren. Det vurderes, at den I den seneste 6-årige tilstandsvurderingsperiode (2019-2024) kunne makroalgeindikatoren estimeres for 29 ud af 42 vandområder med overvågningsdata, dog med stor usikkerhed på indikatoren for mange af disse vandområder. Dette vil påvirke sikkerheden (konfidensen) af den økologiske tilstandsvurdering. Ved at integrere ROV-observationer i overvågningen, vil det være muligt at bestemme makroalgeindikatorer for flere vandområder og samtidig øge sikkerheden i tilstandsvurderingen. Det anbefales at standardisere og inkludere ROV-undersøgelser i overvågningsprogrammet, hvilket også inkluderer overførsel af disse data til Vanda og beregning af indikator for makroalge kumulativ dækning.

## Summary

The Danish WFD assessment method for ecological status needs to be complemented with the macroalgae indicator for cumulative cover, but the estimation of this indicator is impeded by lack of deeper observations in many Danish water bodies. Traditional monitoring of the macroalgae communities at deeper locations is costly and logistically challenging due to reduced diving time with increasing depth. Alternatively, the macroalgae cover at deeper locations can be assessed using footage from ROV surveys that are post-processed through visual inspection. This study shows that ROV observations of macroalgae cover are variable albeit comparable with diver observations of cumulative cover. The major reason for the difference between the two methods is believed to be differences in the areas surveyed. However, it is recommended to do further investigations to confirm this assumption. The study also shows that deeper ROV observations can improve the precision of the macroalgae indicator. For the most recent 6-year assessment period (2019-2024), the macroalgae indicator could be estimated for 29 out of 43 water bodies with monitoring data, although the indicator uncertainty was high for many of these water bodies. Consequently, ecological status assessments have lower confidence. Incorporating ROV observations in the monitoring has the potential to extend the macroalgae indicator to more water bodies and improve confidence in the status assessment. This study provides recommendations for standardizing and incorporating ROV surveys in the monitoring program and for streamlining the data pipeline from monitoring data to indicator estimation.

# 1 Introduction

Macroalgae monitoring is an integrated part of the Danish marine monitoring program (NOVANA). These data are included in the annual reporting of the state of the marine environment but not yet incorporated into the status assessment for the European Water Framework Directive (WFD) and the European Habitats Directive (HD). The WFD requires that the status assessment of the biological quality element (BQE) “benthic vegetation” includes both angiosperms and macroalgae. For the latter, the status should assess macroalgae cover and presence of disturbance-sensitive taxa. So far, status assessment for this BQE has relied on the depth limit of the main eelgrass distribution only. Therefore, the Danish WFD assessment method needs to include status assessment of the macroalgae community as well.

Aarhus University has developed macroalgae indicators intended for assessing the WFD ecological and ecological potential according to the European Habitats Directive (HD). These indicators are based on the decrease of macroalgae cumulative cover and the number of perennial species with depth, parameterized through an attenuation coefficient  $k_{bio}$ , equivalent to a light attenuation coefficient  $k_d$ . The two macroalgae indicators respond to the level of eutrophication, since light conditions are regulating macroalgae cumulative cover and the number of perennial species at deeper depths (Carstensen 2020a). An expert panel under the EU Common Implementation Strategy Working Group ECOSTAT deemed cumulative cover suitable for characterizing macroalgae cover, whereas criticism was raised against the number of perennial species for characterizing reference conditions for the presence of disturbance-sensitive taxa. Therefore, this report only concerns the operationalization of  $k_{bio}$  for cumulative cover. However, this indicator cannot be estimated for all water bodies (only ~30 out of 42) and for several water bodies the indicator is estimated with considerable uncertainty (Carstensen 2020b). The main reason for this is the lack of macroalgae observations at deeper depths, where light limitation becomes prominent. This macroalgae indicator can potentially be estimated for additional water bodies and with greater precision, provided that deeper macroalgae observations are made available. This report will assess the current status of cumulative cover for the Danish water bodies with macroalgae cover and document the estimation procedure in a Python script.

Previous investigations have documented the presence of suitable substrate and macroalgae at deeper depths (Dahl et al. 2023; Carstensen et al. 2025). Including deeper macroalgae observations significantly improves the estimation of  $k_{bio}$  for cumulative cover (Carstensen 2024). However, macroalgae monitoring at deeper depths is more complicated due to dive time restrictions and hence, more resource demanding. Therefore, this report will assess if Remotely Operated Vehicle (ROV) monitoring can provide supplementary data for obtaining more precise, yet unbiased, estimates of  $k_{bio}$  for cumulative cover.

## 2 Materials and methods

The macroalgae indicator for cumulative cover was developed in Carstensen (2020a) and describes three phases regulating cover with depth: 1) physical exposure near the surface, 2) light saturated maximum cover and 3) light-regulated declining cover. Moreover, the macroalgae indicator includes a factor to account for grazing by sea urchins. The theoretical foundation of the macroalgae indicator is found in Carstensen (2020a) and the Python code for implementing the indicator estimation is found in Annex B.

### 2.1 Comparing diver and ROV assessment

During the cruise for monitoring boulder reefs in Denmark in 2025, 16 locations were monitored by diver and ROV, spanning a depth range from 8.0 m at Lønstrup Rødgrund to 21.9 m at Kims Top (Figure 2.1; Table 2.1). The diver recorded total and species-specific cover of the macroalgae species as well as the cover of suitable hard substrate according to the guidelines (Dahl & Lundsteen 2018). Diver surveys are typically within  $\pm 0.25$  m of the site-specific depth. The study sites cover a salinity gradient from  $\sim 10$  near Bornholm to  $\sim 33$  at Lønstrup Rødgrund.



**Figure 2.1.** Location of boulder reefs with both diver and ROV observations. Several depths were monitored at specific locations (see Table 2.1).

ROV observations were carried out with ROV Chasing M2: Standard and Pro Max, both utilize 12 mega-pixel camera with 4k/1080p video, with light provided by a 2 x 2000 and 2 x 4000 lumen LED lights, respectively. The ROVs were deployed immediately after the SCUBA diver had completed the

underwater survey following NOVANA guidelines. The ROV was deployed approximately 10 m behind the stern, following a trajectory away from the ship. The depth sensor of the ROV is located right behind the camera and the ROV was operated to have approximately 1 m distance to the substrate (corresponding to 0.7 m vertical distance with a ROV tilt of 45°), although occasionally driving the ROV for close-up on specific species. The ROV was operated to survey approximately the same depth as the diver, but the actual survey depth was recorded. Each ROV survey was limited to a 10 min survey with the aim to identify and accurately estimate the benthic reef community, this included careful inspection of the macroalgae and fauna, while covering as much survey ground as possible. This was employed to mimic the SCUBA diver NOVANA investigation as closely as possible while providing a standardized approach, allowing comparison between methods. However, due to the differences between the diver and ROV surveys the areas examined are expected to have little spatial overlap.

Observations during the ROV survey revealed that environments with large abundances of *Laminaria* sp. and other kelp species prevented the ROV from getting close to the seafloor, that often the under-canopy and encrusting components of the benthic community could not be readily observed, and drift algae could not be separated from attached algae (at some reef sites this can be critical). ROV surveys did, however, cover an expanded search area within the 10-min duration compared to SCUBA divers and reef fish did seem less disturbed by the ROV presence, allowing easier observation.

**Table 2.1.** Boulder reefs monitored in August 2025 where diver and ROV investigations were employed.

Boulder reef	Date	Depth (m)	Duration	ROV
Herthas Flak	12/08/2025	19.8	00:10:04	M2 Chasing Standard
Herthas Flak	12/08/2025	15.0	00:09:51	M2 Chasing Standard
Lønstrup Rødgrund	13/08/2025	14.8	00:11:03	M2 Chasing Standard
Lønstrup Rødgrund	13/08/2025	12.7	00:10:29	M2 Chasing Standard
Lønstrup Rødgrund	13/08/2025	8.0	00:09:28	M2 Chasing Standard
Kims Top	14/08/2025	21.9	00:10:32	M2 Chasing Standard
Davids Banke	17/08/2025	13.9	00:10:43	M2 Chasing Pro Max
Davids Banke	17/08/2025	16.7	00:11:26	M2 Chasing Pro Max
Davids Banke	17/08/2025	21.0	00:10:14	M2 Chasing Pro Max
Bakkebrædt	18/08/2025	15.0	00:11:14	M2 Chasing Pro Max
Adlers Grund	18/08/2025	20.6	00:10:27	M2 Chasing Pro Max
Møns Klint	19/08/2025	20.5	00:10:24	M2 Chasing Pro Max
Böchers Grund	19/08/2025	15.0	00:10:34	M2 Chasing Pro Max
Knudshoved Odde	20/08/2025	10.1	00:10:32	M2 Chasing Pro Max
Kirkegrund	20/08/2025	12.9	00:10:52	M2 Chasing Pro Max
Broen	20/08/2025	17.0	00:10:08	M2 Chasing Pro Max

After the cruise, the ROV footage was analysed using Media Player. Each video session lasted between 9 minutes 51 seconds and 11 minutes 26 seconds per location (Table 2.1). To assess macroalgal diversity and percentage cover on hard substrates, three independent assessment rounds were conducted for each location. These rounds were spaced seven days apart to ensure independent observations. Within each round, the ROV footage was analysed in a randomized order across locations to reduce potential order effects. An advantage of using ROV is the possibility of revisiting the footage for quality assurance of the assessments.

Observed size distribution of boulders and stones, macroalgae species and their estimated cover on hard substrate were documented using the same methodology as diver-based surveys. When species-level identification was not feasible, macroalgae were categorized into functional groups (e.g., filamentous red algae, leaf-forming red algae). Additional notes documented observed fauna, substratum type, processing time per recording, and methodological observations.

The ROV and diver observations were compared for the hard substrate, cumulative and total macroalgae cover. Hard substrate cover was reported directly from the diver surveys where the diver assesses the stable size of boulders based on the cover of flora and fauna often ranging from 5 and up to 30 cm depending on the physical environment. The cumulative cover of stones larger than 5 cm represented the hard substrate cover from the ROV observations. Cumulative cover was estimated by aggregating all except crust-forming macroalgae species-specific cover assessments for both methods (cf. Carstensen 2020a). Total macroalgae cover was assessed directly by the diver, whereas it was set equal to the cumulative cover for ROV observations, assuming that these represented a one-layer structure. Replicate ROV observations against diver observations were analysed separately to identify any potential effect of increasing experience during the process of analysing the ROV footage.

The paired observations of diver and ROV assessments in 2025 were examined in relation to the overall depth gradient for cumulative cover using data from the previous 6-year period with the estimated model for cumulative cover, including  $k_{bio}$  (see Annex B). This analysis was used to assess potential systematic differences between the two methods in relation to the overall variability along the expected depth gradient.

## 2.2 Status assessment for cumulative cover

Macroalgae data from 42 water bodies were extracted from the database Vanda for the 6-year period 2019-2024. These include species-specific macroalgae cover as well as sea urchin cover. Sea urchins are not consistently monitored in the coastal program despite their significant grazing impact on macroalgae within the NOVANA macroalgae monitoring program, but they are monitored as part of the boulder reef monitoring program. Sea urchins were found at boulder reefs within five water bodies: 1) Skagerrak, 2) Nordlige Kattegat, 3) Kattegat, Nordsjælland, 4) Århus Bugt syd, Samsø og Nordlige Bælthav, 5) Storebælt, syd.

The macroalgae indicator ( $k_{bio}$ ) for cumulative cover was estimated separately for the 6-year period for each of the 42 water bodies.

### 3 Results and discussion

In this section, diver and ROV observations are compared and the effect of substituting diver observations with ROV observation on the macroalgae indicator  $k_{bio}$  is assessed. Finally, the estimation of  $k_{bio}$  and its preliminary status is assessed.

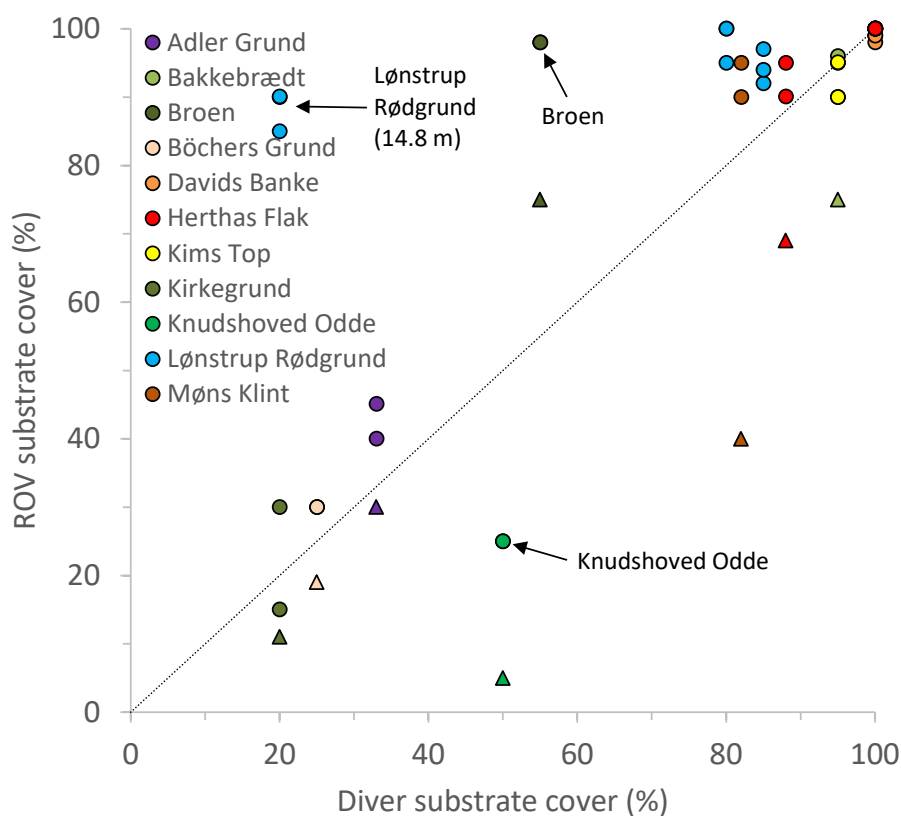
#### 3.1 ROV versus diver observations

ROV and diver observations are paired using their location and depths, although it was not possible for the ROV to cover exactly the same depths and area as the diver. Hence, there can be some variability between the two methods that is due to natural differences in the surveyed area and depth, and not an expression of method uncertainty.

##### 3.1.1 Hard substrate cover

The assessments of suitable hard substrate cover were overall similar for the two methods except for the first round of ROV assessment that had substantially lower substrate cover at specific locations (Figure 3.1). Disregarding the first round of assessment (marked by triangles in Figure 3.1), only Knudshoved Odde, Broen and Lønstrup Rødgrund (only 14.8 m) exhibited larger deviations between ROV and diver assessments. Additionally, in the assessment of the ROV footage at Bakkebrædt, Böchers Grund, Knudshoved Odde and Møns Klint the video showed large cover of mussels or dense macroalgae mats, making the assessment of hard substrate more difficult.

**Figure 3.1.** ROV versus diver observations of hard substrate cover. Boulder reefs are marked with different colours. Dashed line is the 1:1 identity line. The ROV footage from each location and depth was analysed three times. Triangles highlight observations from the first round of ROV footage analyses that deviated substantially from the other rounds.



At Knudshoved Odde, the ROV hard substrate cover was relatively low compared to the diver assessment, which most likely is due to thick macroalgae cover, disallowing a complete substrate assessment (all substrate types accounted for only 43-75% in total). Scaling up this incomplete substrate assessment, assuming that substrate types under the dense macroalgae cover had similar distribution to those parts of the footage that could be assessed, made the hard substrate assessment of the two methods more similar.

At Broen, the distribution of boulders/stones in different size categories were similar (>5 cm: 80% for diver survey and 75-98% for ROV) but the overall diver assessment of hard substrate was only 55%. This is probably due to the diver assessing that only larger stones were suitable substrate. Moreover, since the area surveyed by diver and ROV are not overlapping, the assessment of hard substrate can come out differently.

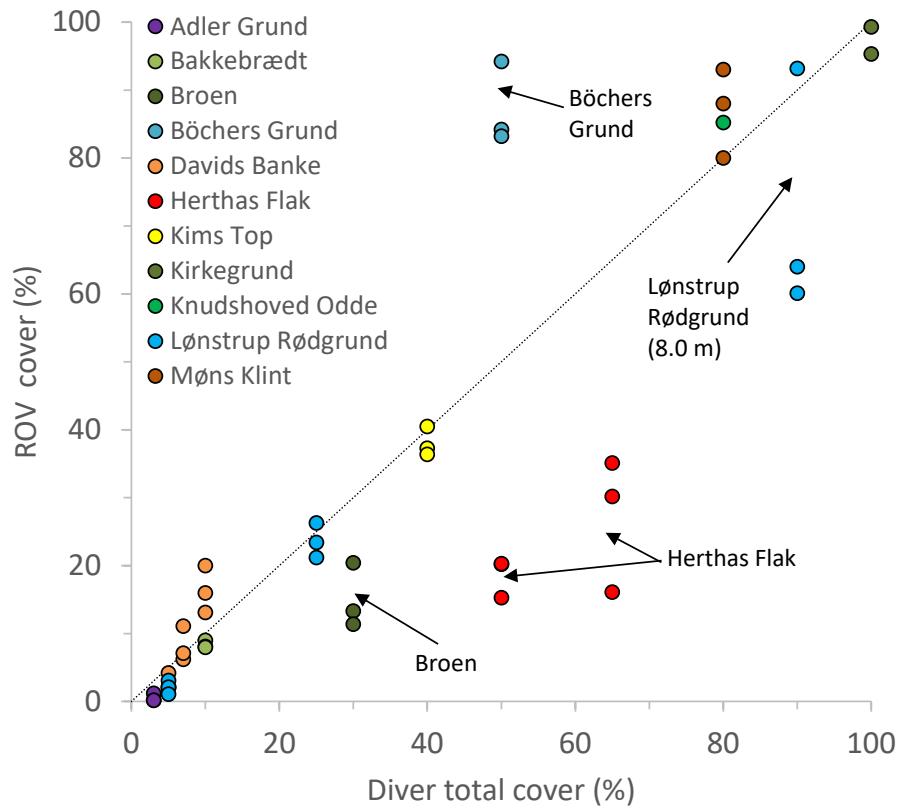
At Lønstrup Rødgrund, the diver assessment of hard substrate matched the size distribution of boulders/stones at the shallower location (8.0 and 12.7 m). At 8.0 m, the diver assessment of 85% hard substrate equalled the cumulative distribution of boulders/stones >5 cm. At 12.7 m depth, the cumulative distribution of boulders/stones >5 cm from the diver assessment was 90%, corresponding to the ROV assessments of 95-100%, whereas the overall diver assessment was somewhat lower (80%). However, at 14.8 m depth, the ROV assessment of hard substrate was 85-90%, which is substantially higher than the overall diver assessment of 20% and the cumulative distribution of boulders/stones >5 cm from the diver (30%). This discrepancy between diver and ROV observations is most likely due to the high patchiness of boulders/stones at this depth, and that there is little overlap between the areas surveyed by the ROV and diver.

The objective of the ROV surveys was to assess the substrate-specific cover of macroalgae communities and not to assess the overall cover of suitable hard substrate. This could imply that ROV surveys yield higher cover of hard substrate if the ROV transects are directed more towards specific areas with high substrate cover. Moreover, the ROV camera is tilted forward (~45°) in contrast to the vertical diver investigations. Hence, larger boulders/stones may hide sand and gravel bottoms to a larger extent on ROV footage, leading to higher cover assessment of hard suitable substrate. Finally, the assessment of suitable hard substrate was incomplete in the presence of dense macroalgae mats and mussel populations. This calls for developing specific guidelines to assess the cover of suitable hard substrate from ROV surveys.

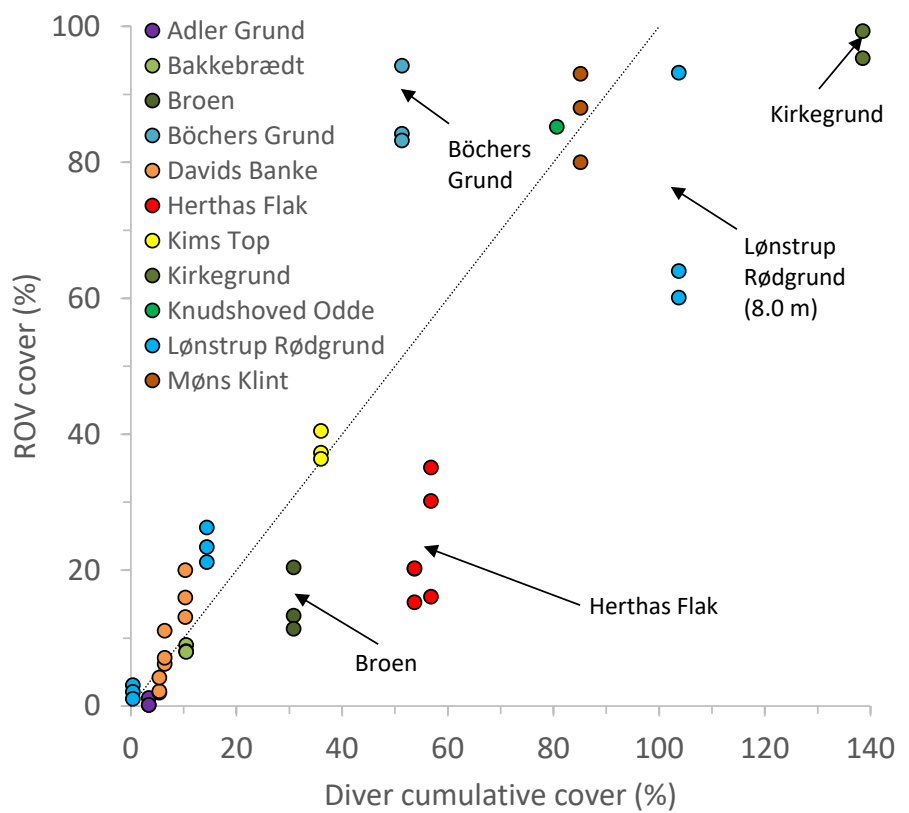
### **3.1.2 Total and cumulative macroalgae cover**

The diver assessment of total macroalgae cover was similar to the ROV assessment of macroalgae cover for 7 out of 11 locations (Figure 3.2). Larger deviations were observed for Broen, Böchers Grund, Herthas Flak (both depths), and Lønstrup Rødgrund at 8.0 m depth. Comparing the ROV assessment of macroalgae cover with the diver assessment of cumulative cover revealed similar patterns with Broen, Böchers Grund, Herthas Flak (both depths), Lønstrup Rødgrund at 8.0 m deviating between the two assessment methods (Figure 3.3). The similarity of these two comparisons is primarily due to the strong and expected agreement between total and cumulative macroalgae cover from diver surveys in most of the depth interval investigated (Figure 3.4), showing small deviations (<10%) for macroalgae communities that are primarily single layered. For multi-layered communities (particularly, cumulative cover >100%), the two cover assessments (cumulative vs total) deviate as total cover approaches 100%.

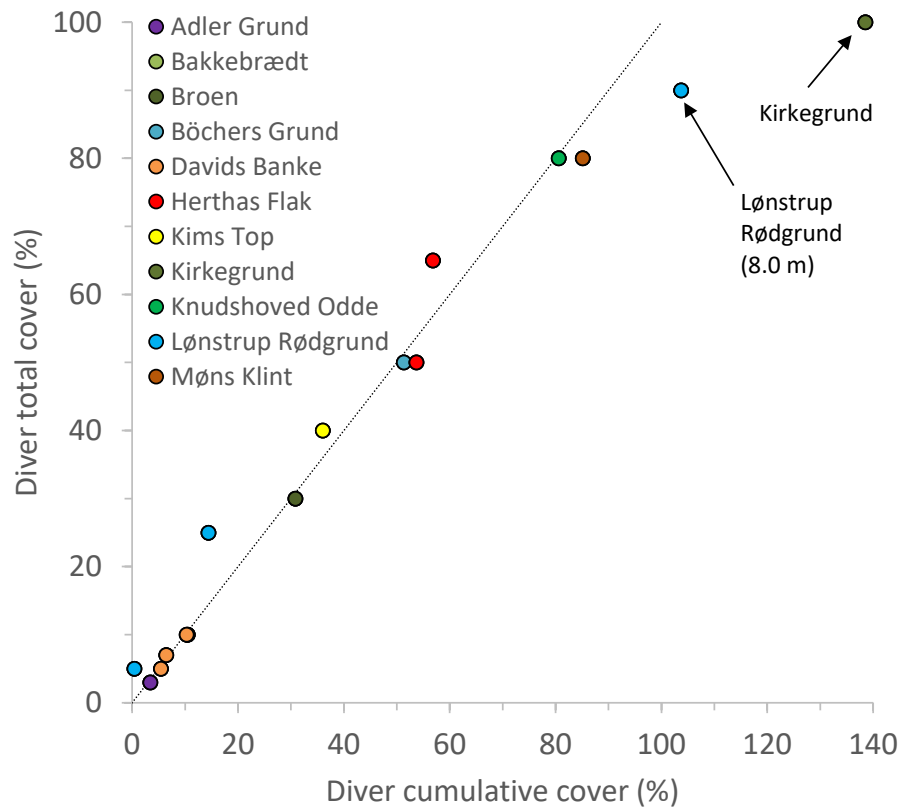
**Figure 3.2.** ROV versus diver observations of total macroalgae cover. Boulder reefs are marked with different colours. Dashed line is the 1:1 identity line. The ROV footage from each location and depth was analysed three times.



**Figure 3.3.** ROV versus diver observations of cumulative macroalgae cover. Boulder reefs are marked with different colours. Dashed line is the 1:1 identity line. The ROV footage from each location and depth was analysed three times.



**Figure 3.4.** Total versus cumulative macroalgae cover from diver surveys. Boulder reefs are marked with different colours. Dashed line is the 1:1 identity line.



At Broen, the macroalgae cover assessed from the ROV was 10-20% lower than the diver assessments. Both diver and ROV investigations found that the macroalgae community was dominated by *Phycodryis rubens*, but the diver assessed the cover of this species to be 29%, whereas ROV assessments were lower (11-20%). Epiphyte growth of *Ciona intestinalis* and filamentous macroalgae on the *Phycodryis rubens* may have exerted a hiding effect on *Phycodryis rubens*, resulting in an underestimation of its actual coverage in the ROV observations.

At Böchers Grund, the ROV assessment was much higher (83-94%) than the diver's assessment of total cover (50%) and cumulative cover (51%). In the ROV assessment, the macroalgae community was mainly comprised of filamentous brown and red algae as well as *Delessaeria sanguinea*, although with clear dominance of filamentous red algae (70-90%). In the diver assessment, *Delessaeria sanguinea* was also identified with similar cover as for ROV and other identified species (*Leptosiphonia fibrillosa*, *Coccotylus brodiei*, *Furcellaria lumbricalis*, *Rhodomela confervoides*, *Vertebrata fucoides*) covered 41%. Insufficient ROV camera quality may explain the absence of red crust detection in the ROV survey. Overexposure of light-coloured substrates and sessile invertebrates (e.g., barnacles, bryozoans, sponges, corals) frequently resulted in poor colour contrast, preventing accurate identification. Furthermore, blurred images from close-up ROV footage also hindered the identification of macroalgae and small fauna species.

At Herthas Flak, the diver assessments of total and cumulative cover were around 50-65% in comparison to much lower macroalgae cover with the ROV. The ROV assessments identified leaf-forming red algae (15-35%), categorized as most likely *Phycodryis rubens* in addition to red and brown crusts that are not included in the macroalgae cover. In the diver assessments, *Dasysiphonia japonica* had the largest cover at both depths (30-40%), and they also included

*Vertebrata byssoides*, *Desmarestia aculeata*, *Delesseria sanguinea*, *Phycodryis rubens* and *Bonnemaisonia hamifera* (at least 1% cover). During the ROV survey at Herthas Flak, visibility was strongly reduced due to marine snow, and filamentous red algae were partially covered by it, causing them to blend with the surrounding substratum. The combination of reduced visibility and marine snow sedimentation on macroalgae complicated coverage estimation at this location, and it is plausible that the cumulative macroalgal cover in the ROV assessment was underestimated.

At 8 m depth at Lønstrup Rødgrund, there was a large difference in the ROV assessment of *Laminaria* sp. from the first round (80%) to the second and third rounds (30%). The diver assessment for *Laminaria hyperborea* was 60%, suggesting that the cover of *Laminaria* sp. was underestimated in the second and third round. In fact, if the cover assessment of *Laminaria* sp. in the second and third round had been 60%, there would be little difference between the two methods. The manoeuvrability of the ROV was limited by the dense population of *Laminaria* sp. at this location. At Lønstrup Rødgrund, footage was obtained either very close to the substratum or above the canopy of *Laminaria* sp., producing markedly different observations of *Laminaria* sp. coverage. Near-substratum perspectives suggested approximately 30% cover of *Laminaria* sp., whereas above the canopy, the coverage appeared closer to 80%. This variation in *Laminaria* sp. coverage, correlating with the ROV's observational angle, complicates coverage estimation and is a plausible explanation for the large variation in *Laminaria* sp. coverage observed between the two assessments and within the ROV assessment.

### 3.1.3 Cover of sea urchins

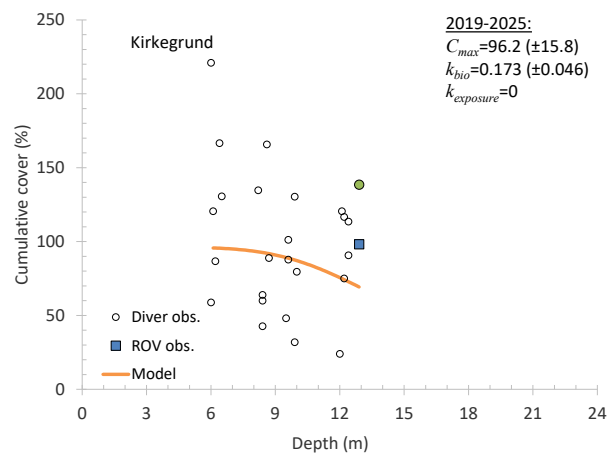
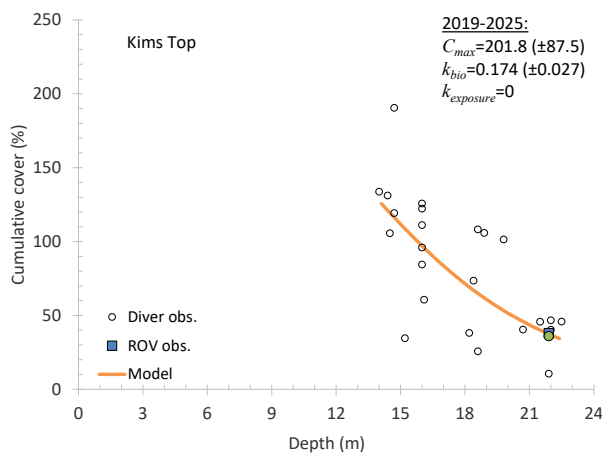
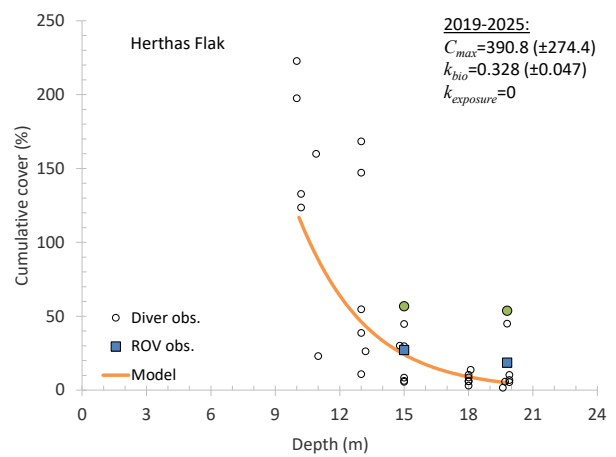
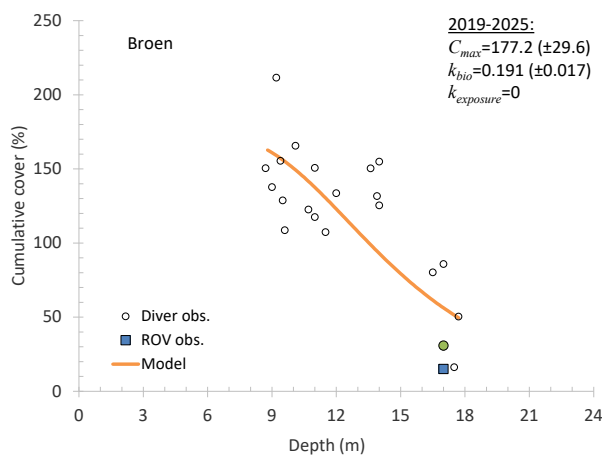
Sea urchins were observed by the diver at Broen (15 individuals of *Strongylocentrotus droebachiensis* corresponding to 0.1%), Kims Top at 21.9 m depth (three individuals of *Echinus esculentus* corresponding to 0.2%), and Lønstrup Rødgrund at 12.7 m depth (two individuals of *Echinus esculentus* corresponding to 0.1%). In the ROV analysis, sea urchins were only recorded at Broen at (1% *Strongylocentrotus droebachiensis*), whereas there were no sea urchin recordings at Kims Top or Lønstrup Rødgrund. Sea urchins were abundant at Broen, leading to identification for both diver and ROV surveys, although the cover assessment from the ROV was higher. This is due to the difficulty of assessing low cover from the ROV footage, where 1 % was used as a minimum value. Counting the number of sea urchins to get a more precise cover estimate from ROV is difficult, as sea urchins can be hidden under macroalgae and stones, and assessing their sizes from the video is difficult. At Kims Top and Lønstrup Rødgrund, sea urchins were found more sporadically and the difference between the two methods could be due to sheer randomness. However, it is possible that sea urchins can be overlooked by ROV surveys, if individuals are hidden below macroalgae or behind stones. Nevertheless, the presence of sea urchins was limited (3 out of 16 sampling locations) and with low cover, suggesting that a potential underestimation from including ROV surveys would have a relatively small effect on the estimation of  $k_{bio}$  for these specific investigations, but methods to improve the estimation of sea urchin cover should be considered.

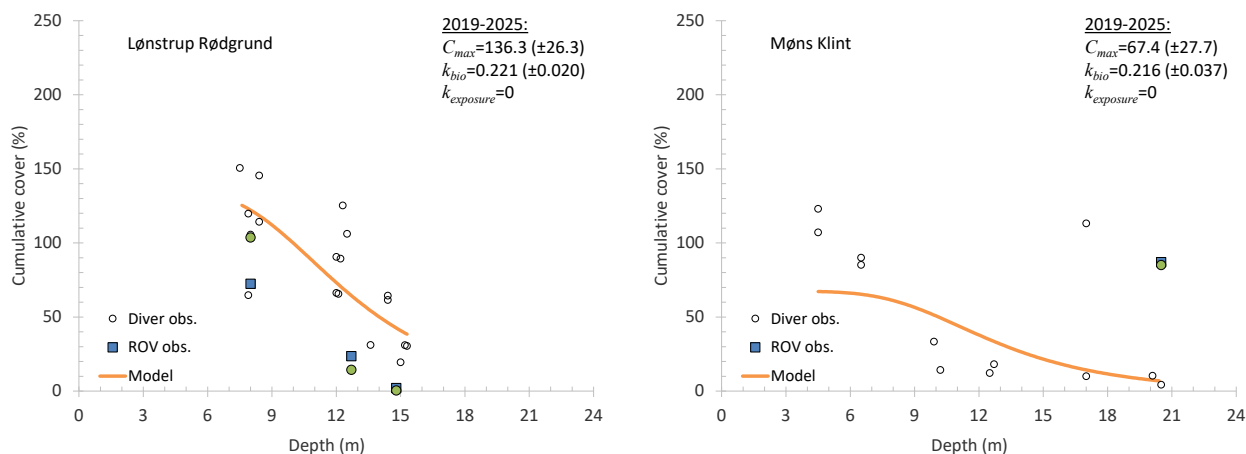
### 3.1.4 ROV observations along depth gradients

Only 6 of the 11 locations had sufficient data for analysing the depth gradient and estimating the model for cumulative cover (Figure 3.5). Adler Grund,

Bakkebrædt, Bøchers Grund, Davids Banke and Knudshoved Odde had too few observations to assess the depth gradient and for estimating  $k_{bio}$  (4 observations or less). Five locations (Adler Grund, Bakkebrædt, Bøchers Grund, Davids Banke, Møns Klint) are also characterised by high cover of *Mytilus* (60-100 %) that likely affect the vegetation cover.

The diver and ROV observations for 2025 were within the expected range of variability along the depth gradient for all six locations, except for Møns Klint where both diver and ROV observations in 2025, as well as a diver observation from 2020, were higher than expected. This specific pattern is observed in several years at Møns Klint, where filamentous red algae with an annual life cycle (typically *Phyllophora spp.* and *Polysiphonia spp.*) may proliferate over summer using blue mussels attached to boulders as substrate and contribute to substantial cover during the surveys in August (data not shown). Although blue mussels are not considered suitable substrate according to the technical guidelines, it can be difficult to assess if the macroalgae are attached to or intertwined with the mussels, particularly from ROV footage. Nevertheless, the depth gradient of cumulative cover at Møns Klint deviates from those observed at other boulder/stone reef locations, which could be due to the substrate at this location as well as the faunal community that also occupies the hard substrate.





**Figure 3.5.** Macroalgae cumulative cover versus depth for the period 2019-2025 with diver and ROV observations from 2025 highlighted (green = diver assessment 2025). The model for cumulative cover was estimated based on diver observations only. Parameter estimates from the model are inserted in the graphs.

At Broen, the relatively low cover in 2025, for both diver and ROV, can only partly be explained by the presence of sea urchins, which according to the model reduces the cumulative cover by ~5-10%. Nevertheless, there was a good agreement between diver and ROV observations in 2025 that overall fit within the expected depth gradient. Noteworthy, the variability between these two observations is small relative to the overall variability.

At Herthas Flak, the ROV observations correspond well to the model expectation, whereas diver observations in 2025 are relatively high, which could be due to spatial differences in the areas surveyed. Overestimation of the cover of some species (e.g. *Dasysiphonia japonica*) by the diver is also a possibility. Even though the differences between diver and ROV assessments were high at Herthas Flak, these differences were still within the usual variability along the depth gradient.

At Kims Top, the diver and ROV assessments in 2025 were almost identical and corresponded to the model expectation.

At Kirkegrund, the diver assessment of cumulative cover in 2025 (138.5%) was relatively high compared to observations from other years, whereas the ROV observation was better aligned with the general pattern. The difference between the two assessments was small compared to the overall variability. However, it should be stressed that at this depth (12.9 m) the macroalgae community is mostly multi-layered and therefore, the two assessment methods are not strictly comparable.

At Lønstrup Rødgrund, macroalgae cover in 2025 from both assessment methods were relatively low, particularly at the deeper sampling sites. On the other hand, the agreement between the two assessment methods at the two deeper sampling sites, and to some extent also at 8 m depth, suggests that the macroalgae community experienced less favourable conditions in 2025. Overall, the differences between the two assessment methods were small compared to the overall variability along the depth gradient.

### 3.1.5 Considerations for ROV assessments

This section summarises the observations and considerations derived from the ROV surveys across the 16 boulder reef locations.

#### **Image resolution and detection of small taxa.**

The ROV camera produced blurred footage during close-up observations, a resolution where identification of fauna and macroalgae species was not possible. Species requiring fine morphological detail for identification – such as leaf-forming and filamentous red algae and small sessile invertebrates – were particularly affected in the ROV assessment. Small specimens are also likely to be frequently overlooked, or their presence underestimated in ROV surveys due to limited camera resolution. This represents a major limitation compared to diver-based assessments.

#### **Overexposure of light-coloured objects**

Overexposure of light-coloured objects (e.g., rocks, corals, barnacles, bryozoans, sponges, and algal crusts) was common in ROV footage, resulting in poor colour contrast and thereby limiting the species-level identification and the detection of light-coloured objects (e.g., the red algae crust) on the substratum in the ROV assessment.

#### **Manoeuvrability in kelp**

Dense kelp populations restricted ROV manoeuvrability due to an increased risk of entanglement and equipment damage. Consequently, the ROV surveys are confined to open canopy areas, limiting spatial coverage and potential, leading to an underestimation of macroalgal and faunal species occurring beneath the kelp canopy. By comparison, divers are less restricted by the kelp, allowing for more detailed examination of the substrata.

#### **Depth registration**

Registration of depth are less accurate as the ROV are located at various depth above the seabed and needs to be corrected in the post processing of the footage.

#### **ROV operational angle**

The ROV is operated with a fixed tilt angle and limited flexibility for moving around the substrate. This can affect the assessment of species and their cover relative to diver surveys, where the diver can better obtain a 3-dimensional picture of the macroalgae community. These challenges can partly be overcome using ROV with multiple cameras capturing the macroalgae community from several angles.

#### **Macroalgal mats**

At sites dominated by dense macroalgal mats (e.g., Bøchers Grund, Bakkebrædt, Kirkegrunden, Møns Klint), examination of the underlying substratum was not possible using an ROV. Less abundant macroalgal and faunal species occurring within the macroalgal mat were often difficult to detect and could not be identified from ROV footage. This limitation resulted in incomplete substratum characterisation and potentially underestimated species diversity. In contrast, divers can physically manipulate macroalgal mats to reveal hidden species and substratum composition, yielding more accurate assessments. This phenomenon becomes more pronounced as the macroalgae community becomes more developed at shallower depths.

### **Sedimentation of POM on macroalgae**

Sedimentation of marine snow on macroalgae reduced species identification accuracy in ROV footage. At distances, macroalgae also blended with the underlying substratum due to surface coverage by particulate organic matter (POM), further complicating species discrimination for coverage estimation. Diver-based surveys offer an advantage, as divers can remove POM from macroalgae to facilitate more accurate species identification.

### **Absence of size reference**

ROV footage lacked scale indicators, precluding accurate size estimation of macroalgae, fauna, and rocks, whereas diver-based assessments are considered more reliable for quantitative size measurements. For ROV surveys exploring substratum composition, the use of fewer and broader size categories (e.g., sand, gravel, rock, boulder) is therefore recommended for obtaining more relevant data. Use of twin preferable green fan shaped laser pointers mounted on the ROV is also an option.

### **Optimal comparison between methods.**

Observational variation was present between the two assessment methods. The difference was most pronounced in the interval between 20 and 80% cover. Part of this variation may be attributed to the lower camera resolution of the ROV, which provides less visual detail than diver-based observations. Furthermore, incomplete spatial overlap between ROV and diver surveys conducted at the same locations may have contributed to discrepancies in observations. To improve comparability between methods, ROV and diver surveys should be conducted in overlapping survey areas.

### **Protocols for ROV surveys**

ROV speed and direction varied throughout the 10-minute surveys, which facilitated species detection but introduced bias in coverage estimation. Fast and abrupt movements also produced blurred footage unsuitable for analysis. Conducting ROV surveys at a slow, constant speed would improve footage quality; while conducting surveys along linear transects at controlled speed and altitude may reduce the risk of bias in coverage estimation.

## **3.2 Macroalgae cumulative cover status assessment**

The macroalgae cumulative cover indicator has been estimated for the period 2019–2024 and tested for potential inclusion in the VP4 status assessment. Of the 42 assessed water bodies, cumulative cover estimation was feasible for 30 sites (see Table A.1). Calculations were performed using Python (Jupyter notebook; version 7.4.5) code translated from the original SAS implementation.

The resulting  $k_{bio}$  values across Danish estuaries and coastal systems reveal a broad spectrum of ecological conditions when evaluated against established classification boundaries. Water bodies such as Østersøen, Bornholm (T.5), Storebælt, SV (T.20) and Lillebælt, syd (T.23) exhibit low  $k_{bio}$  values between 0.135 and 0.159, corresponding to high EQR scores (between 0.855 and 1.000) and High ecological status, even approaching Reference Condition for Lillebælt, syd (T.23) (EQR=1.000). However, except for Østersøen, Bornholm (T.5), the standard error of  $k_{bio}$  was considerable due to lack of deeper observations expressing light regulation of the macroalgae community. In contrast, water bodies like Løgstør Bredning (T.17), Thisted Bredning (T.34) and Bjørnholms Bugt (T.32) present elevated  $k_{bio}$  values exceeding 1.0, with EQRs below 0.2, suggesting Bad ecological status and significant degradation.

Transitional zones, including Storebælt, NV (T.20) and Det sydfynske Øhav (T.13), fall within intermediate  $k_{bio}$  ranges (0.218–0.227), corresponding to Moderate ecological quality.

The probability of achieving Good Ecological Status (pGES) further reinforces these classifications, with high-performing water bodies like Østersøen, Bornholm (T.5), Storebælt, SV (T.20), Kattegat, Læsø (T.21) and Lillebælt, syd (T.23) showing pGES values exceeding 0.95, whereas many water bodies exhibit pGES values of 0.000, indicating virtually no probability of meeting GES targets under current conditions. Of the 30 assessed water bodies, 9 (30%) achieved High or Good status, 10 exhibited Moderate status (33%), while 11 (37%) were classified as Poor or Bad. Overall, the  $k_{bio}$  metric proves to be a reliable indicator for ecological classification of most water bodies across Danish coastal waters. However, improving the precision of  $k_{bio}$  through obtaining deeper observations would improve the confidence of the classification.

## 4 Conclusion and recommendation

Analysis of ROV footage can provide complementary information to diver observation on macroalgae cumulative cover at deeper locations, where the macroalgae community is predominantly one-layered. ROV surveys cannot replace diver observations of species-specific cover, due to poorer taxonomical resolution, but it can provide observations of macroalgae cover at deeper locations where diver observations are not possible for logistical and financial reasons and it has the advantage that ROV footage is stored and can be re-analysed for quality assurance. Macroalgae cover from ROV observations do exhibit large, but no clear systematic bias compared to diver observations. The major cause for difference between the two methods in this study is most likely due to spatial variability due to the limited overlap in the two surveys. However, this assumption should be tested in a well-planned study with exactly the same area being surveyed by both methods. ROV observations of macroalgae cover aligned well along the depth gradient with long-term diver observations of cumulative macroalgae cover. These results suggest that including macroalgae cover from ROV at deeper locations can improve the precision of the  $k_{bio}$  indicator without introducing systematic bias.

However, based on the experience of using ROV footage for assessing macroalgae cover there are some recommendations to improve the quality and standardisation of such data:

- *Survey paths should be predetermined.* In this study, the ROV survey paths were random with occasional revisits to the same spots within the surveyed location. For monitoring and the consistency of monitoring data, the same survey path for a given sampling location should be followed across years. Survey paths should be laid out to cover most sides of boulders/stones to the extent possible. The survey path is confirmed with GPS positioning.
- *Standard operating procedures for ROV surveys.* In order to get the best possible footage of the macroalgae while manoeuvring the ROV to avoid obstacles, the ROV's camera should be tilted at an angle held constant throughout the survey (between 30° and 60°, e.g. 45°). The highest angle will best mimic the vertical diver observations. The ROV should be operated at constant and lowest possible speed to ensure comparable areal coverage across time lapses of the footage. Changing ROV speed may cause blurred images.
- *Improving optical resolution.* The ROVs employed in the study were equipped with 12 mega-pixel camera with 4k/1080p video, representing state-of-the-art technology without excessive costs. The camera should also be capable of adjusting to varying light conditions. A market survey should be carried out to identify the best price-performance camera/video applicable to routine monitoring. The potential of using ROVs with several cameras/videos should also be investigated.
- *Harmonised post-processing of ROV footage.* To ensure consistency across ROV footage analysed by different people, intercalibration of these analysts should take place regularly. Automated analysis of the footage may be considered as a development project, although the potential benefits of such algorithm should be evaluated against visual inspection in terms of data quality and resources.

- *Integration of ROV data with existing monitoring data.* Data from the analysis of ROV footage should be ingested into Vanda as the method is already in use for larger fauna organism on very deep boulder reefs in Skagerrak (>30m depth). Separate tables for this data type should be established, such that the data are not confused with diver observations. Moreover, the taxonomical resolution obtained from ROV footage (for a large number of species aggregated to functional groups) does not comply with the taxonomical tables in Vanda, so data reporting and taxonomical resolution need to be decided. Routines for extracting and displaying ROV data, together with diver observations, should be established.

These recommendations are included as suggested amendments to the technical guidelines (Annex C).

This study and previous studies (Carstensen 2024; Carstensen et al. 2025) have demonstrated that ROV data can improve the precision of the macroalgae indicator  $k_{bio}$  for cumulative cover. Standardising the collection and analysis of ROV observations through a data pipeline feeding into the database Vanda will allow for routine procedures for estimating  $k_{bio}$ . Statistical procedures for calculating  $k_{bio}$  have been developed in Python (Annex B) and can be integrated with Vanda for routine indicator assessment. It is recommended that the data pipeline for diver and ROV observations and the estimation routine for  $k_{bio}$  are integrated into the standard WFD reporting.

## References

Carstensen J. 2020a. Macroalgae indicators for assessing ecological status in Danish WFD water bodies. Aarhus University, DCE – Danish Centre for Environment and Energy, 74 pp. Technical Report No. 170. <https://dce2.au.dk/pub/TR170.pdf>

Carstensen J. 2020b. Makroalgeindikatorer og deres anvendelse til VRD tilstandsvurdering. Notat fra DCE, 30. september 2020. [https://dce.au.dk/fileadmin/dce.au.dk/Udgivelser/Notater\\_2020/MST\\_spoergsmaal\\_om\\_makroalger.pdf](https://dce.au.dk/fileadmin/dce.au.dk/Udgivelser/Notater_2020/MST_spoergsmaal_om_makroalger.pdf)

Carstensen, J. 2024. Validation of macroalgae indicators with observations from deeper depths. Sampling diver observations at deeper depths and estimation of macroalgae indicators. Aarhus University, DCE - Danish Centre for Environment and Energy, 18 s. – Scientific note no. 2024|3. [https://dce.au.dk/fileadmin/dce.au.dk/Udgivelser/Notater\\_2024/N2024\\_03.pdf](https://dce.au.dk/fileadmin/dce.au.dk/Udgivelser/Notater_2024/N2024_03.pdf)

Carstensen J, Al-Hamdani Z, Larsen EG. 2025. Validation of macroalgae indicators through deeper sampling. Identification of suitable substrate and macroalgae observations for indicator improvement. Aarhus University, DCE – Danish Centre for Environment and Energy, 51 pp. Scientific Report No. 647. [https://dce.au.dk/fileadmin/dce.au.dk/Udgivelser/Videnskabelige\\_rapporter\\_600-699/SR647.pdf](https://dce.au.dk/fileadmin/dce.au.dk/Udgivelser/Videnskabelige_rapporter_600-699/SR647.pdf)

Dahl K, Lundsteen S. 2018. Makroalger og hårdbundsfauna på sten- og boblerev. Aarhus University, DCE - Danish Centre for Environment and Energy, 28 s – Teknisk Anvisning M14. [https://ecos.au.dk/fileadmin/ecos/Fagdatacentre/Marin/TA\\_M14\\_Makroalger\\_og\\_bundfauna\\_paa\\_sten-og\\_boblerev\\_ver1.pdf](https://ecos.au.dk/fileadmin/ecos/Fagdatacentre/Marin/TA_M14_Makroalger_og_bundfauna_paa_sten-og_boblerev_ver1.pdf)

Dahl K, Göke C, Carstensen J, Gai F, Petersen P, Nielsen S. 2023. Identifikation af dybe stenforekomster i Lillebælt og Flensborg Fjord. Technical Report No. 276. <https://dce2.au.dk/pub/TR276.pdf>

Høgslund S, Dahl K, Krause-Jensen D, Lundsteen S, Rasmussen MB, Windelin A (2014) Makroalger på kystnær hårdbund. Teknisk Anvisning M12. [https://ecos.au.dk/fileadmin/ecos/Fagdatacentre/Marin/TA\\_M12\\_Makroalger\\_paa\\_kystnaer\\_haardbund\\_ver3.pdf](https://ecos.au.dk/fileadmin/ecos/Fagdatacentre/Marin/TA_M12_Makroalger_paa_kystnaer_haardbund_ver3.pdf)

## Annex A: Draft status assessment

Table A.1: Parameter estimate for  $k_{bio}$  and the associated standard error (Std.Err.) for cumulative coverage in the WFD period (2019–2024) with the ecological quality ratio (EQR; median) and probability of achieving Good Ecological Status (pGES). Classification is based on threshold values proposed in Carstensen (2020): blue denotes High status, green denotes Good status, yellow denotes Moderate status, orange denotes Poor status, and red denotes Bad status. #NS:  $k_{bio}$  was not significant ( $P > 0.01$ ). #NA:  $k_{bio}$  could not be estimated.

Type	Water body	Cumulative Cover				
		$k_{bio}$	Std.Err.	G/M	EQR	pGES
T.5	Østersøen, Bornholm	0.159	0.004	0.206	0.914	1.000
T.9	Smålandsfarvandet, syd	0.308	0.035	0.304	0.594	0.452
T.10	Horsens Fjord, ydre	0.624	0.069	0.182	0.195	0.000
T.12	Køge Bugt	#NS		0.221		0.000
T.12	Hjelm Bugt	#NA		0.214		
T.13	Det sydfynske Øhav	0.218	0.021	0.187	0.530	0.064
T.13	Lillebælt, Bredningen	0.332	0.008	0.182	0.354	0.000
T.14	Rødsand og Bredningen	0.377	0.022	0.381	0.614	0.584
T.17	Løgstør Bredning	1.132	0.045	0.322	0.188	0.000
T.17	Roskilde Fjord, ydre	#NS		0.279		0.000
T.17	Isefjord, ydre	0.356	0.050	0.279	0.485	0.062
T.17	Isefjord, indre	#NS		0.403		0.000
T.19	Kås Bredning og Venø Bugt	0.823	0.054	0.269	0.198	0.000
T.19	Nissum Bredning	0.618	0.028	0.338	0.353	0.000
T.20	Djursland Øst	0.235	0.006	0.202	0.532	0.000
T.20	Nordlige Øresund	#NS		0.246		0.000
T.20	Storebælt, NV	0.227	0.025	0.200	0.544	0.138
T.20	Jammerland Bugt og Musholm Bugt	#NS		0.161		0.000
T.20	Storebælt, SV	0.158	0.019	0.198	0.858	0.979
T.20	Kalundborg Fjord	#NS		0.191		0.000
T.21	Kattegat, Læsø	0.187	0.004	0.210	0.719	1.000
T.21	Nordlige Kattegat, Ålbæk Bugt	0.220	0.012	0.164	0.456	0.000
T.21	Kattegat, Nordsjælland	0.169	0.016	0.169	0.600	0.497
T.21	Kattegat, Nordsjælland >20 m	0.150	0.016	0.181	0.789	0.969
T.22	Smålandsfarvandet, åbne del	0.181	0.016	0.221	0.801	0.994
T.22	Århus Bugt og Begtrup Vig	#NS		0.223		0.000
T.22	Århus Bugt syd, Samsø og Nordlige Bælthav	0.243	0.007	0.182	0.462	0.000
T.22	Vejle Fjord, ydre	0.292	0.012	0.212	0.443	0.000
T.22	Kalø Vig	0.338	0.039	0.221	0.393	0.001
T.22	Langelandssund	#NS		0.221		0.000
T.22	Sejerø Bugt	0.146	0.033	0.158	0.686	0.644
T.22	Ebeltoft Vig	#NS		0.212		0.000
T.23	Flensborg Fjord, ydre	0.169	0.007	0.161	0.580	0.145
T.23	Lillebælt, syd	0.135	0.022	0.218	1.000	1.000
T.23	Åbenrå Fjord	0.276	0.028	0.163	0.370	0.000
T.23	Flensborg Fjord, indre	#NS		0.236		0.000
T.23	Als Fjord	0.335	0.022	0.200	0.373	0.000
T.27	Roskilde Fjord, indre	0.606	0.056	0.428	0.427	0.001
T.28	Vejle Fjord, indre	#NS		0.276		0.000
T.31	Odense Fjord, ydre	0.674	0.039	0.374	0.357	0.000
T.32	Bjørnholms Bugt, Riisgårde Bredning, Skive Fjord og Lovns Bredning	1.272	0.081	0.381	0.190	0.000
T.34	Thisted Bredning	1.284	0.089	0.322	0.178	0.000

## Annex B: Python code for cumulative cover indicator

### B.1 Background and Purpose

A Python-based computational routine was developed to calculate the biological quality indicator ( $k_{bio}$ ) for macroalgae cumulative cover in Danish coastal waters (presented in Table 1 of Annex A). This implementation translates the existing SAS code into open-source Python (tested in version 3.9-3.11; Jupyter Notebook version 7.4.5), maintaining full compatibility with the operational Water Framework Directive (WFD) assessment framework.

The implementation consists of two main components: (I) non-linear model fitting to estimate macroalgae attenuation coefficients ( $k_{bio}$ ) and (II) Monte Carlo simulation for uncertainty propagation and Ecological Quality Ratio (EQR) classification.

### B.2 Part I: Non-linear Model Fitting

#### B.2.1 Ecological Model

The macroalgae cumulative cover is modelled as a function of multiple environmental factors including depth, light availability, physical exposure, and grazing pressure, representing the key ecological processes that limit macroalgae growth in Danish coastal waters. The model structure follows a multiplicative framework where each factor acts as a potential limiting constraint on macroalgae coverage.

The potential coverage component is expressed as:

$$\text{pot\_cover} = \text{maxC} \times \tanh(I_{0\_sat} \times \exp(-k_{bio} \times \text{depth}))$$

where light availability decreases exponentially with depth according to the Beer-Lambert law ( $\exp(-k_{bio} \times \text{depth})$ ), with  $k_{bio}$  representing the light attenuation coefficient that quantifies water turbidity. The hyperbolic tangent function ( $\tanh$ ) introduces a saturation effect, ensuring that predicted coverage asymptotically approaches the maximum potential coverage ( $\text{maxC}$ ) under optimal light conditions, which reflects the physiological response of macroalgae to light intensity. This formulation captures both the exponential decay of light with depth and the saturating relationship between light and photosynthetic capacity.

Physical exposure from wave action and currents is modelled as:

$$\text{phys\_exposure} = \text{parm\_physexp} \times \text{depth}^{-2}$$

where the inverse square relationship with depth reflects the stronger hydrodynamic disturbance in shallow waters that can physically dislodge macroalgae or prevent their establishment. The exposure effect is incorporated as a divisor ( $1 + \text{phys\_exposure}$ ) that reduces potential coverage in proportion to the intensity of physical disturbance, with the effect diminishing rapidly with increasing depth.

Sea urchin grazing pressure is represented as:

$$\text{sea\_urchin\_effect} = 1 / (1 + K\_seaurchin \times (\exp(\log\_seaurchin) - 0.01))$$

where  $K\_seaurchin$  determines the strength of the grazing effect and the subtraction of 0.01 centres the effect around a baseline sea urchin density. This formulation allows grazing to progressively reduce macroalgae coverage as urchin density increases, with the effect saturating at high densities to reflect biological constraints on grazing rates.

The combined model integrates all three limiting factors through multiplication:

$$\text{kum\_daek} = (\text{pot\_cover} / (1 + \text{phys\_exposure})) \times \text{sea\_urchin\_effect}$$

following the ecological principle of limiting factors where each constraint independently reduces the coverage below the potential maximum. The model is fitted to log-transformed observations ( $\log(\text{kum\_daek} + 1)$ ) to stabilize variance across the coverage range and ensure that residuals approximate a normal distribution, which is required for valid statistical inference. The addition of 1 before log-transformation prevents undefined values at zero coverage while maintaining the interpretability of the model parameters.

Prior to model fitting, a quality control filter was applied to exclude observations where the cumulative macroalgae cover was more than 20 percentage points lower than the total recorded cover percentage ( $\text{cumcover} < \text{totcover\_daekpct} - 20$ ). Such observations are considered unreliable as they represent an implausibly large discrepancy between cumulative and total cover, likely reflecting recording errors or data inconsistencies. These observations were excluded by setting their log-transformed values to missing prior to model fitting, consistent with the quality control procedure applied in the original SAS implementation.

### B.2.2 Model Parameters

The model parameters included fixed parameters- light saturation parameter ( $I_0\_sat = 8.5$ ), grazing effect coefficient ( $K\_seaurchin = 0.543604881414991$ ), and sea urchin reference density of  $\log(0.01)$ , and estimated parameters, which included two- and three-parameter models.

Model selection between the two- and three-parameter formulations followed a two-step procedure. In the first step, the full three-parameter model was fitted to all water bodies, including the physical exposure coefficient ( $\text{parm\_physexp}$ ). The significance of  $\text{parm\_physexp}$  was then evaluated using a t-test ( $p < 0.05$ ). In the second step, water bodies where  $\text{parm\_physexp}$  was not statistically significant were refitted using the reduced two-parameter model with physical exposure fixed at zero ( $\text{parm\_physexp} = 0$ ), as the exposure effect could not be distinguished from zero given the available data. This stepwise approach ensures that physical exposure is only included in the model where there is sufficient statistical evidence for its effect, avoiding overfitting in sheltered water bodies where exposure is not a meaningful constraint on macroalgae distribution.

The two-parameter model was applied to 22 water bodies with non-significant exposure effects and involved estimation of the light attenuation

coefficient ( $k_{bio}$ ,  $m^{-1}$ ) and maximum cumulative cover ( $maxC$ , %). The three-parameter model was applied to the remaining 19 water bodies where physical exposure was statistically significant, additionally estimating the physical exposure coefficient ( $parm\_physexp$ ). Initial values for the iteration were set as:  $k_{bio} = 0.1$ ,  $maxC = 100$ ,  $parm\_physexp = 0$ .

### B.2.3 Statistical Estimation

Parameter estimation uses non-linear least squares optimization (SciPy `least_squares` with Trust Region Reflective algorithm). Uncertainty quantification includes standard errors calculated from Hessian matrix, t-statistics ( $t = \text{estimate} / SE$ ), and p-values from t-distribution.

The Python implementation uses Trust Region Reflective optimization while the original SAS code uses Gauss-Newton method (PROC MODEL). Both methods minimize the same objective function and produce statistically equivalent results, with minor numerical differences (typically  $<0.001\%$ ) due to algorithmic implementation. Parameter estimates, standard errors, and statistical conclusions remain identical within numerical precision.

## B.3 Part II: Monte Carlo Simulation and EQR Classification

### B.3.1 Uncertainty Propagation

Monte Carlo simulation with 10,000 iterations per water body is used to propagate parameter uncertainty from  $k_{bio}$  estimates to ecological quality classifications. The simulation process begins by generating  $k_{bio}$  values from a normal distribution ( $k_{bio\_sim} \sim N(k_{bio\_mean}, k_{bio\_stderr})$ ), where each simulated value represents a plausible estimate given the parameter uncertainty. For each simulated  $k_{bio}$  value, the Ecological Quality Ratio (EQR) is calculated using piecewise linear interpolation through water body-specific quality class boundaries. The simulated EQR is then used to assign a quality class ranging from 0 to 4, representing Bad through High ecological status. After completing all iterations, distribution statistics including mean, median, percentiles, and class proportions are calculated to characterize the uncertainty in the ecological quality assessment. Parameters with p-values greater than 0.05 or non-positive  $k_{bio}$  values ( $k_{bio} \leq 0$ ) are excluded from classification following WFD statistical requirements for ensuring reliable assessments based only on statistically significant parameter estimates.

### B.3.2 EQR Calculation

The Ecological Quality Ratio (EQR) is calculated using piecewise linear interpolation between water body-specific class boundaries, which divide the  $k_{bio}$  gradient into five quality classes. High quality (Class 4) corresponds to EQR values between 0.8 and 1.0, Good quality (Class 3) ranges from 0.6 to 0.8, Moderate quality (Class 2) spans 0.4 to 0.6, Poor quality (Class 1) covers 0.2 to 0.4, and Bad quality (Class 0) represents EQR values below 0.2. The interpolation is performed using the following formula, illustrated here for the High/Good boundary region: when  $k_{bio\_RC} \leq k_{bio} < k_{bio\_HG}$ , then  $EQR = -(k_{bio} - k_{bio\_RC}) / (k_{bio\_HG} - k_{bio\_RC}) \times 0.2 + 1.0$ . In this formula,  $k_{bio\_RC}$  represents the Reference Condition boundary (the lowest  $k_{bio}$  value corresponding to best quality),  $k_{bio\_HG}$  is the High/Good boundary,  $k_{bio\_GM}$  is the Good/Moderate boundary,  $k_{bio\_MP}$  is the Moderate/Poor boundary, and  $k_{bio\_PB}$  is the Poor/Bad boundary. Similar linear interpolations are applied

for each adjacent class boundary pair, ensuring that EQR decreases linearly with increasing kbio within each quality class range.

### **B.3.3 Output Structure**

The classification routine produces three primary outputs. First, class proportions for each water body represent the probability distribution over five quality classes (High, Good, Moderate, Poor, Bad), quantifying uncertainty in classification due to parameter estimation uncertainty. Second, EQR statistics including mean, median, and percentiles (P5, P95) characterize the central tendency and variability of the EQR distribution for each water body. Third, all individual Monte Carlo samples are retained for verification and sensitivity analysis purposes.

### **B.4 Input Data Requirements**

The Python routine requires two primary data inputs for ecological quality assessment. The monitoring data includes water body identifiers specifying the type, area name, and WFD monitoring period, along with depth-stratified macroalgae cumulative cover observations collected across the depth gradient and sea urchin density data provided in log-transformed format. A typical dataset encompasses approximately 42 water bodies with 20 to 120 depth-stratified observations per water body, providing sufficient data density for robust parameter estimation across the Danish coastal gradient.

The classification boundaries consist of predefined water body-specific quality class thresholds that define the transitions between ecological status classes, including kbio\_RC (Reference Condition), kbio\_HG (High/Good boundary), kbio\_GM (Good/Moderate boundary), kbio\_MP (Moderate/Poor boundary), and kbio\_PB (Poor/Bad boundary).

### **B.5 Implementation and Validation**

The Python implementation was developed using open-source scientific computing packages (NumPy, SciPy, Pandas) and executed in Jupyter Notebook (notebook version 7.4.5) format for transparency and reproducibility. The code was validated against the existing SAS implementation, with minor numerical differences arising from different optimization algorithms and do not affect ecological interpretation or quality class assignments. The validated Python code provides a robust foundation for routine ecological status classification of Danish coastal waters under the Water Framework Directive.

## Annex C: Amendments to Technical Guidance (TA)

This annex lists suggested amendments to the Danish Technical Guidance for 'Makroalger og hårdbundsfauna på kystnær hårdbund, sten- og boblerev' that is currently under revision (cf. Teis Boderskov, DCE). The Technical Guidance is in Danish. Suggested amendments are shown in red.

### 1 Indledning (last paragraph)

Undersøgelserne er baseret på en artskyndig dykkers visuelle bedømmelse af arter og sediment og deres dækningsprocent, kombineret med indsamling af artsmateriale og efterfølgende bestemmelse i laboratoriet. Ved ekstensive undersøgelser benyttes det indsamlede materiale til verifikation af dykkerens artsbestemmelser, hvorimod der ved intensive undersøgelser foretages en detaljeret artsbestemmelse i laboratoriet. **Denne TA inkluderer desuden undersøgelser med ROV til bestemmelse af samlet dækningsprocent for dybere forekomster af makroalger uden fler-laget struktur.**

### 2 Metode (added paragraph at the end)

**Ved større dybder med et-laget makroalgensamfund, som ikke er mulige at undersøge med dykker, kan undersøgelsen suppleres med ROV video undersøgelser fra båden. Disse videoundersøgelser analyseres efterfølgende af en person med erfaring i alge- og faunaorganismer på og omkring sten.**

#### 2.1 Tid, sted og periode (added paragraph at the end)

**Ved ROV-undersøgelser på dybere lokaliteter defineres et fast transekt, som dækker et område svarende til punktdyk.**

#### 2.2 Undersøgelsestype (added paragraph at the end)

**Ved ROV-undersøgelser bestemmes makroalge-og faunasammensætningen på og omkring sten alene på videomateriale, og der indsamles ikke prøver til yderligere bestemmelse i laboratoriet.**

#### 2.3 Udstyr - Feltudstyr (added bullet point to list)

- ROV udstyret med kamera/video med tilstrækkelig opløsning (minimum 12 mega-pixel), dybdemåler og GPS sender**

#### 2.4 Procedure (added paragraph before 'Feltskema for ...')

**Videomateriale fra ROV'en screenes samtidig på skibet for at sikre tilstrækkelig kvalitet til bestemmelse af makroalger og fauna efterfølgende.**

##### 2.4.1.1 Kystnær hårdbund/Stenrev (first paragraph)

På en station placeres målestederne/undersøgelserne inden for dybdeintervaller i punkter af 25 m<sup>2</sup> (cirkler med radius på ca. 2,8 m) med >10% hårdbund, dog hvor områder med >25 % stabil hårdbund prioriteres. Hvis undersøgelsen udføres i dybdeintervaller med <25 % stabil hårdbund foretages 3

undersøgelser indenfor dybdeintervallet. For dybdeintervaller hvor stabil hårdbund udgør mere end 25 % kan der nøjes med 1 undersøgelse pr dybdeinterval. Såfremt dykkertider ikke tillader, at der gennemføres 3 undersøgelser i dybdeintervaller med <25 % hårdbund, kan der afviges fra ovenstående og foretages 1 undersøgelse. **For lokaliteter, hvor dykkertider begrænser undersøgelserne omfang og hvor makroalgesamfundet formodes at være et-laget, kan dykkerobservationer suppleres med ROV undersøgelser.**

#### **2.4.2.2 Registrering af dybde (first paragraph)**

På kystnær hårdbund og stenrev måles dybden for en undersøgelse på havbunden mellem stenene. På boblerev måles dybden for de områder, der bliver undersøgt. **For ROV undersøgelser anslås dybden ud fra den målte dybde på ROV og estimeret dybdeafstand fra ROV til substrat.** Dybden angives i meter med 1 decimals nøjagtighed. Det er vigtigt at være opmærksom på den aktuelle vandstand og korrigerer dybderne i forhold til Dansk Vertikal Reference 1990 (DVR90).

#### **2.4.2.3 Bedømmelse af substratet (added paragraph at the end)**

**ROV undersøgelser opgøres på samme vis som punktdyk for dybere stationer, dog kun for den del af videoen, hvor makroalgesamfundet tillader, at substratet kan vurderes med tilpas sikkerhed.**

#### **2.4.2.5 Samlet dækningsgrad af makroalger på stabil hårdbund (first paragraph)**

I hvert punktdyk vurderer dykkeren den samlede dækningsgrad for fasthæftede oprette alger (ikke skorpeformede alger), som helhed på den stabile hårde bund. **Dette gøres på tilsvarende vis ved analyse af video fra ROV.**

#### **2.4.2.6 Angivelse af drivende algers dækning (added paragraph at the end)**

**Dækning af drivende alger opgøres ikke for ROV undersøgelser, da det ikke er muligt ud fra videoen at afgøre, i hvilket omfang algerne er fastsiddende eller drivende.**

#### **2.4.2.8 Udbredelse af søpindsvin (added paragraph at the end)**

**Ved ROV undersøgelser vurderes kun samlet dækningsgrad, da det ikke er muligt at bestemme størrelsesfordelingen ud fra videomaterialet.**

#### **2.4.2.9 Artsregistrering af makroalger og fauna (added paragraph at the end)**

**For ROV undersøgelser er det ikke muligt at artsbestemme alle makroalger og fauna på samme niveau som dykkerobservationer. Der registreres efter artsgrupper, som kan identificeres på videoobservationerne.**

#### **2.6 Navne for vanskeligt bestemmelige artskomplekser (added bullet list at the end)**

**For ROV undersøgelser anvendes desuden følgende algegrupper:**

- Filamentous red algae: Det omfatter slægterne *Rhodomela*, *Carradoriella*, *Leptosiphonia*, *Melanothamnus*, *Vertebrata* og *Ceramium*, samt familierne: *Dasyaceae*, *Cystocloniaceae* og *Bonnemaisoniaceae*.
- Leaf-forming red algae: Det omfatter familierne *Phyllophoraceae*, *Gigartinaeae*, *Dumontiaceae*, *Cystocloniaceae*, *Delesseriaceae*, og *Palmariaceae*
- Filamentous brown- or red algae: Det omfatter genus *Sphacelaria*, slægterne *Rhodomela*, *Carradoriella*, *Leptosiphonia*, *Melanothamnus*, *Vertebrata* og *Ceramium*, samt familierne: *Dasyaceae*, *Cystocloniaceae*, *Desmarestiaceae*, *Ectocarpaceae* og *Bobbemaisoniaceae*.
- Branched Red or Brown algae: Det omfatter slægten: *Carradoriella* og familierne *Desmarestiaceae*, *Furcellariaceae* og *Ahnfeltiaceae*
- *Laminaria* sp: Det omfatter arterne *Laminaria digitata*, *Laminaria hyperborea* og *Saccharina latissima*
- Red calcified crust: Det omfatter familierne *Lithophyllaceae*, *Hapalidiaceae* og *Hydrolithaceae*.

### 3 Særlige forholdsregler og faldgruber (last paragraph)

Det henstilles, at der løbende afholdes interkalibreringskurser, som imødekommer eventuelle udfordringer, der kan opstå i forbindelse med bestemmelse af arter for både dykker, videoanalytiker og laboratiemandskab.

### 5 Kvalitetssikring (third paragraph)

Videoptagelser og samtaler fra de enkelte undersøgelser kan gemmes som dokumentation og evt. verifikation i forbindelse med uklarheder mellem feltbestemte og laboratoriebestemte arter. Videoptagelser fra ROV skal gemmes, da de udgør råmaterialet til opgørelse af alger og fauna.

## OPERATIONALIZATION OF MACROALGAE MONITORING FOR WFD CUMULATIVE COVER INDICATOR

The potential for complementing diver observations of macroalgae cumulative cover with ROV surveys at deeper locations was investigated. When the macroalgae community is one-layered, there is no systematic deviation between diver and ROV observations. Including ROV observations at deeper locations will improve the macroalgae indicator precision and allow for estimating the indicator for more water bodies. It is recommended to incorporate ROV surveys for deeper locations in the national monitoring program and streamlining data flows for indicator estimation.