



MACROALGAL INDICATORS FOR DANISH NATURA 2000 HABITATS

Technical Report from DCE – Danish Centre for Environment and Energy

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Data sheet

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Abstract: This report investigates the potential for assessing good ecological potential according to the Habitats Directive for 12 stone reefs and 21 coastal habitats in Denmark. Three different macroalgal indicators were investigated and they were shown to be regulated by light availability, salinity, physical exposure and the presence of sea urchins. The indicators allow separation of anthropogenic influence from natural variations. Thresholds for good ecological potential at stone reefs are proposed and an approach for similar values in coastal habitats is proposed.

Keywords: Habitats Directive, eutrophication, sea urchins, physical exposure, salinity, light regulation, good ecological potential

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Preface

This report is the outcome of a project investigating the applicability of using macroalgal indicators for assessing good ecological potential according to the European Habitats Directive. This report addresses two different types of Natura 2000 habitats: *Large shallow inlets and bays (type 1160)* and *Reefs (type 1170)*. Financial support was provided by the Danish Environmental Protection Agency (Miljøstyrelsen).

Summary

The applicability of using macroalgal indicators for assessing the ecological potential according to the European Habitats Directive was investigated. Three indicators have been developed and applied to macroalgal monitoring data from 12 stone reefs and 21 coastal habitats. Ecological indicators must reflect ecological status as affected by human activities. Therefore, a key component of indicator development is to separate natural variations from variations caused by anthropogenic pressures. Observations of macroalgal cover, distilled into three indicators (cumulative cover, proportion of opportunists, and number of perennial species), are influenced by physical exposure, salinity, grazing by sea urchins and light limitation, with light limitation partly reflecting nutrient pressure from land.

These different sources of variability affect the indicators to varying extent in different types of areas. Physical exposure is most pronounced at shallow depth (less than 2-4 m). Sea urchins can completely graze down the entire macroalgal community, stressing the importance of recording sea urchins in the monitoring programme. The macroalgal community structure is strongly controlled by salinity, limiting the number of species in brackish water and shifting the composition towards more opportunistic species. In deeper waters, the macroalgal community changes from light-saturated to light-limited growth, and this change is linked to the attenuation of light in the water column, which varies between sites and over time, primarily reflecting differences in eutrophication. This report presents statistical models that can separate these differences sources of variation and provide a "cleansed measure" (indicator) of anthropogenic disturbance.

Using Secchi depth as a proxy for light attenuation, we show that the macroalgal indicators respond to variations in Secchi depth over time at the open-water stone reefs, and that the spatial variation in macroalgal indicators in coastal habitats is correlated with mean Secchi depths for the habitats. Historical data are not available for guiding a target setting of the macroalgal indicators, but ecological targets for Secchi depth from the Baltic Sea Action Plan and the implementation of the Water Framework Directive (WFD) can be translated into targets for the macroalgal indicators.

Sammenfatning

Denne rapport undersøger muligheden for at vurdere det økologiske potentiale i henhold til det europæiske habitatdirektiv ved hjælp af makroalgeindikatorer. Tre indikatorer er blevet udviklet og anvendt på makroalgeovervågningsdata fra 12 stenrev og 21 kystnære habitater. Indikatorer skal afspejle den økologiske tilstand, og hvordan mennesker påvirker denne. En vigtig komponent i indikatorudviklingen er derfor at adskille naturlig variation fra variationer forårsaget af menneskelig påvirkning. Derved fås et klarere signal om, hvordan menneskelige aktiviteter påvirker økosystemerne. Makroalgeobservationer, sammenfattet til tre indeks (kumulativ dækning, andelen af opportunister og antallet af flerårige arter), er påvirket af fysisk eksponering, saltholdighed, græsning fra søpindsvin og lysbegrænsning, hvoraf lysbegrænsning er delvist påvirket af næringsstoffer fra land.

Disse kilder til variation påvirker de foreslåede indikatorer i varierende grad. Fysisk eksponering er størst på små dybder (mindre end 2-4 m). Søpindsvin kan græsse makroalger helt væk, hvilket understreger behovet for at få denne information opgjort konsekvent. Samfundsstrukturen af makroalgerne er stærkt påvirket af saltholdigheden, idet antallet af arter falder markant i brakvandsområder og samfundet skifter mod flere opportunister. På større dybder skifter makroalgesamfundet fra at være lysmættet til at være lysbegrænset, og dette skift er koblet til lyssvækkelsen i vandsøjlen, som igen er koblet til eutrofiering. Rapporten præsenterer statistiske modeller, som kan adskille disse forskellige kilder til variation i data, hvilket resulterer i et "renset signal" (indikator) for den menneskeskabte påvirkning.

Ved at anvende sigtdybde som alternativt mål for lyssvækkelsen viser vi, at makroalgeindikatorerne responderer på variationer i sigtdybde over tid på stenrevne i de indre åbne farvande, og at der er en rumlig korrelation mellem makroalgeindikatorerne og sigtdybden i de kystnære habitater. Det har ikke været muligt at anvende historiske data til at finde passende grænseværdier for indikatorerne, men det er muligt at bestemme sådanne ud fra tilsvarende grænseværdier for sigtdybde, som er bestemt i Østersøhandlingsplanen og vandrammedirektivet.

1. Introduction

The objective of this report is to investigate the applicability of three macroalgal indicators proposed for the European Habitats Directive (HD) to the two Natura 2000 habitats *Large shallow inlets and bays (type 1160)* and *Reefs (type 1170)*. The three tested algal indicators can be included in the tools to be developed for assessing conservation status for habitat type *Reefs*, except those too deep for hosting macroalgal vegetation, and for the habitat type *Large shallow inlets and bays*, where these include sufficient hard substrate in the photic zone to host macroalgal vegetation.

Coastal Natura 2000 sites: Some coastal Natura 2000 areas designated due to the presence of the habitat type *Large shallow inlets and bays* also host *Reef* sites as part of the designation and the reefs are in many cases also located within the bays themselves. Despite this technical separation, these coastal reefs are an ecological subset of the biological habitats in bays, and macroalgal indicators are relevant in both cases. In this exercise, we have not been able to separate coastal algal data sampled in Natura 2000 sites designated due to *Large shallow inlets and bays* from *Reefs* within the same areas.

Macroalgal data are also sampled in *Large shallow inlets and bays* as part of the national monitoring programme (NOVANA), in areas not fulfilling the Danish definition of reefs. The NOVANA programme sets a minimum level of 10 % hard stable substrate for a sampling location for macroalgal vegetation, whereas the Danish reef definition states that a core area having 25 % cover of hard stable substrate must be present. The analyses of this report are done for the inlets or bays as a whole based on vegetation data collected on sites with at least 10 % hard substrate.

Open water Reefs: Natura 2000 sites designated as *Reefs* are also present in open waters outside the coastal waterbodies. In such areas, monitoring differs to some degree; however, we aim at identifying common macroalgal indicators and principles for target setting for both coastal and open water *Reefs* and *Large shallow inlets and bays* and highly recommend this approach.

2. Macroalgal monitoring data and methods

Macroalgal data were extracted from the national database (ODA) and aggregated to three indicators (Carstensen et al. 2014):

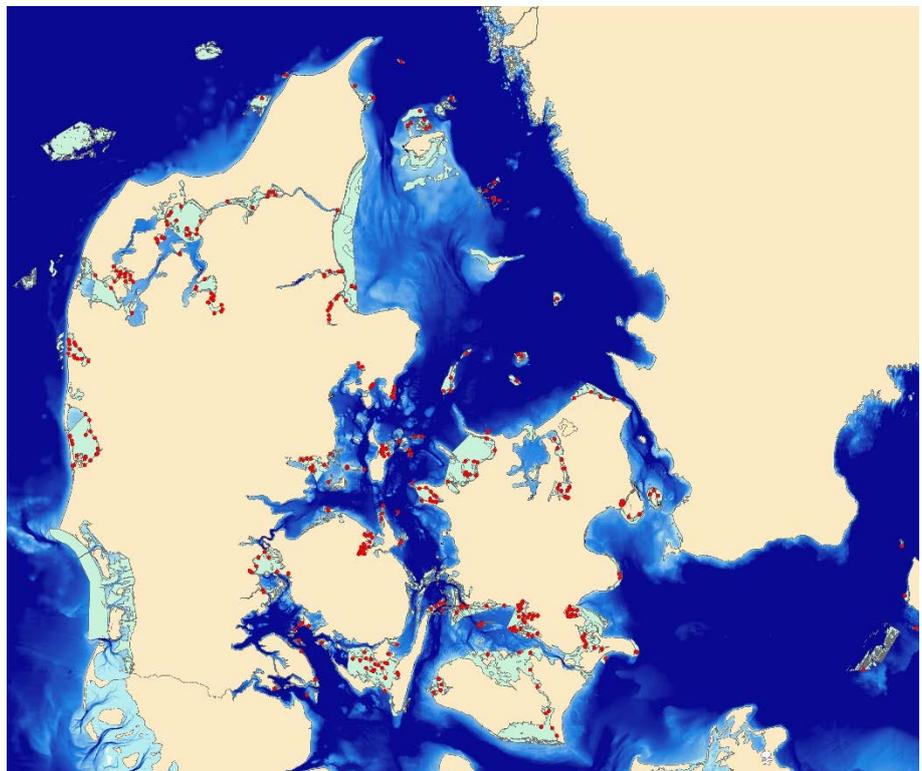
- *Cumulative cover*: The sum of species-specific cover of all erect macroalgal species in each subsample (depth-specific), i.e. all macroalgae except crust-forming algae.
- *Proportion of opportunists*: The cumulative cover of opportunistic species divided by the cumulative cover of all erect macroalgal species for each subsample.
- *Perennial species richness*: The number of perennial species in each subsample having a cover of at least 1 %.

The data were separated into two groups:

- Open-water reefs (habitat type 1170), where the cover of sea urchins was also monitored with the macroalgae, and
- Coastal sites covering two habitat types: *Reefs* (type 1170) and *Large shallow inlets and bays* (type 1160).

Only data located within Natura 2000 sites were analysed. Further, within the two groups of data, only habitats with sufficient data were selected for the analyses. The selected sites for the two groups of data are shown in Fig. 2.1.

Figure 2.1. Selected Natura 2000 sites for analysis of macroalgal indicators grouped into open-water reefs and coastal sites. Macroalgal transects used in the study are shown as red dots



Pelagic monitoring stations were associated with the selected sites to provide information on environmental conditions representative for the macroalgae. From these pelagic stations, the average salinity profile with depth was calculated and combined with the depth-specific indicators for macroalgae. Furthermore, site-specific and annual means for Secchi depth, total nitrogen (TN)

and total phosphorus (TP) were computed following the methodology in Hansen et al. (2018).

2.1 Open-water Reefs

Macroalgal monitoring at stone reefs in the open waters started in 1989, and monitoring of species-specific cover was introduced in 1991, but only at a few sites. Since 1994, species-specific cover has been monitored more consistently and these data are included in the analysis, although not all sites have been monitored every single year (*Table 2.1*). Consequently, the stone reef monitoring data are not homogeneously sampled in time and space, and the subsamples cover different depth ranges.

Table 2.1. Overview of data used for analysing open-water reefs. HD no. refers to the Danish numbering system for the Habitats Directive.

Site	HD no.	Years	Depths (m)	#sub-samples
Broen	175	1994-2015	7.0 - 17.2	47
Hatter Barn	174	1994-2016	6.3 - 17.3	33
Havet omkring Nordre Rønner	176	1998-2017	0.4 - 12.8	394
Herthas Flak	166	1994-2016	9.0 - 20.5	193
Kims Top og den Kinesiske Mur	165	1994-2016	13.0 - 26.5	156
Kirkegrund	149	1994-2016	5.6 - 14.5	103
Klinteskov og Klinteskov Kalkgrund	207	1995-2016	0.4 - 20.9	122
Knudegrund	203	2004-2017	168	126
Læsø Trindel og Tønneberg Banke	168	1994-2016	10.0 - 18.6	114
Lønstrup Rødgrund	202	2004-2016	7.9 - 15.3	25
Schultz og Hastens Grund samt Briseis Flak	204	1994-2016	1.5 - 19.1	359
Store Middelgrund	169	1994-2016	7.0 - 24.0	238

2.2 Coastal Natura 2000 sites with Reefs and Large shallow inlets and bays

Macroalgal monitoring in coastal habitats started in 1989 on a national level, although some sporadic regional monitoring data are available from 1981 to 1988. Before 2001, macroalgal cover was assessed as average values over depth intervals, and in 2001, the monitoring guidelines were changed so that macroalgal cover was assessed at discrete depths. Previous analyses have shown that this change of monitoring method also introduced changes in the trends and, consequently, the analyses of macroalgae in coastal habitats were restricted to data after 2001. Furthermore, data from Ringkøbing Fjord and Nissum Fjord were not included, since macroalgae were only recorded down to 1.5 m due to lack of substrate at deeper depths. A total of 21 coastal habitats with sufficient data for analysing the macroalgal indicators were used (*Table 2.2*).

Table 2.2. Overview of data used for analysing coastal Natura 2000 areas with habitat types 1160 and 1170. HD no. refers to the Danish numbering system for the Habitats Directive.

Site	HD no.	Habitat type	Years	Depths (m)	#sub-samples
Agger Tange, Nissum Bredning, Skibsted Fjord og Aggersund	28	1160, 1170	2001-2017	0.2 - 5.8	674
Flensborg Fjord, Bredgrund og farvandet omkring Als	173	1170	2001-2017	0.1 - 14.5	894
Fyns Hoved, Lillegrund og Lillestrand	91	1160, 1170	2001-2016	0.3 - 11.4	314
Gilleleje Flak og Tragten	171	1170	2001-2016	0.4 - 14.0	303
Havet og kysten mellem Karrebæk Fjord og Knudshoved Odde	148	1160, 1170	2001-2017	0.1 - 11.5	692
Hesselø med omliggende stenrev	112	1170	2001-2016	0.5 - 13.7	219
Hirsholmene, havet vest herfor og Ellinge Å's udløb	4	1170	2004-2017	0.3 - 11.8	210
Horsens Fjord, havet øst for og Endelave	52	1160, 1170	2001-2017	0.1 - 18.0	335
Kaløskovene og Kaløvig	230	1160, 1170	2001-2016	0.4 - 9.1	108
Lillebælt	96	1160, 1170	2001-2016	0.1 - 13.0	594
Lovns Bredning, Hjarbæk Fjord og Skals, Simested	30	1160, 1170	2001-2017	0.3 - 4.6	153
Løgstør Bredning, Vejlerne og Bulbjerg	15	1160, 1170	2001-2017	0.2 - 4.8	289
Mols Bjerge med kystvande	186	1160, 1170	2005-2016	0.3 - 9.9	263
Roskilde Fjord	120	1160	2003-2017	0.2 - 15.0	493
Røsnæs, Røsnæs Rev og Kalundborg Fjord	195	1160, 1170	2001-2016	0.3 - 15.1	695
Saltholm og omliggende hav	126	1160, 1170	2001-2017	0.3 - 11.4	259
Sejerø Bugt og Saltbæk Vig	135	1160, 1170	2001-2016	0.5 - 8.9	387
Stavns Fjord, Samsø Østerflak og Nordby Hede	51	1160, 1170	2001-2017	0.3 - 21.2	107
Sydfynske Øhav	111	1160, 1170	2005-2017	0.2 - 9.8	502
Vestamager og havet syd for	127	1160	2001-2010	0.8 - 9.2	58
Æbelø, havet syd for og Næstå Strand	92	1160, 1170	2003-2016	0.5 - 9.6	139

2.3 Statistical analyses

The objective of the statistical analyses was to model variations in the three macroalgal indicators as functions of location and depth, time, salinity, and cover of sea urchins (only open-water stone reefs). The indicator observations were analysed using two different modelling approaches: 1) a descriptive linear model for partitioning variations between location and depth, years, and the cover of sea urchins, and 2) a semi-mechanistic non-linear model encapsulating the main physico-chemical drivers of variations in the three macroalgal indicators.

2.3.1 Descriptive linear model

The macroalgal indicators were unevenly distributed across years and *Reefs/Large shallow inlet and bays* (Tables 2.1 and 2.2). In order to compare different years and sites, a model was formulated to encapsulate this heterogeneity in the sampling. Macroalgal indicators were transformed to obtain normal distributed variates (y_i) and, for coastal Natura 2000 sites hosting *Large shallow inlets and bays* as well as *Reefs*, these were modelled as:

$$\begin{aligned}
 y_i = & \text{year}_i + \text{site}_j + \text{depth} + \text{year}_i \times \text{depth} + \text{site}_j \times \text{depth} \\
 & + \text{YEAR}_i \times \text{SITE}_j + \text{YEAR}_i \times \text{SITE}_j \times \text{DEPTH} \\
 & + \text{STATION}_k(\text{SITE}_j) + \text{STATION}_k(\text{SITE}_j) \times \text{DEPTH} + e_{ijkl}
 \end{aligned}
 \tag{Eq. 2.1}$$

where year_i described variations between years, site_j described variations between coastal Natura 2000 sites, depth described the common depth relationship for all years and habitats, $\text{year}_i \times \text{depth}$ described the year-specific depth relationships, and $\text{site}_j \times \text{depth}$ described the Natura 2000 site-specific depth

relationships (all fixed factors). In addition to these effects, the model included a number of random variations, i.e. $YEAR_i \times SITE_j$ describing the interannual variations among Natura 2000 sites, $YEAR_i \times SITE_j \times DEPTH$ describing the interannual variations in the site-specific depth relationships, $STATION_k(SITE_j)$ describing the variation among monitoring transects within a Natura 2000 site, $STATION_k(SITE_j) \times DEPTH$ describing variations in the depth relationship among transects within a site, and e_{ijkl} describing the residual variation. Two higher-order factors ($STATION_k(SITE_j) \times YEAR_i$ and $STATION_k(SITE_j) \times YEAR_i \times DEPTH$) were not included in Eq. 2.1, because there were too few observations for their estimation. The analysis of Eq. 2.1 was restricted to depths where physical exposure was considered small (see Carstensen et al. 2008 for details).

For *Reefs* in Natura 2000 sites in open waters, the potential effect of sea urchins was included as an additional covariate, after log-transformation of the cover (adding 1 % to account for zero observations).

$$\begin{aligned}
y_t = & year_i + site_j + depth + year_i \times depth + site_j \times depth \\
& + \log(cover_{seaurchin} + 1) + \log(cover_{seaurchin} + 1) \times depth \\
& + YEAR_i \times SITE_j + YEAR_i \times SITE_j \times DEPTH \\
& + STATION_k(SITE_j) + STATION_k(SITE_j) \times DEPTH + e_{ijkl}
\end{aligned} \tag{Eq. 2.2}$$

where $\log(cover_{seaurchin} + 1)$ described the overall effect of sea urchins and $\log(cover_{seaurchin} + 1) \times depth$ described if this effect changed linearly with depth. It was not possible to investigate interactions between sea urchin cover and habitats/transects as well as interactions between sea urchin cover and year, because of the general lack of variability in sea urchin cover (many zero observations with sporadic occurrences of sea urchins recorded). Since the model aimed at describing the declining cumulative cover with increasing depth, i.e. where macroalgae were light regulated, the analysis of Eq. (2.2) was restricted to depths deeper than 8 m.

The descriptive model (Eq. 2.1 and 2.2) was used to examine the significance of the different sources of variation as well as to describe temporal and spatial variations for at a given depth and a given sea urchin cover. These latter variations were calculated as marginal means from the model, using a standard depth of 4 and 15 m for coastal habitats and stone reefs, respectively, and a standard sea urchin cover of 0 % (stone reefs only).

2.3.2 Semi-mechanistic non-linear model for stone reef macroalgae

Variations in macroalgal indicators with depth are not always well-described using linear models. Macroalgal growth, and consequently macroalgal cover, depends on light availability, which decreases with depth. Similarly, physical exposure from waves can reduce macroalgal cover, but the physical exposure also decreases with depth. Macroalgal cover and community composition may also depend on salinity, because species diversity increases with salinity allowing more complex communities with higher cumulative cover to develop at high salinities.

Light availability decreases exponentially with depth (d) as described by Lambert-Beer's law with a light attenuation coefficient (k_d), which can vary spatially (among sites) and temporally (e.g. among years).

$$I = I_0 \cdot \exp(-k_d \cdot d) \tag{Eq. 2.3}$$

where I_0 is the irradiance at the surface. However, macroalgal cover does not respond proportionally to light availability, because of light saturation of macroalgal growth and reduced growth by self-shading. Using the light-limited growth curve by Platt & Jassby (1976), the potential macroalgal cover C_{pot} can be described as:

$$C_{pot} = C_{max} \cdot \tanh\left(\frac{I_0}{I_{sat}} \exp(-k_{bio} \cdot d)\right) \quad (\text{Eq. 2.4})$$

where C_{max} describes the maximum cover at irradiance levels sustaining maximum growth, k_{bio} describes the attenuation of macroalgal indicators with depth (as opposed to k_d describing the attenuation of light, Duarte 1991) and I_{sat} is a parameter describing the light saturation ($I = I_{sat}$ is the light level corresponding to 76 % of C_{max} , i.e. $\tanh(1)$). However, the macroalgal cover potential may not be fully exploited due to physical exposure and grazing by sea urchins.

Furthermore, C_{max} depends on the diversity of the community, implying that species-rich communities have a higher maximum cover, since there can be several layers of different species overlaying each other, whereas the cumulative cover of species-poor communities is limited by the low number of species. This effect of species diversity on cumulative cover was modelled using a linear function (defined by parameters a_{max} and b_{max}) for salinity (S), since species richness generally increase with salinity from the brackish Baltic Sea to saline North Sea:

$$C_{max} = a_{max} + b_{max} \cdot S \quad (\text{Eq. 2.5})$$

The advantage of using the salinity relationship for C_{max} , in contrast to a site-specific estimate, is that the maximum cover can change over the depth gradient at stone reefs where the salinity gradient is large (typically around 10 across the halocline).

The physical exposure from wave action generally decreases with the square of the depth and the effect of physical exposure on macroalgal cover can be described using Michaelis-Menten kinetics.

$$f_{exposure} = \frac{1}{1 + k_{exposure} \cdot d^{-2}} \quad (\text{Eq. 2.6})$$

where $f_{exposure}$ is a scaling factor for the depth-specific physical exposure on macroalgal cover (approaching 1 at deeper depths) and $k_{exposure}$ is a parameter describing how fast the physical exposure decreases with depth. Similarly, the grazing effect from sea urchins can be modelled using Michaelis-Menten kinetics for the cover of sea urchins:

$$f_{grazing} = \frac{1}{1 + k_{seaurchin} \cdot C_{seaurchin}} \quad (\text{Eq. 2.7})$$

where $1/k_{seaurchin}$ describes the sea urchin cover, where grazing reduces macroalgal cover by 50 % ($k_{seaurchin} \cdot C_{seaurchin} = 1$).

Combining the potential macroalgal cover with the limitations imposed by physical exposure and sea urchin grazing, and the salinity dependence of $C_{max} = a_{max} + b_{max} \cdot S$ yields:

$$C_{pot} = (a_{max} + b_{max} \cdot S) \cdot \tanh\left(\frac{I_0}{I_{sat}} \exp(-k_{bio} \cdot d)\right) \cdot \frac{1}{1 + k_{exposure} \cdot d^{-2}} \cdot \frac{1}{1 + k_{seaurchin} \cdot C_{seaurchin}} \quad (\text{Eq. 2.8})$$

This model (Eq. 2.8) was estimated on observations of the cumulative cover of macroalgae as well as on the number of perennial species (both log-transformed) using non-linear regression with a least squares criterion.

For the proportion of opportunists, a modification of the model was employed, taking into account that this community indicator is not regulated by light in the same way as the cumulative cover and the number of perennial species. Instead, in the lack of theoretical underpinning, the depth relationship for the proportion of opportunists (P_S) was modelled with site-specific slopes (P_{site}) and intercepts that varied across years. Finally, it was found from exploring the data that the proportion of opportunists varied with salinity in a non-linear manner, showing a decreasing proportion at salinities (defined by the slope b_S) up to a salinity threshold (S_T) and a constant proportion at higher salinities:

$$P_S = \begin{cases} b_S \cdot (S - S_T) + P_{site} & S < S_T \\ P_{site} & S \geq S_T \end{cases} \quad (\text{Eq. 2.9})$$

Thus, the model for the proportion of opportunists (P), using the logit-transformation was (shown prior to taking the log):

$$\frac{P}{1-P} = P_S \cdot \frac{1}{1 + k_{exposure} \cdot d^{-2}} \cdot \frac{1}{1 + k_{seaurchin} \cdot C_{seaurchin}} \cdot k_{year} \cdot \exp(-k_{bio} \cdot d) \quad (\text{Eq. 2.10})$$

From the parameter estimates of the non-linear model (Eq. 2.8), the site- and year-specific estimates of k_{bio} were analysed in relation to site- and year-specific means of Secchi depths. These relationships between macroalgal indicators and Secchi depth, on both spatial and temporal scale, were examined for the cumulative cover and the number of perennial species, whereas this analysis was not done for the proportion of opportunists due to the lack of consistency in k_{bio} estimates, since the proportion of opportunists could both increase and decrease with depth.

2.3.3 Semi-mechanistic non-linear model for macroalgae in *Large shallow inlets and bays and Reefs* in coastal Natura 2000 areas

Variations in macroalgal indicators for coastal Natura 2000 areas were analysed with a model based on the same principles as for reefs in open waters (see above), but slightly modified to accommodate differences in data. Firstly, the cover of sea urchins has not been monitored with the macroalgal cover in the coastal monitoring data and consequently, it was not possible to include the grazing factor (Eq. 2.7). Secondly, the depth gradient in macroalgal cover was not always as well defined for the shallower coastal habitats and, therefore, k_{bio} values could not be estimated independently for both sites and years. As a consequence, only spatial variations in k_{bio} were considered, assuming that spatial variations were substantially larger than temporal. Thirdly, the variability among coastal habitats was large and to test the assumption that C_{max} was a simple linear function of salinity, site-specific estimates of C_{max} were estimated and analysed in relation to salinity and similarly, site-specific estimates of k_{bio} were estimated and analysed in relation to Secchi depth.

Incorporating these simplifications led to the following model for cumulative cover and number of perennial species, where both C_{max} and k_{bio} values were site-specific. The additional parameter ($k_{exposure}$) was used to explain the effect of physical exposure.

$$C_{pot} = C_{max} \cdot \tanh\left(\frac{I_0}{I_{sat}} \exp(-k_{bio} \cdot d)\right) \cdot \frac{1}{1 + k_{exposure} \cdot d^{-2}} \quad (\text{Eq. 2.11})$$

For the proportion of opportunists, site-specific factors were estimated for P_{site} and k_{bio} , in addition to the parameter describing the physical exposure ($k_{exposure}$).

$$\frac{P}{1 - P} = P_{site} \cdot \frac{1}{1 + k_{exposure} \cdot d^{-2}} \cdot \exp(-k_{bio} \cdot d) \quad (\text{Eq. 2.12})$$

The estimated parameters C_{max} and k_{bio} were related to mean salinity and Secchi depth, respectively, for the different coastal habitats.

3. Results

3.1 Variations in macroalgal indicators over time and space from the linear model

Variations in the three macroalgal indicators (cumulative cover, proportion of opportunists and number of perennial species) were partitioned into temporal, spatial and depth variations for *Reefs* in open waters (Eq. 2.1) and the two coastal habitat types *Large shallow inlets and bays* and *Reefs* (Eq. 2.2). For all estimated models, the largest sources of random variation were differences among stations within a site ($STATION_k(SITE_j)$) and residual variation (e_{ijkl}) describing variations around the depth gradient (Table 3.1). For *Reefs* the residual variation was larger than the variation among different stations within the stone reef, whereas variation among stations within coastal habitats was larger than the residual variation, highlighting the large spatial heterogeneity within the coastal habitats. Variations in depth-related gradients in macroalgal indicators among stations within sites ($STATION_k(SITE_j) \times DEPTH$) were very small for stone reefs in open water and somewhat larger for the coastal habitats, although still relatively small. This implied that the three macroalgal indicators exhibited similar depth gradients for all monitoring stations within the sites. Differences in the interannual variation ($YEAR_i \times SITE_j$ and $YEAR_i \times SITE_j \times DEPTH$) were indeed small for *Reefs* in open water, indicating that the different stone reefs had similar trends over time. However, trends in the macroalgal indicators were more variable for coastal habitats, i.e. larger variances in the temporal components (Table 3.1).

Table 3.1. Estimated variances of the random factors in the descriptive mixed model (Eq. 2.1 and 2.2) for the three macroalgal indicators (C.Cov. = cumulative cover, P.Opp. = proportion of opportunists, #Peren = number of perennial species). Variances are for the transformed indicators. The coastal habitats included *Reefs* as well as *Large shallow inlets and bays*.

Random factor	Reefs in open waters			Coastal habitats		
	C.Cov.	P.Opp.	#Peren	C.Cov.	P.Opp.	#Peren
$YEAR_i \times SITE_j$	0.0035	0.0000	0.0090	0.0726	0.4553	0.0230
$YEAR_i \times SITE_j \times DEPTH$	0.0004	0.0034	0.0000	0.0031	0.0141	0.0004
$STATION_k(SITE_j)$	0.1908	0.3931	0.0620	0.5050	1.7049	0.1129
$STATION_k(SITE_j) \times DEPTH$	0.0002	0.0006	0.0005	0.0167	0.0772	0.0034
e_{ijkl}	0.5295	1.6728	0.1484	0.1623	1.0878	0.0698

Reefs in open water

Interannual variations for cumulative cover and proportion of opportunists were not significant for *Reefs* (Table 3.2), although cumulative cover displayed somewhat higher values in later years (2012-2016) (Fig. 3.1). The proportion of opportunists was low (~10 %) throughout the study period. However, interannual variations in the number of perennial species were significant, increasing from 2-3 at 15 m depth in the first years to 3-4 in the last 9 years.

Variations among sites were significant for all macroalgal indicators (Table 3.2), with low cumulative cover at Knudegrund and Lønstrup Rødgrund in Skagerrak and high values at Kims Top and Kirkegrund (Fig. 3.1). The proportion of opportunists did not display similar spatial gradient and varied from ~1 % at Kirkegrund to 57 % at Klinteskov. However, the number of perennial species showed a spatial gradient similar to the cumulative cover, increasing from ~2 at Knudegrund to ~6 at Kims Top (Fig. 3.1).

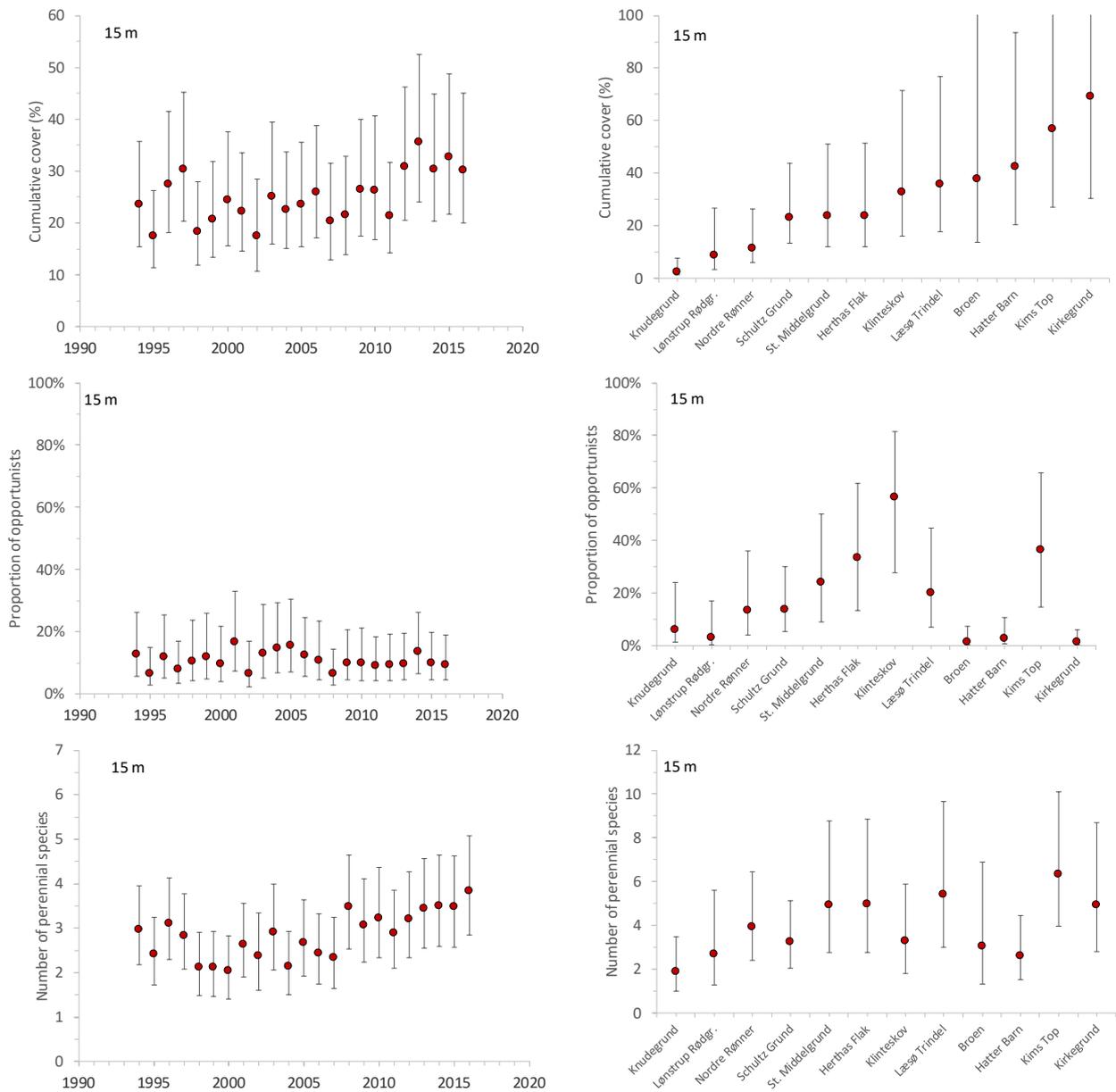


Figure 3.1. Interannual variation (left panel) and variation among open-water reef habitats (right panel) estimated from the mixed model (Eq. 2.2) and predicted for a standard depth of 15 m without presence of sea urchins ($cover_{seaurchin} = 0$). Error bars show the 95 % confidence interval for the annual and site-specific means. Site-specific means are ranked after the cumulative cover.

Table 3.2. P-values for the fixed factors in the descriptive mixed model (Eq. 2.1 and 2.2) for the three macroalgal indicators (C.Cov. = cumulative cover, P.Opp. = proportion of opportunists, #Peren = number of perennial species). Significant P-values (<0.05) are highlighted in bold. Note that sea urchins were not included in the mixed model for the coastal habitats.

Random factor Variances	Stone reefs			Coastal habitats		
	C.Cov.	P.Opp.	#Peren	C.Cov.	P.Opp.	#Peren
$year_i$	0.1199	0.2777	0.0022	0.0153	0.1179	0.0861
$site_j$	0.0003	0.0001	<0.0001	0.0268	0.0005	<0.0001
$depth$	<0.0001	0.5259	<0.0001	<0.0001	0.7552	<0.0001
$year_i \times depth$	0.0094	0.2785	0.0041	0.0006	0.5265	0.0005
$site_j \times depth$	<0.0001	0.0001	0.0011	<0.0001	0.0001	<0.0001
$\log(cover_{seaurchin} + 1)$	0.0002	0.4839	0.6240			
$\log(cover_{seaurchin} + 1) \times depth$	0.1361	0.0705	0.1054			

Variations with depth were significant for the cumulative cover and the number of perennial species, and the depth gradient varied significantly among years and sites (Table 3.2). The proportion of opportunists also showed significant depth variations among sites, but not between years, and the overall mean opportunist cover did not show significant variation with depth. The cover of sea urchins only affected cumulative cover and the relationship was not depth-dependent.

Coastal habitats

Interannual variations in the macroalgal indicators for the coastal habitats were significant only for cumulative cover (Table 3.2), and the significance was most likely driven by low numbers in 2008 (Fig. 3.2). However, a tendency for an increasing number of perennial species was observed for coastal habitats as well, similar to that observed for Reefs in open waters. Variations among coastal habitats were significant for all macroalgal indicators (Table 3.2), although most significant for the proportion of opportunists and the number of perennial species.

Lovns Bredning, Nissum Bredning, Roskilde Fjord and Løgstør Bredning had low cumulative cover, whereas Hirsholmene, Mols Bjerge kyst and Stavns Fjord had high cumulative cover when comparing these data for the same standard depth (4 m, Fig. 3.2). The proportion of opportunists ranged from 9 % in Løgstør Bredning to 98 % in Roskilde Fjord, without any pronounced spatial gradients similar to cumulative cover. The number of perennial species also varied significantly across sites, increasing from an expected value around 1 in Lovns Bredning and Roskilde Fjord to almost 20 in Stavns Fjord (Fig. 3.2). It should be observed that the Stavns Fjord habitat includes both shallow macroalgal populations in Stavns Fjord itself and deep macroalgal populations in the northern Belt Sea (similar to stone reef habitats). However, due to physical exposure imposing depth restrictions to the data used for the mixed model, the estimates for Stavns Fjord included the deep samples only. Consequently, the habitat means for Stavns Fjord were extrapolated from deep samples to 4 m, assuming that cumulative cover and number of perennial species increase linearly towards shallower depths. In fact, macroalgal samples used for the mixed model span highly different depth intervals (Table 2.2), which with the depth restriction due to physical exposure are not overlapping and therefore extrapolation with depth is needed to produce estimates comparable across coastal habitats. However, it is possible that extrapolation with depth may introduce artefacts, because the assumption of linearity does not hold.

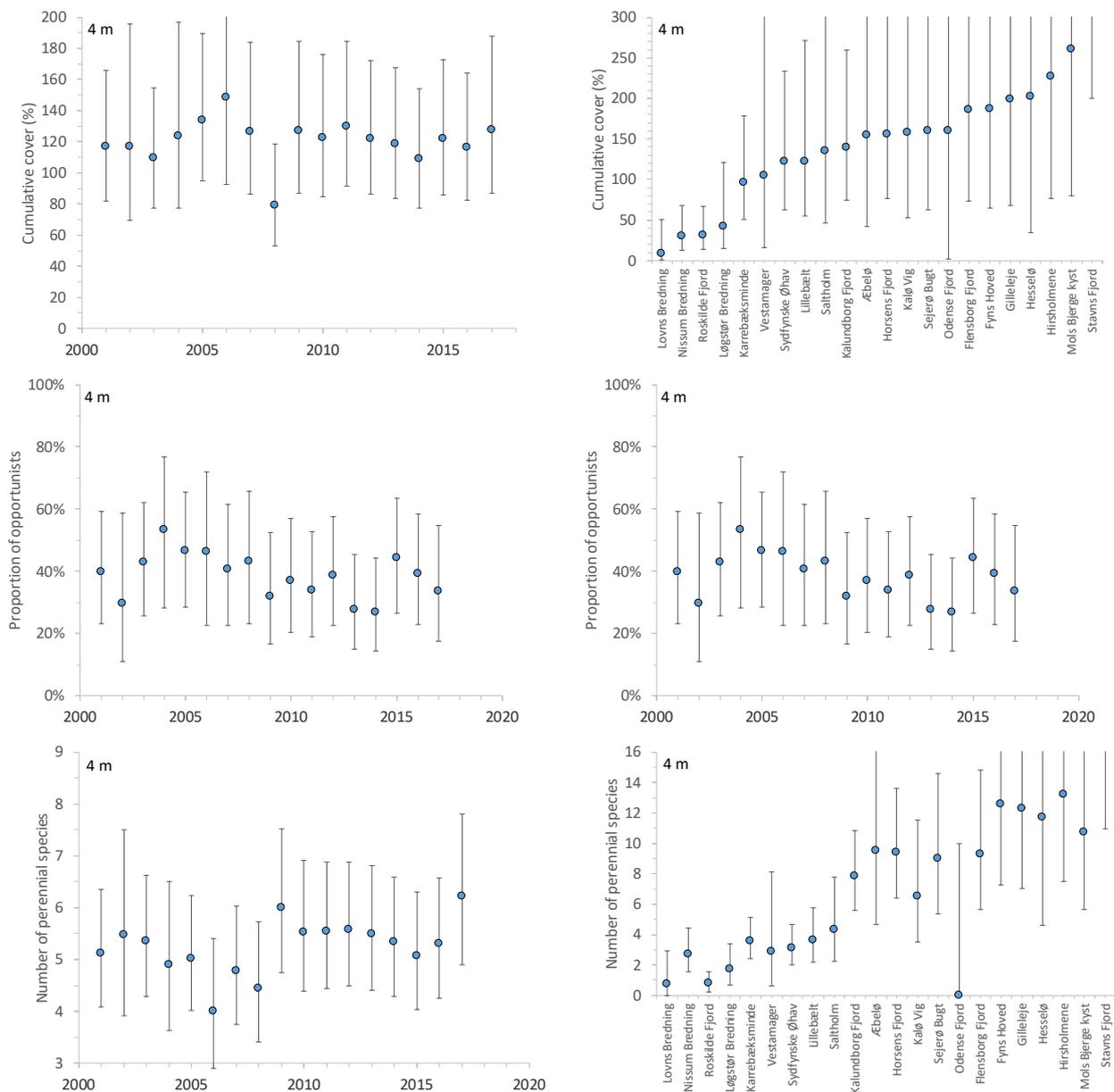


Figure 3.2. Interannual variation (left panel) and variation among coastal habitats (right panel) estimated from the mixed model (Eq. 2.1) and predicted for a standard depth of 4 m. Note that the presence of sea urchins was not included. Error bars show the 95 % confidence interval for the annual and site-specific means. Site-specific means are ranked after the cumulative cover. Note that the estimated cumulative cover for Stavns Fjord (638 %) and number of perennial species ($n = 19.9$) exceeded the scale on the y-axis.

The cumulative cover and the number of perennial species varied significantly with depth, and the depth variation was different among years and Natura 2000 sites (Table 3.2). Decreases in the cumulative cover were steeper for the coastal habitats in Limfjorden (Nissum Bredning, Løgstør Bredning, Lovns Bredning), i.e. areas more strongly affected by eutrophication, whereas the cumulative cover decreased rather slowly with depth for Hesselø and Sejerø Bugt. The proportion of opportunists also had significant site-specific variations with depth, but the overall depth relationship and the year-specific depth relationships were not different from zero. This pattern of depth relationships was similar to that observed for *Reefs* in open waters (Table 3.2).

3.2 Non-linear macroalgal models for open water Reefs

3.2.1 Cumulative cover

The model for cumulative cover explained 62 % of the observed variation, although considerable variation (± 100 %) around the regression lines remained (Fig. 3.3), highlighting the inherent variability in the data. There were large differences in the estimated maximum cumulative cover across stone reefs, with the highest values observed at Knudegrund and Lønstrup Rødgrund and lowest values observed at Kirkegrund and Klinteskov. At some reefs, no data were available to support the modelled maximum cumulative cover as the top of the reef is located in deeper waters where light levels were not sufficient to saturate macroalgal cover levels (e.g. Kims Top and Herthas Flak). There were significant differences in the k_{bio} -values ($P < 0.0001$), i.e. the steepness of the declines in cumulative cover with depth. The largest declines were observed for Knudegrund ($k_{bio} = 0.404$) and Lønstrup Rødgrund ($k_{bio} = 0.354$), whereas cumulative cover decreased more slowly at Kirkegrund ($k_{bio} = 0.196$) and Klinteskov ($k_d = 0.118$).

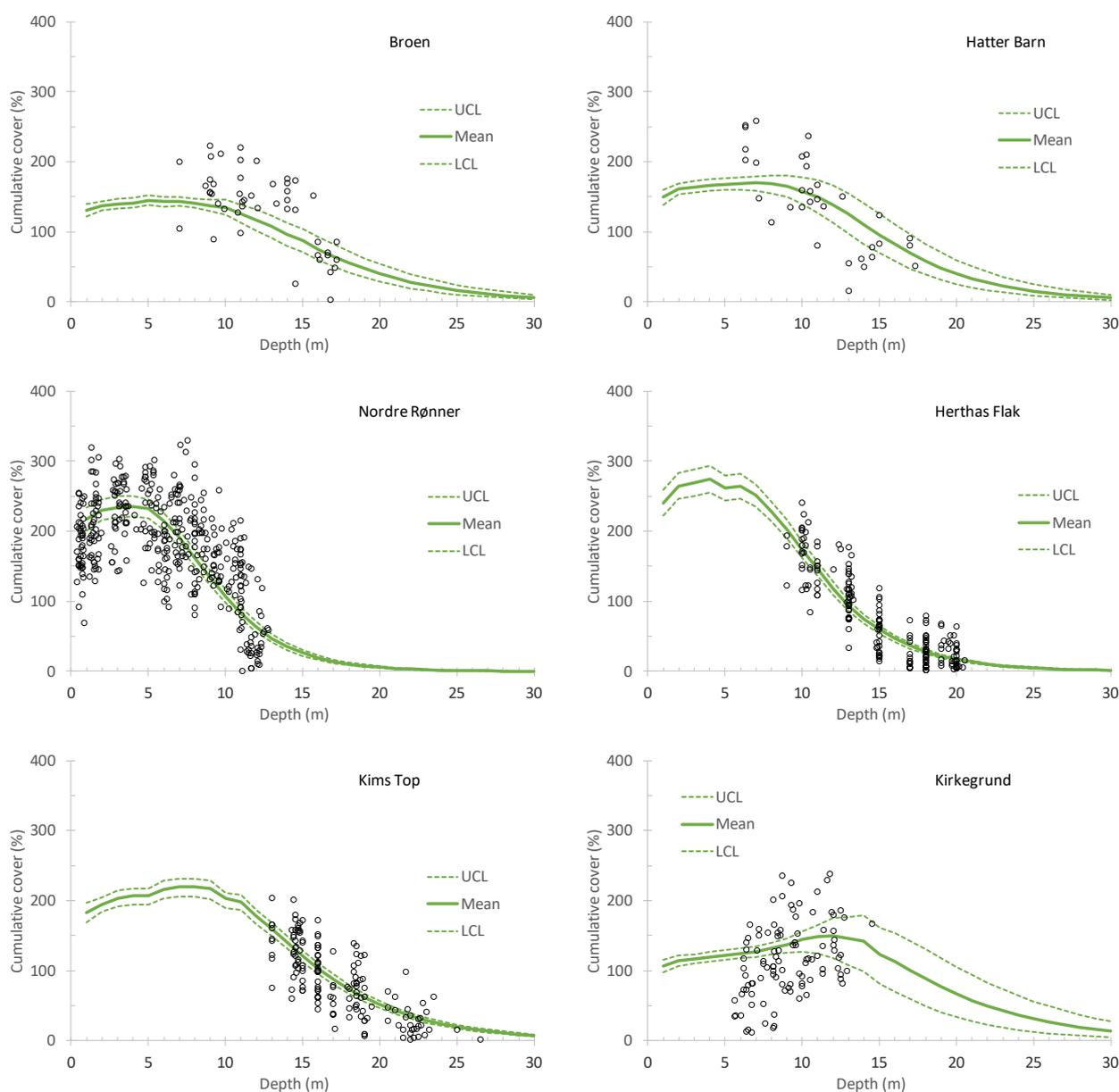


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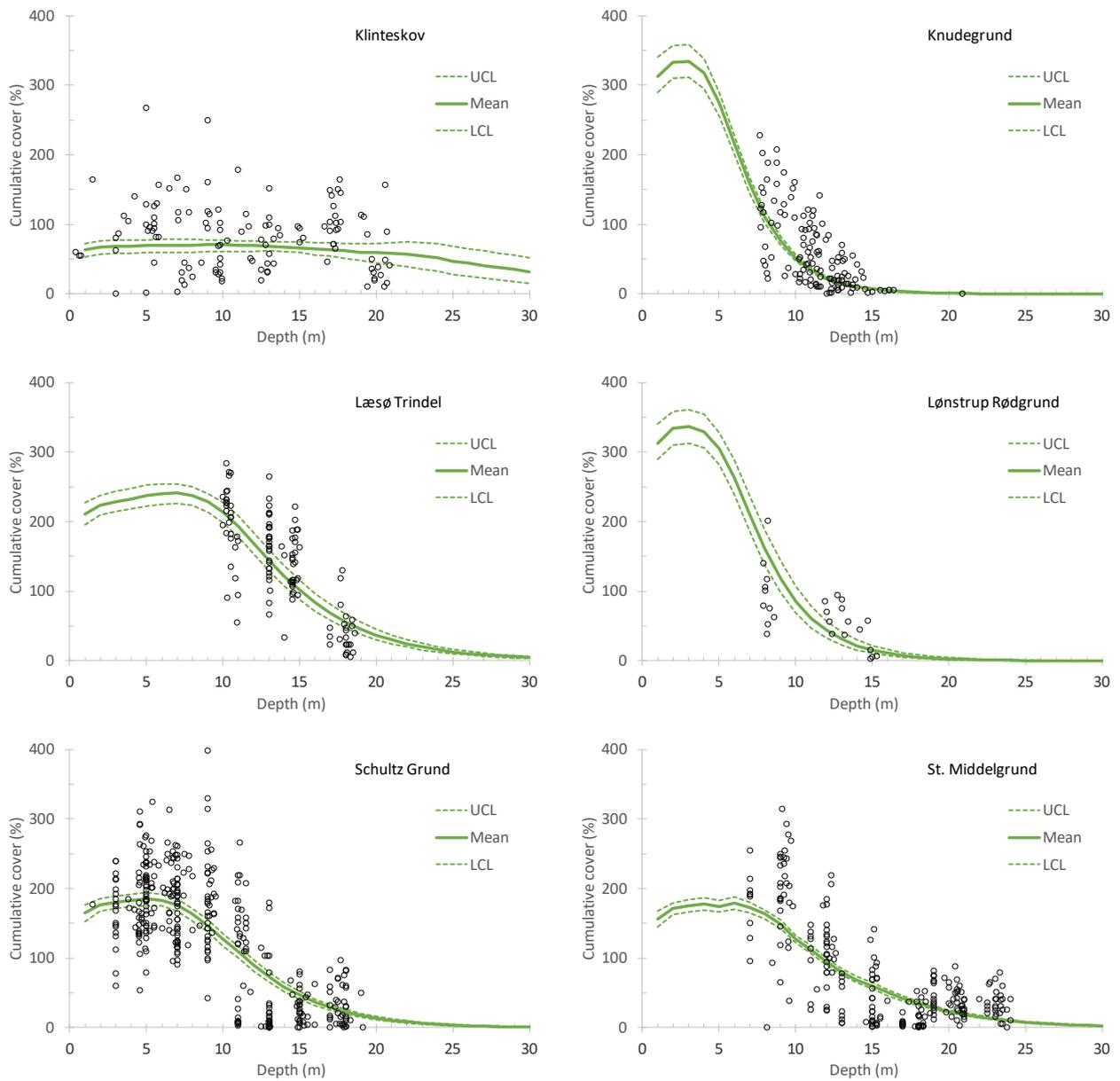


Figure 3.3. Observed cumulative cover versus depth for the 12 investigated reef areas in open waters. For each reef the estimated depth relationship from the non-linear model (Eq. 2.8) without sea urchins ($cover_{seaurchin} = 0$) is shown with the 95 % confidence interval of the model. The estimated model and confidence interval represent the geometric mean, corresponding to the median distribution.

The effect of sea urchins was highly significant ($P < 0.0001$), showing that a sea urchin cover of 1 % reduced the cumulative cover by 35 % and a sea urchin cover of 10 % reduced the cumulative cover by 84 % (Fig. 3.4a). There was an apparent strong effect of physical exposure at depths below 2 m and the effect was less than 10 % for depths above 4 m (Fig. 3.4b). However, this effect was only marginally significant ($P = 0.0829$), mainly due to the relatively few observations recorded at these shallow depths.

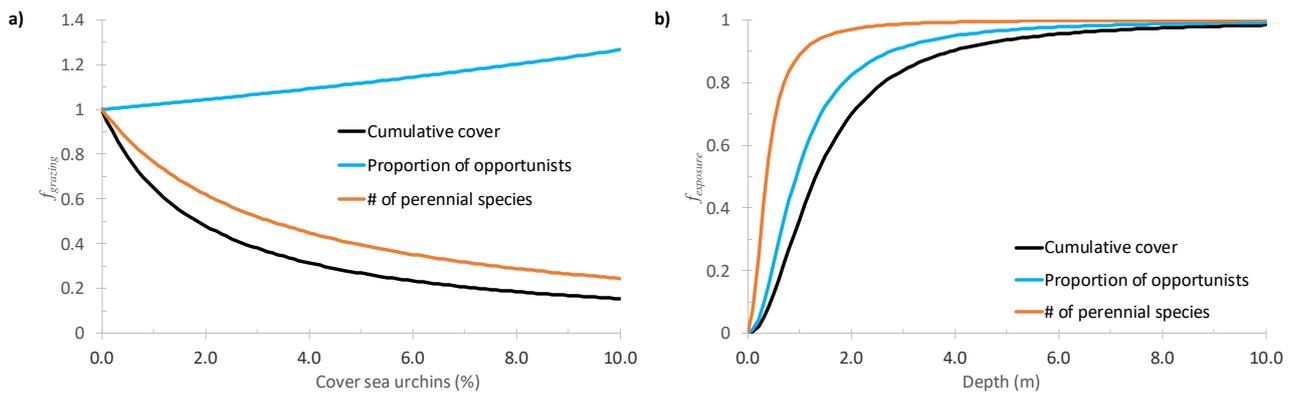


Figure 3.4. Estimated effects of sea urchins (a) and physical exposure (b) for modelling cumulative cover, proportion of opportunists and number of perennial species at reef sites in open waters (Eq. 2.8).

3.2.2 Proportion of opportunists

The model for the proportion of opportunists explained 44 % of the observed variation with a considerable variation around the regression lines remaining (Fig. 3.5). There were large differences among stone reefs in the overall proportion of opportunists as well as the slopes. In fact, there was no consistent pattern with respect to the depth relationship, i.e. eight stone reefs displayed decreasing tendencies whereas four stone reefs had increasing proportion of opportunists with depth. Even the two stone reefs in the Skagerrak (Knudegrund and Lønstrup Rødgrund) displayed opposite relationships with depth, although the relationship for Knudegrund was only borderline significant ($P = 0.0348$). The same phenomenon was observed for Nordre Rønner ($k_{bio} < 0$) and Herthas Flak ($k_{bio} > 0$). The most systematic depth relationship was observed for Kirkegrund, clearly separating a community dominated by opportunists and one dominated by perennial species around 10 m depth. This pattern was consistent with the location of the halocline between 7 and 14 m, separating a surface layer with salinity around 12-13 from deeper waters with salinity above 24. The estimated effect of salinity (Eq. 2.9) showed decreasing proportion of opportunists with increasing salinity up to 20.5 with no effect on the proportion of opportunists for higher salinity (data not shown).

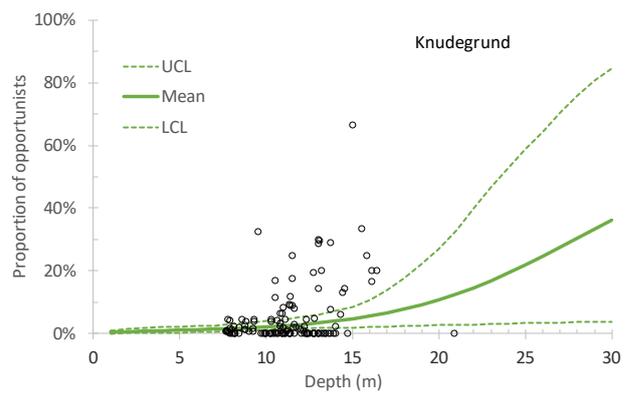
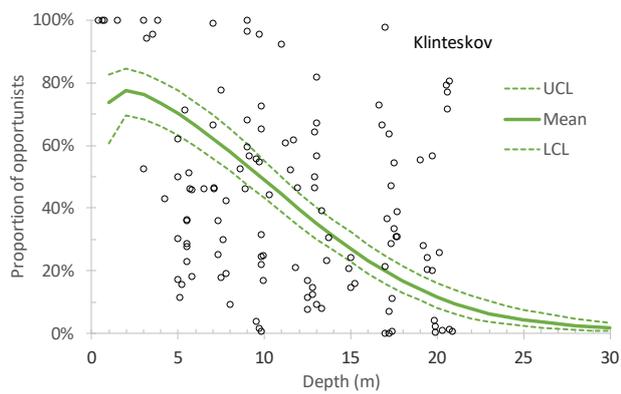
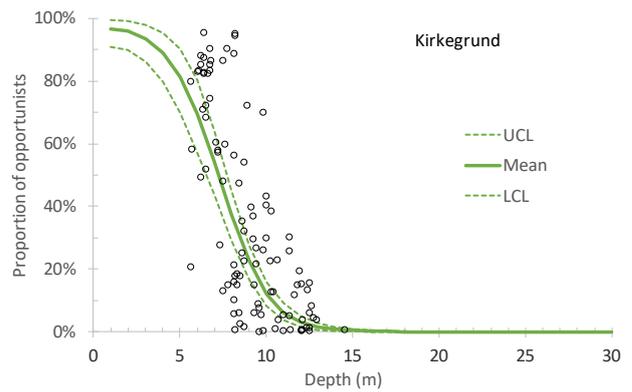
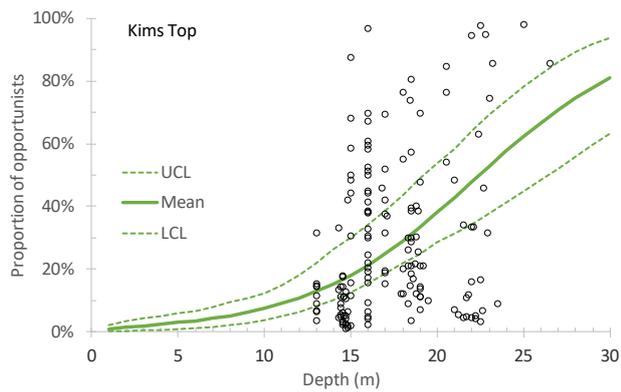
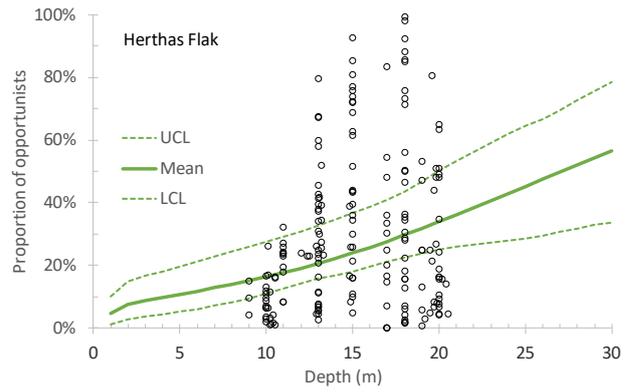
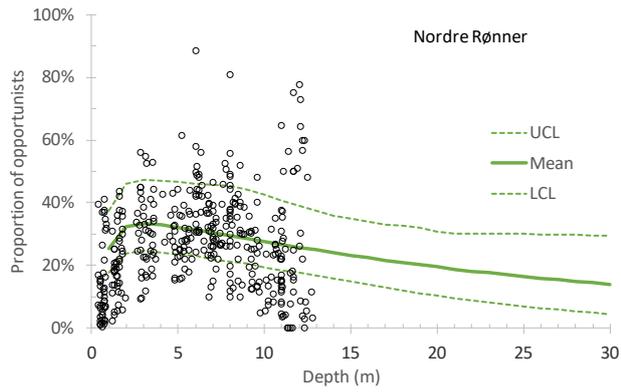
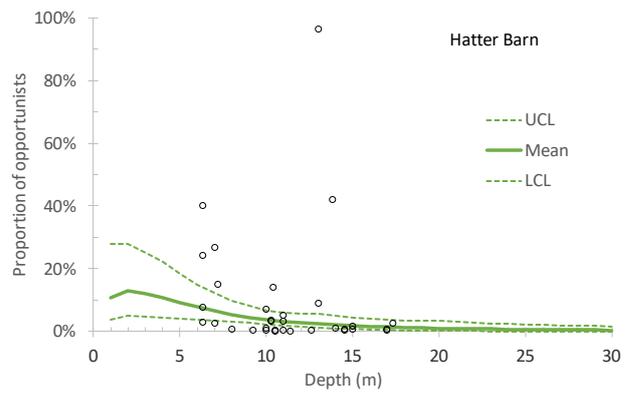
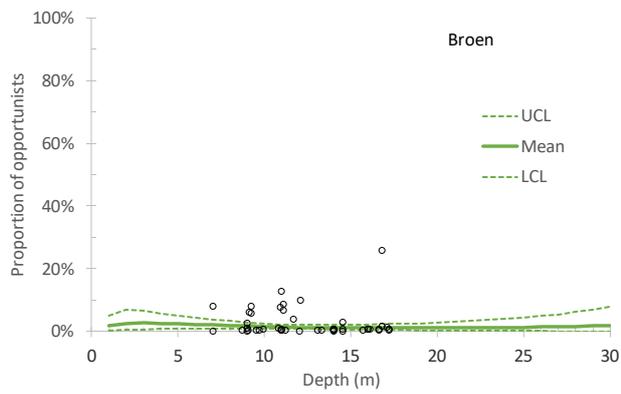


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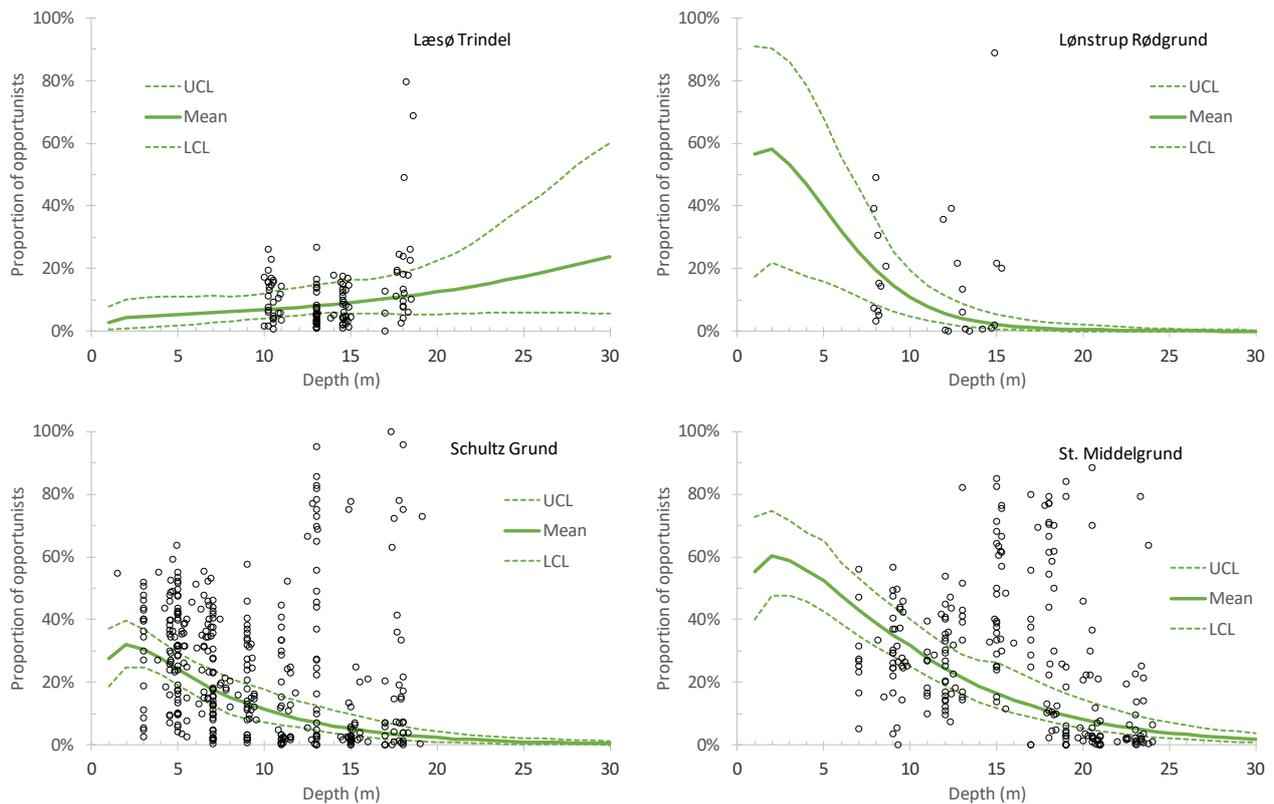


Figure 3.5. Proportion of opportunistic macroalgal species versus depth for the 12 investigated stone reefs. For each stone reef the estimated depth relationship from the non-linear model (Eq. 2.10) without sea urchins ($cover_{seaurchin} = 0$) is shown with the 95 % confidence interval of the model. The estimated model and confidence interval represent the geometric mean, corresponding to the median distribution.

The effect of sea urchins was not significant ($P = 0.1761$), although it suggested that the presence of sea urchins could promote opportunists by up to 20 % (Fig. 3.4a). The effect of physical exposure was significant ($P = 0.0192$), suggesting that physical exposure can constrain opportunists in the top 2-3 m, although this effect was mainly based on data from Nordre Rønner. This reduced proportion of opportunists in shallow waters was also reported in earlier analyses of macroalgal data (Carstensen et al. 2014).

3.2.3 Number of perennial species

The model for the number of perennial species explained 65 % of the observed variation, but there was still considerable variation (± 50 %) around the regression lines (Fig. 3.6). The highest number of perennial species was found at Nordre Rønner ($n = 23$), whereas Klinteskov had the lowest number ($n = 8$ species recorded at most). However, due to the combination of effects of salinity (high salinity supporting more perennial species) and depth relationships (more illuminated depths supporting more species), the theoretically highest number of perennial species would occur at 4-5 m depth at Knudegrund and Lønstrup Rødgrund (expected median of 18-19 perennial species), but no observations have been obtained from such shallow depths. There were significant differences in the k_{bio} -values ($P < 0.0001$), i.e. the steepness of the declines in the number of perennial species with depth. The largest declines were observed for Knudegrund ($k_{bio} = 0.326$) and Lønstrup Rødgrund ($k_{bio} = 0.298$), whereas the cumulative cover decreased more slowly at Klinteskov ($k_{bio} = 0.066$), although the latter estimate was more uncertain (0.066 ± 0.040).

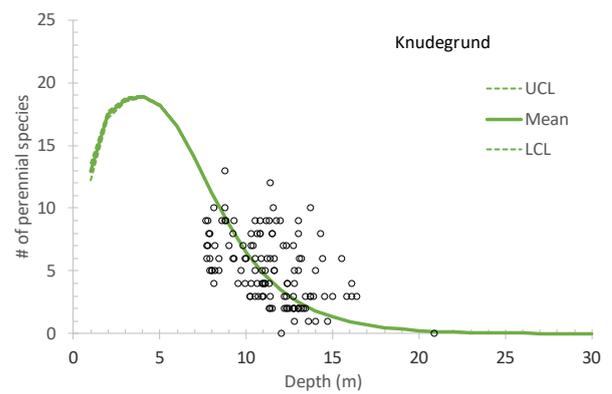
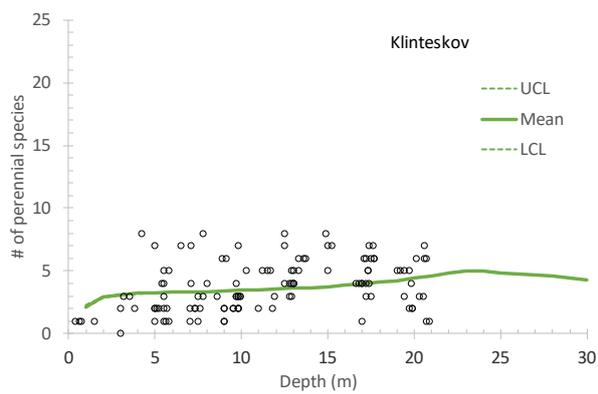
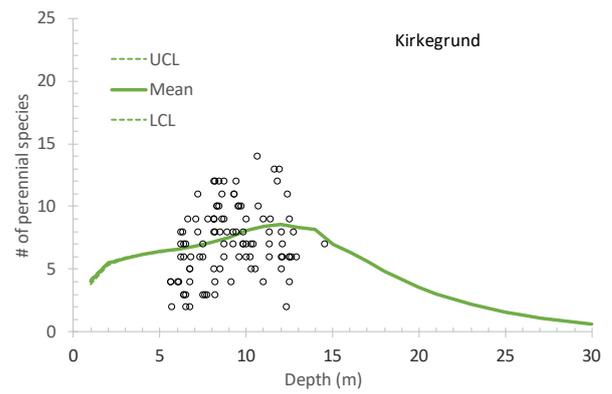
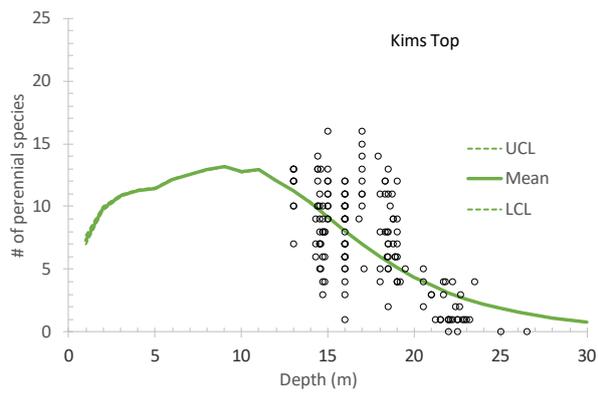
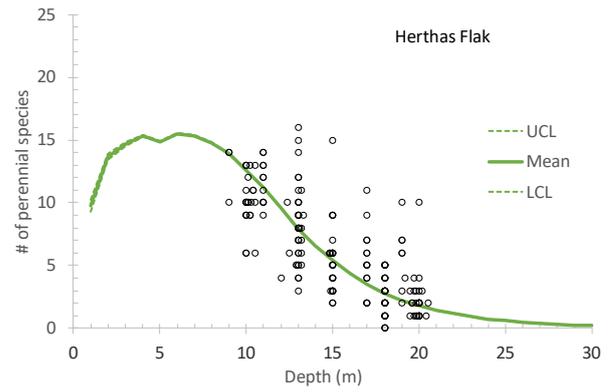
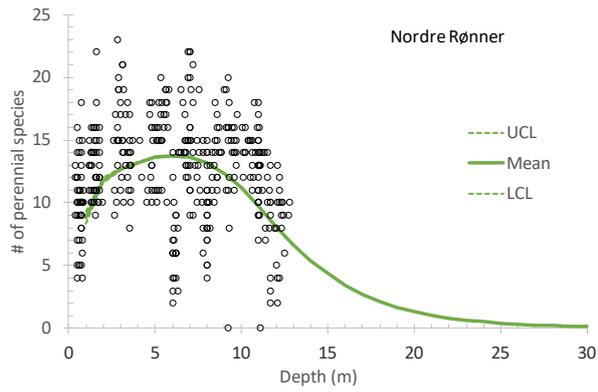
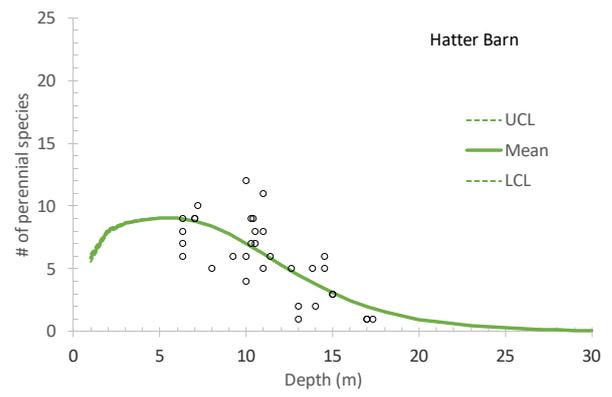
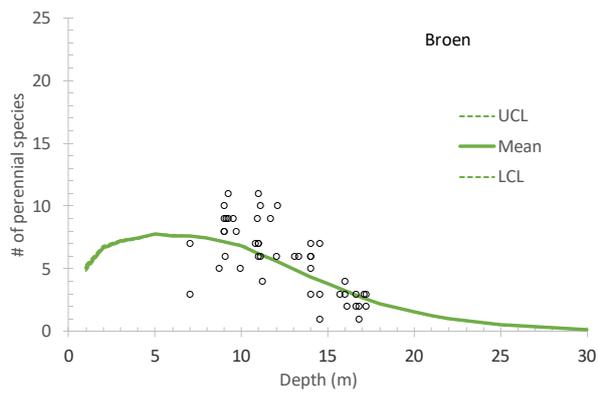


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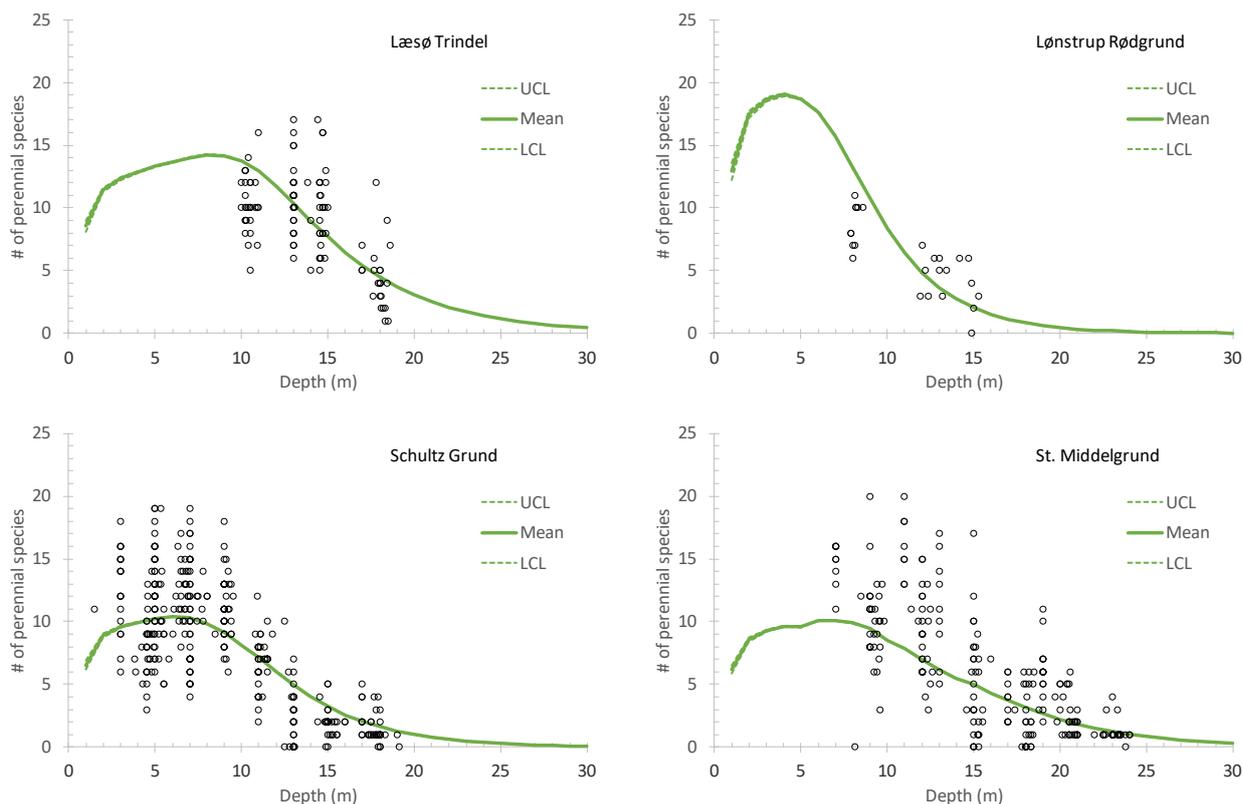


Figure 3.6. Number of perennial species versus depth for the 12 investigated Natura 2000 sites with reefs in open water. For each site the estimated depth relationship from the non-linear model (Eq. 2.8) without sea urchins ($cover_{seaurchin} = 0$) is shown with the 95 % confidence interval of the model. The estimated model and confidence interval represent the geometric mean, corresponding to the median distribution.

The effect of sea urchins was highly significant ($P < 0.0001$), showing that a sea urchin cover of 1 % reduced the number of perennial species by 21 % and a sea urchin cover of 10 % reduced the cumulative cover by 73 % (Fig. 3.4a). There was a significant effect of physical exposure ($P < 0.0001$) at depths below 1 m, but the effect vanished quickly at deeper depths (Fig. 3.4b). Apparently, perennial species are more resistant than opportunistic species to physical exposure.

3.2.4 Variations in depth attenuation parameter k_{bio}

For cumulative cover and number of perennial species the k_{bio} estimates were separated into a temporal and spatial component, and these were analysed in relation to Secchi depth. For the proportion of opportunists, consistent depth-relationships were not obtained and, therefore, this indicator was not analysed in relation to Secchi depth.

Spatial variations

The k_{bio} estimates for the 12 Natura 2000 sites in open water with reefs were not related to Secchi depth (Fig. 3.7), neither for the cumulative cover nor for the number of perennial species, suggesting that the spatial variation among k_{bio} estimates is not only governed by light availability but most likely by other unknown factors as well. Hence, k_{bio} estimates depend on a combination of different factors, which could include Secchi depth, and thus the model cannot be further simplified by expressing k_{bio} from Secchi depth alone.

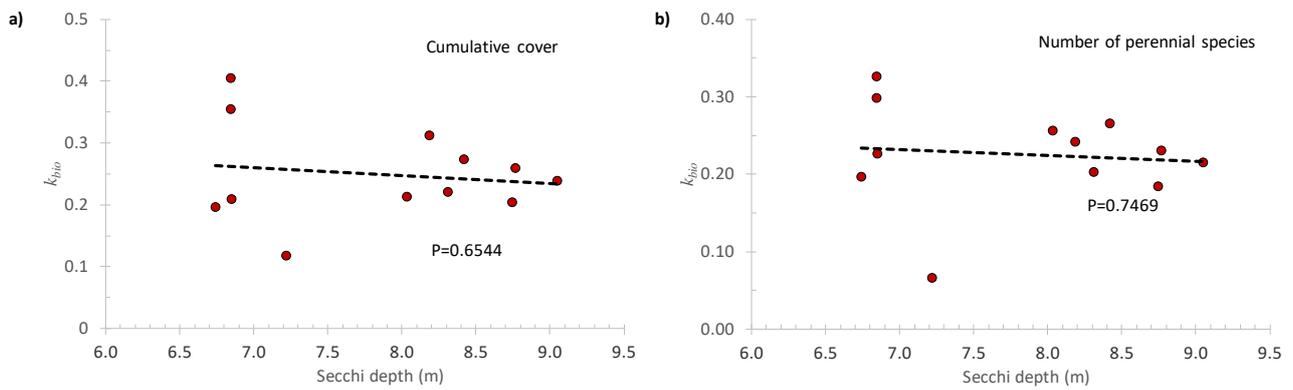


Figure 3.7. Site-specific estimates for the depth relationship (k_{bio}) versus Secchi depth for cumulative cover (a) and number of perennial species (b).

Interannual variations

The k_{bio} estimates for the interannual variation across all stone reefs were significantly related to Secchi depth for cumulative cover (Fig. 3.8a), but the relationship for number of perennial species was not significant although the same tendency as for cumulative cover was observed (Fig. 3.8b). The relationship between k_{bio} estimates and Secchi depth demonstrate that macroalgae grow denser and deeper in years with relatively good light conditions.

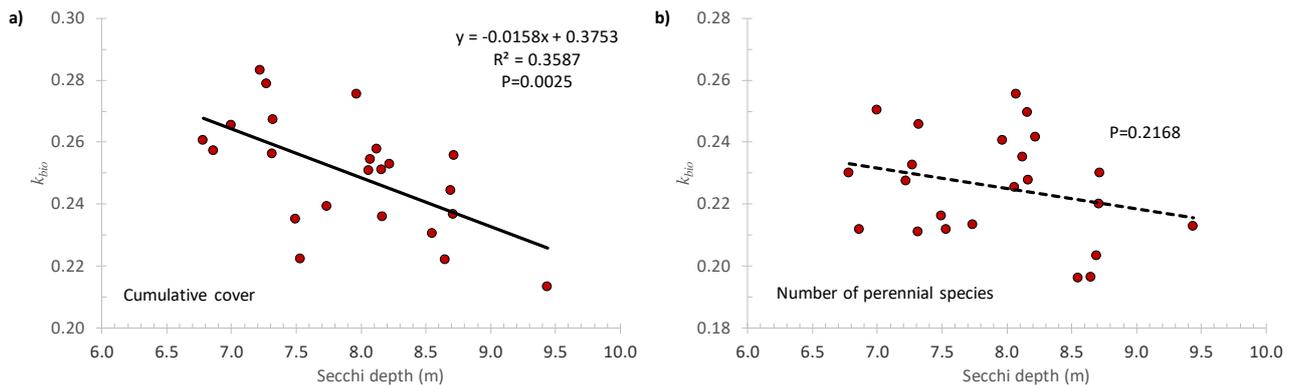


Figure 3.8. Annual estimates (1994-2016) for the depth relationship (k_{bio}) versus Secchi depth for cumulative cover (a) and number of perennial species (b).

3.2.5 Possible target setting for cumulative cover

The cumulative cover was described as a function of salinity, depth, sea urchin cover as well as a parameter k_{bio} that was specific to stone reef and year. Given that the interannual variation in k_{bio} was significantly related to Secchi depth (Fig. 3.8a), the interannual variation in k_{bio} could be expressed as function of Secchi depth. Consequently, targets for cumulative cover, specific to each stone reef, could be calculated, provided that targets for Secchi depth are found.

Estimation of the site-specific Secchi depth target is not available at the moment. As a demonstration of the methodology, we have used basin-wide Secchi depth targets from the Baltic Sea Action Plan (HELCOM 2013), knowing that spatial heterogeneity is important within the basins with higher water transparency off the coasts and off the frontal zones. Targets for cumulative cover were calculated as functions of depth and the mean salinity profile at the given reef site. The cover of sea urchins was set at zero, assuming that

observations of cumulative cover, used for status assessment, are adjusted using Eq. 2.6 to account for the grazing effect of sea urchins. Secchi depth targets (summer) were 7.56 m for the Kattegat, 7.75 m for the Great Belt and 7.60 m for the Arkona Basin. Note that Secchi depth targets for the Skagerrak (Knudegrund and Lønstrup Rødgrund) are not available and therefore it was not possible to translate these into targets for cumulative cover. It should be noted that these targets covered June-September, whereas the mean Secchi depth in Fig. 3.8 covered March-September.

The target profiles (Fig. 3.9) were similar to the estimated depth profiles (Fig. 3.3), as the only difference was substituting the year-specific k_{bio} value with an estimate calculated from the regression of k_{bio} versus Secchi depth (Fig. 3.8a). Status assessment based on observations relative to the target profile can be assessed using different approaches sensu the WFD: a) face-value, b) benefit-of-doubt, and c) fail-safe. These correspond to uncertainty equally shared, benefitting the polluters, or benefitting the environment, respectively, and imply operating with different confidence levels. The face-value approach considers whether the median of deviations from the target profile is above or below the target. The benefit-of-doubt approach considers the target to be met, unless there is sufficient confidence (e.g. 90 %) that the distribution is below the target profile, i.e. more than 90 % of the observations are below the target. The fail-safe approach considers the target not to be met, unless there is sufficient confidence that the distribution is above the target profile, i.e. more than 90 % of the observations are above the target profile. Thus, the choice of assessment approach can lead to highly different conclusions.

In the present example, the majority of stone reefs had more than 50 % of observations above the target profile and therefore favourable conservation status is achieved with the face-value approach. This is not surprising, since the basin-wide targets for Secchi depth (7.56-7.75) are slightly below the basin-wide average Secchi depth (1994-2016: 7.84 m). The example illustrates that it is possible to derive targets for cumulative cover from Secchi depth targets.

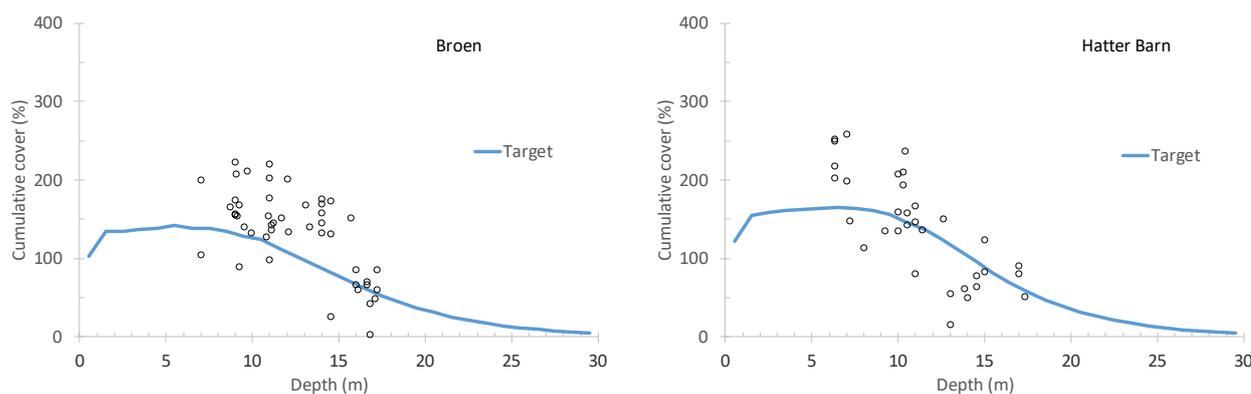


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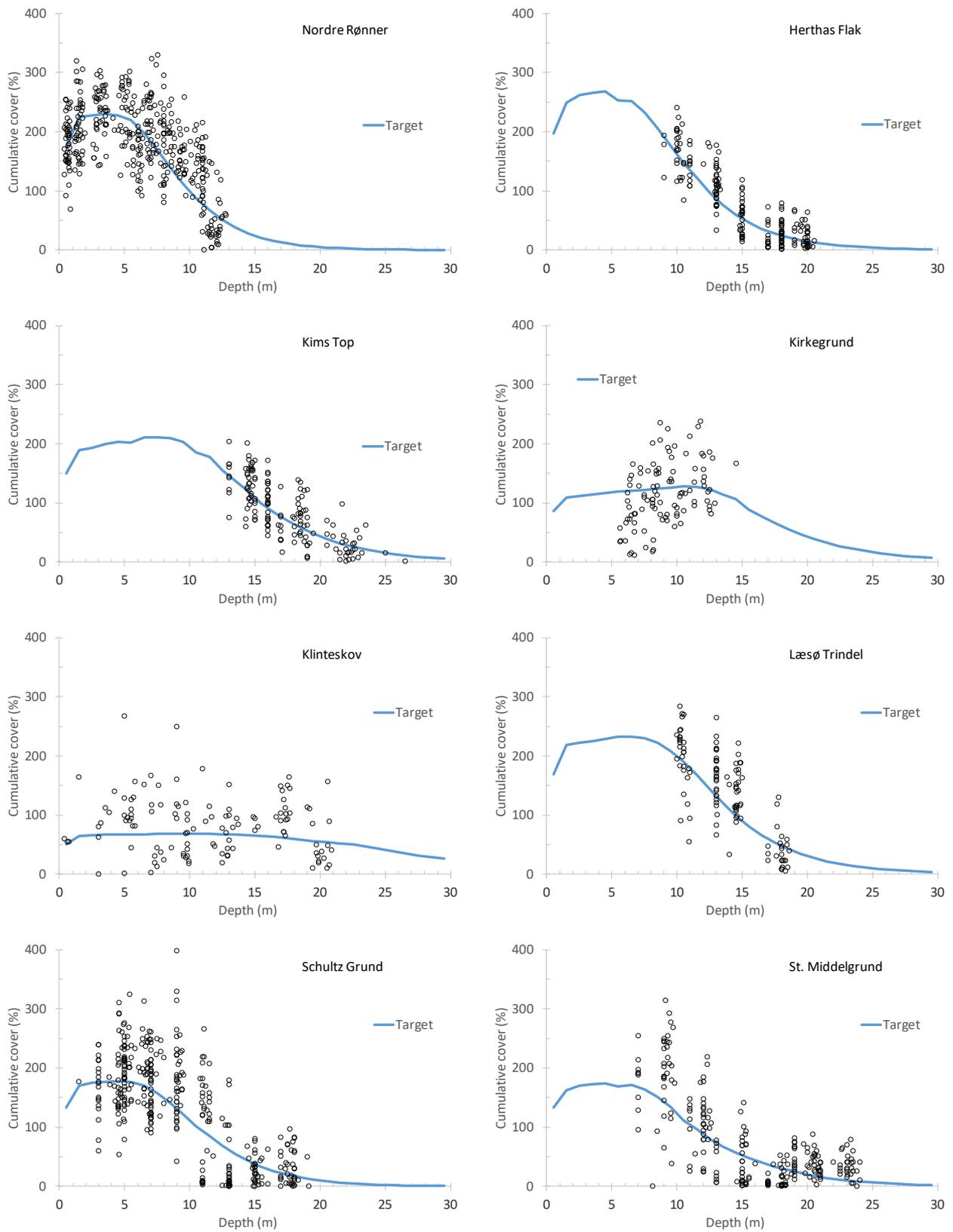


Figure 3.9. Observations of cumulative cover versus proposed site-specific targets, calculated as function of depth and salinity without sea urchins ($cover_{searchin} = 0$).

3.3 Non-linear models for macroalgal vegetation in coastal type 1160 and 1170 habitats

3.3.1 Cumulative cover

The model for cumulative cover explained 46 % of the observed variation, although considerable variation (± 70 %) around the regression lines remained (Fig. 3.10), highlighting the inherent variability in the data. There were large and significant differences ($P < 0.0001$) in the estimated maximum cumulative cover across stone reefs, with the highest values observed at Kalø Vig and Samsø nordøst and lowest values observed at Lovns Bredning and Vestamager. In contrast to the stone reefs, the estimated peak of cumulative cover was supported by observations but the decrease with depth was not always captured with the observations. For example, Gilleleje and Sydfynske Øhav showed increasing tendencies throughout the entire depth range and consequently, reliable k_{bio} estimates could not be obtained, and the model did not predict decreasing cumulative cover for depths deeper than 10 m (Fig. 3.10). With the exception of these two coastal habitats, the k_{bio} estimates were significantly different from zero ranging from 0.088 at Kalundborg Fjord to 0.971 in Lovns Bredning – a span much broader than for stone reefs.

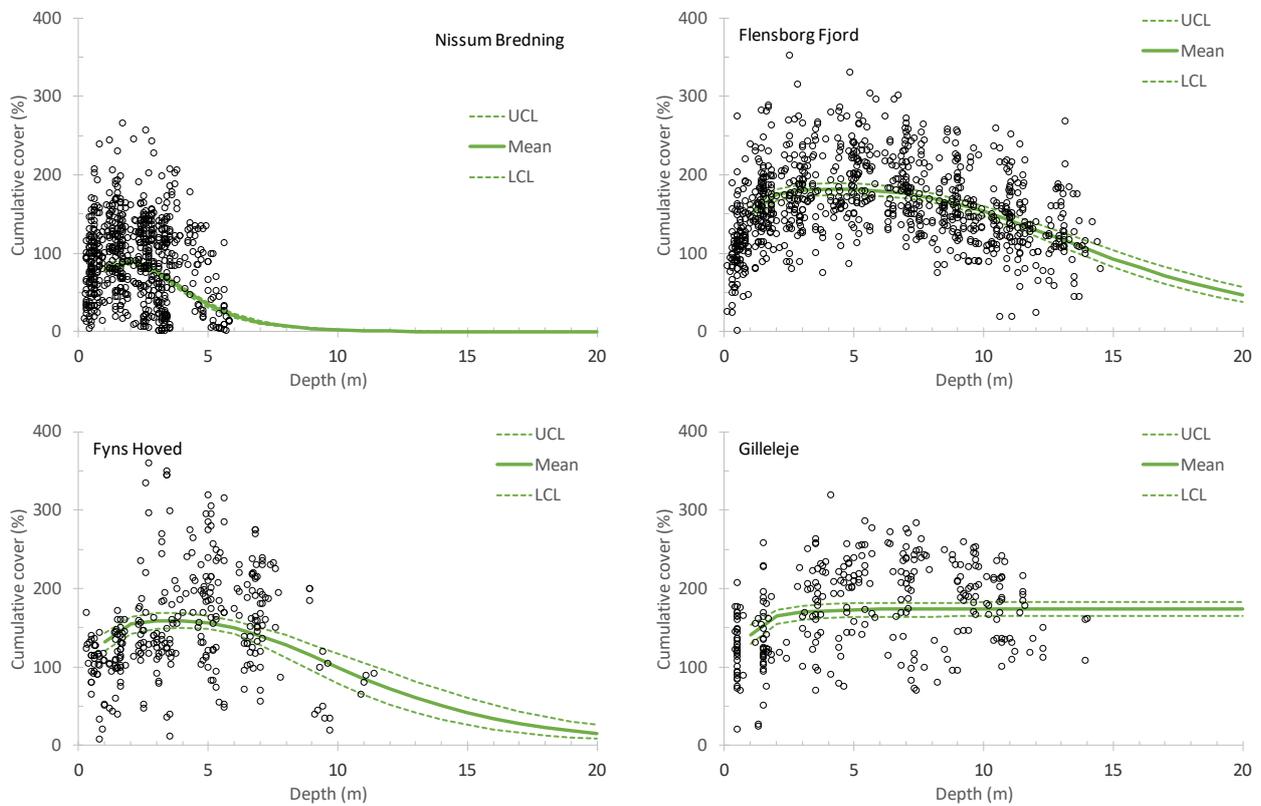


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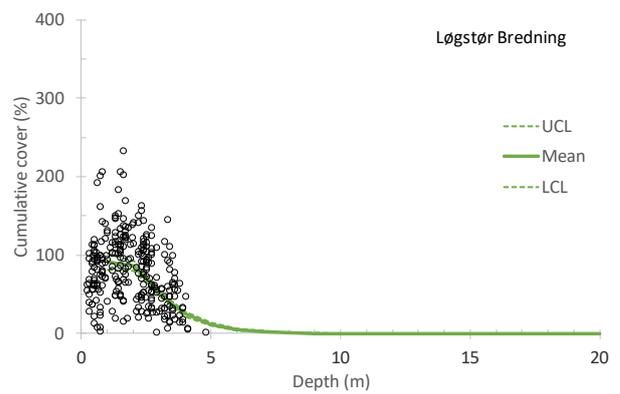
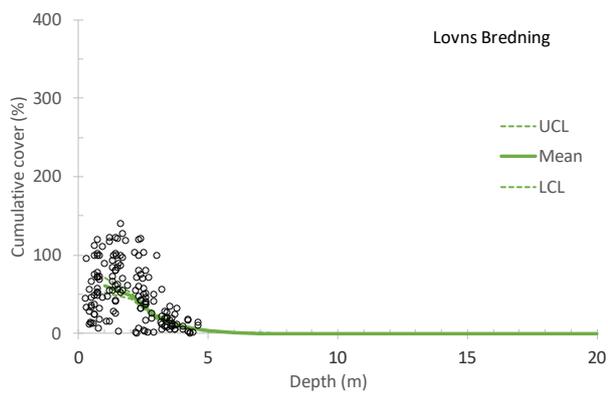
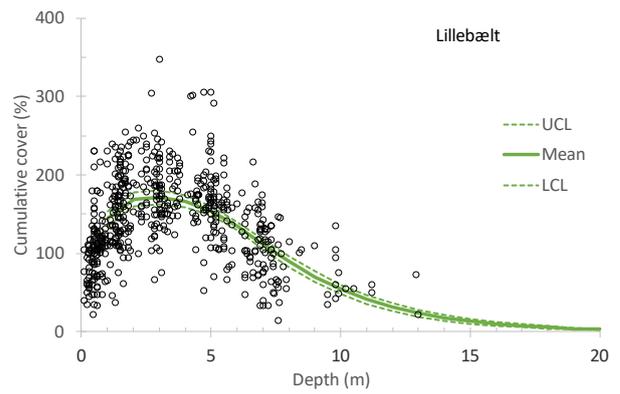
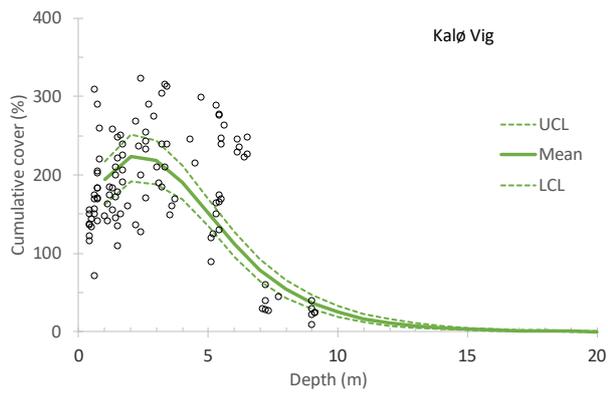
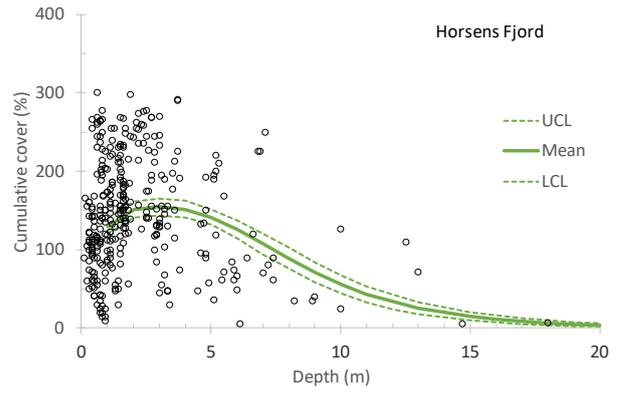
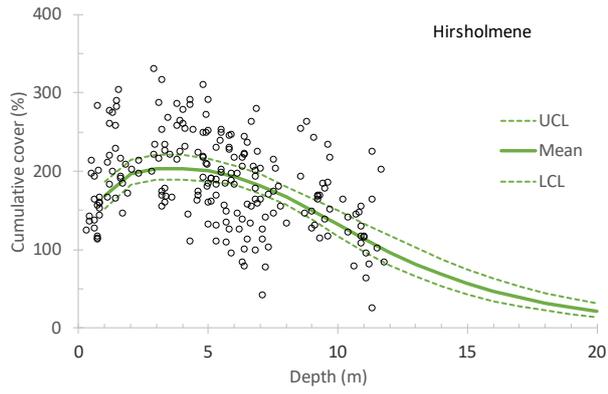
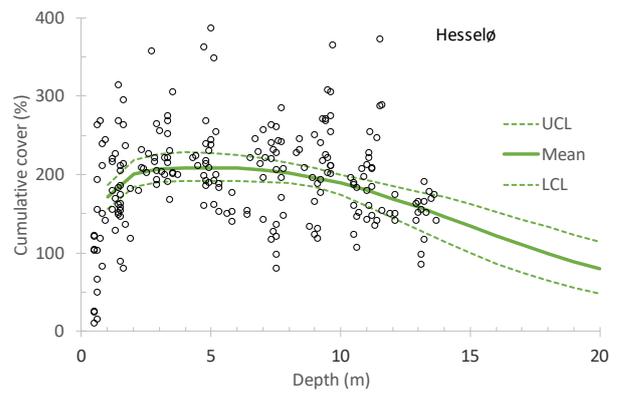
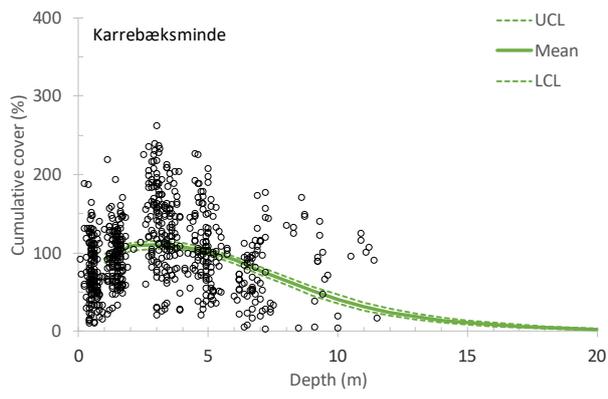


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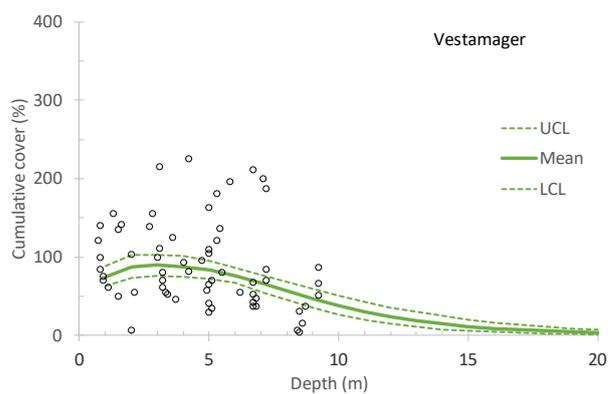
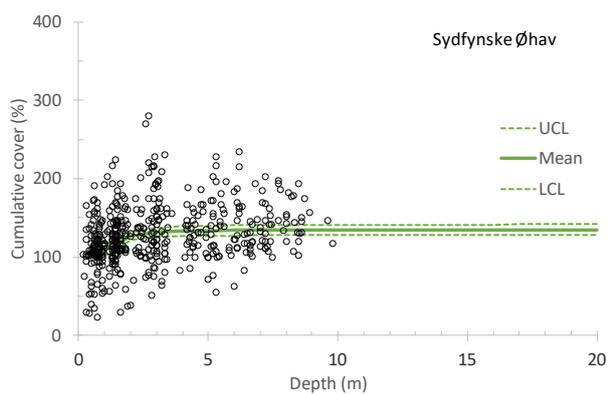
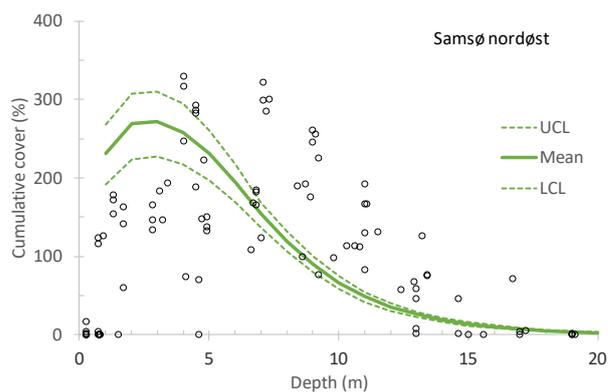
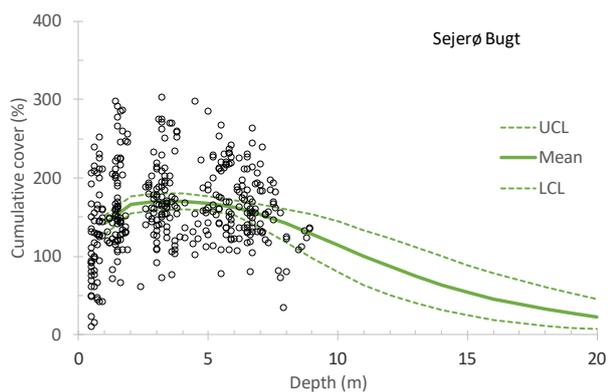
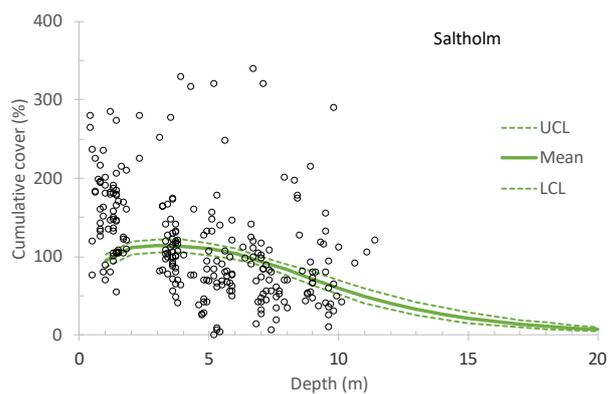
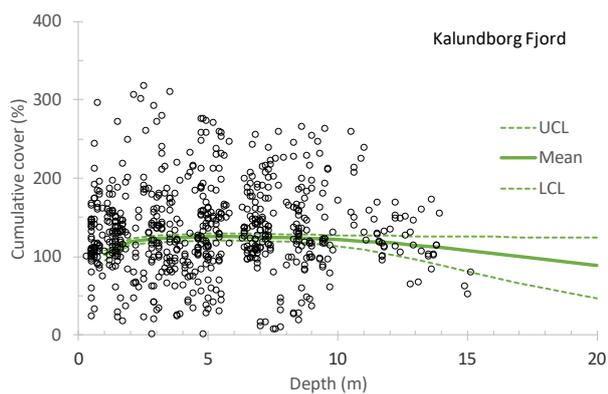
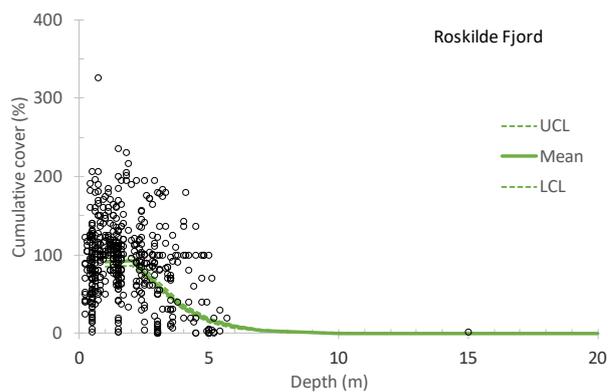
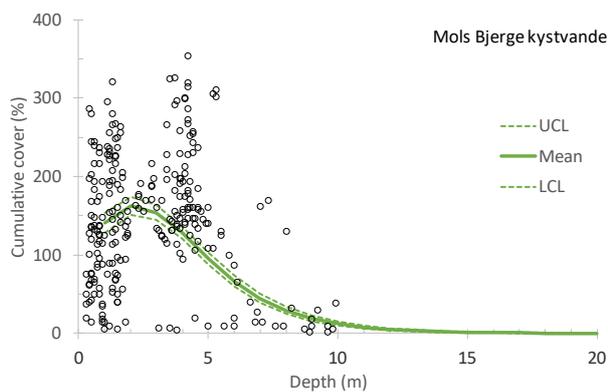


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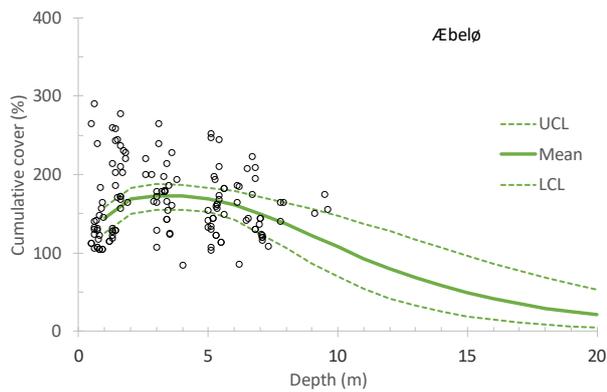
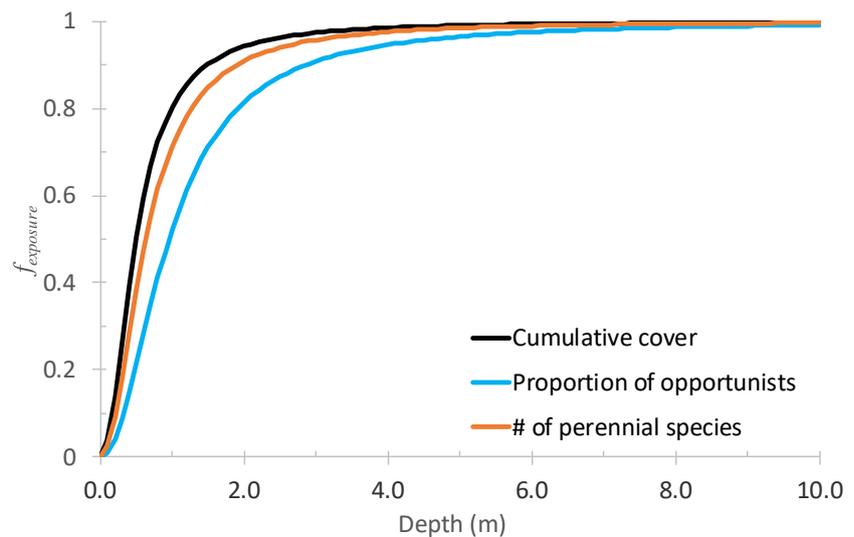


Figure 3.10. Cumulative cover versus depth for the 21 investigated coastal habitats. For each coastal habitat, the estimated depth relationship from the non-linear model (Eq. 2.11) is shown with the 95 % confidence interval of the model. The estimated model and confidence interval represent the geometric mean, corresponding to the median distribution.

The effect of physical exposure was highly significant ($P < 0.0001$), indicating a strong reduction in cumulative cover for depths shallower than 1 m, whereas the effect was small for depth deeper than 2 m (Fig. 3.11). The effect of physical exposure vanished faster with depth for coastal habitats compared to the stone reefs (Fig. 3.4).

Figure 3.11. Estimated effect of physical exposure for modelling the cumulative cover and the number of perennial species (Eq. 2.11) as well as the proportion of opportunists (Eq. 2.12) for coastal sites.



3.3.2 Proportion of opportunists

The model for the proportion of opportunists explained 53 % of the observed variation, although with considerable variation around the regression lines remaining (Fig. 3.12). For many coastal habitats, the proportion of opportunists could span the entire possible range (0-100 %) across a broad depth range. With the exception of Lovns Bredning and Roskilde Fjord, the proportion of opportunists decreased with depth for all coastal habitats. Lovns Bredning did not have observations deeper than 4.6 m, and the tendency of increasing proportion of opportunists with depth could be an artefact from lack of deeper data, whereas Roskilde Fjord was so strongly dominated by opportunists that it was difficult to estimate a depth relationship. Hesselø exhibited the weakest decline in proportion of opportunists with depth ($k_{bio} = 0.021$), whereas Hirschholmene had the steepest decline ($k_{bio} = 0.321$). Variations in k_{bio} among coastal habitats were significant ($P < 0.0001$). Disregarding Lovns Bredning

and Roskilde Fjord, the factor P_5 , expressing the overall proportion of opportunists, could be translated into values ranging from 28 % in Nissum Bredning to 95 % for Vestamager, using the inverse of the logit transformation. Although these values do not exactly represent mean proportions, they do provide a relative measure for comparing the proportion of opportunists across coastal habitats.

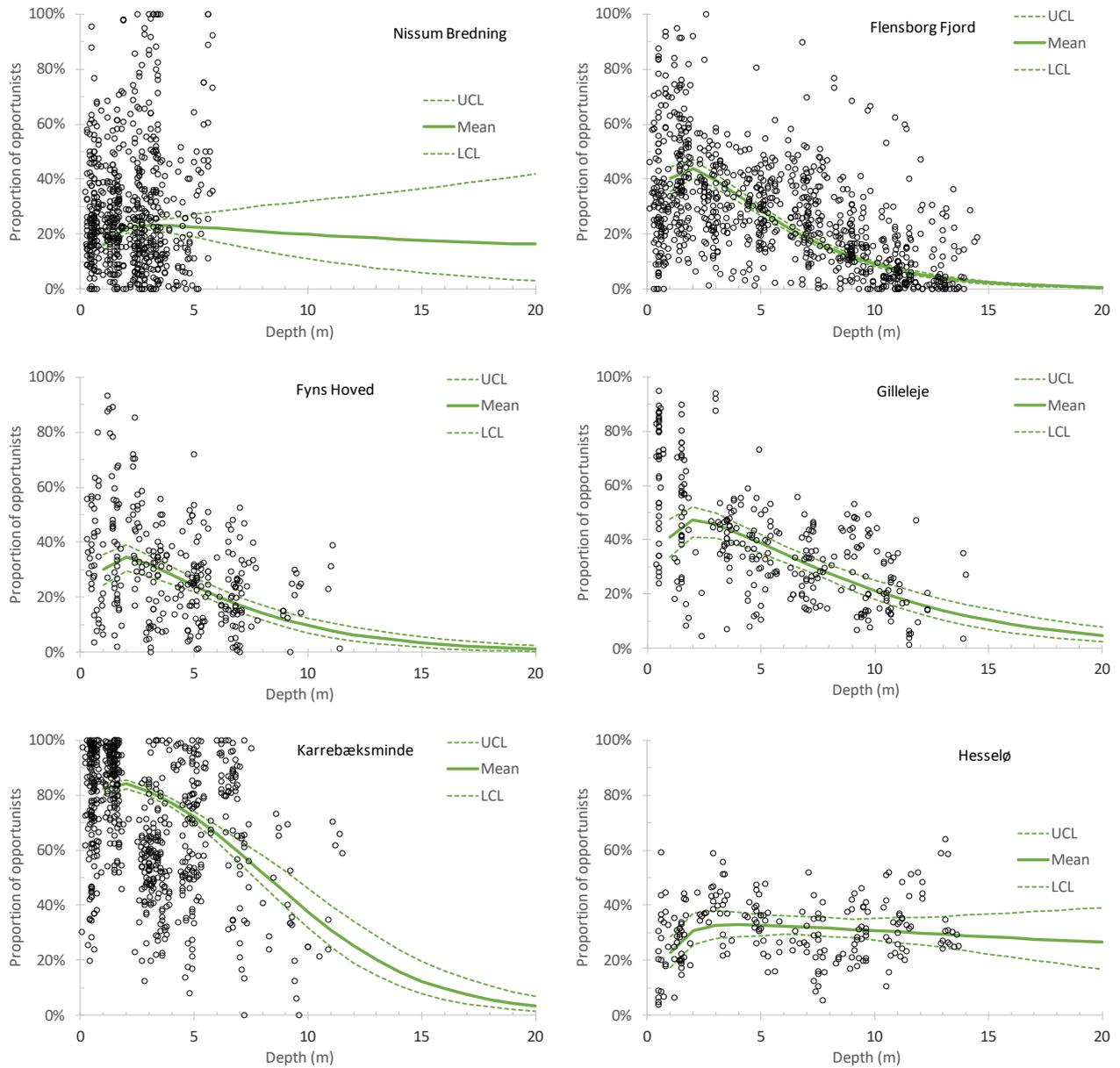


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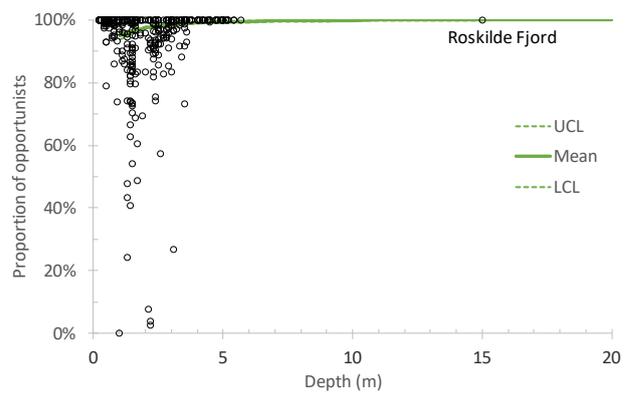
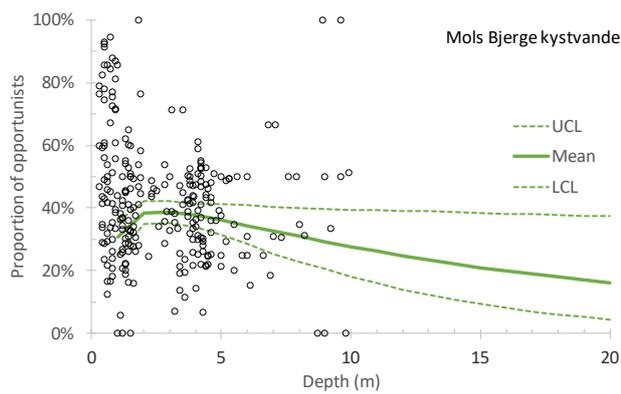
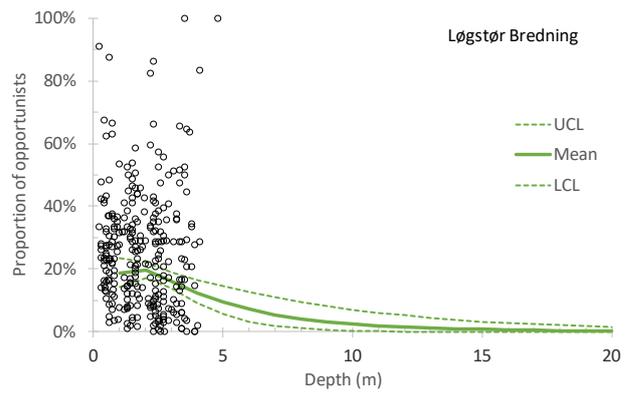
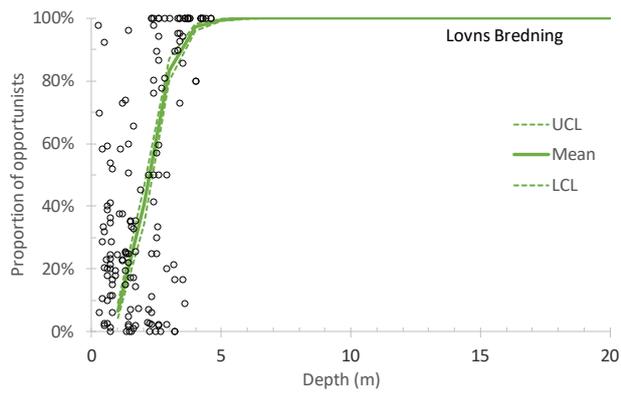
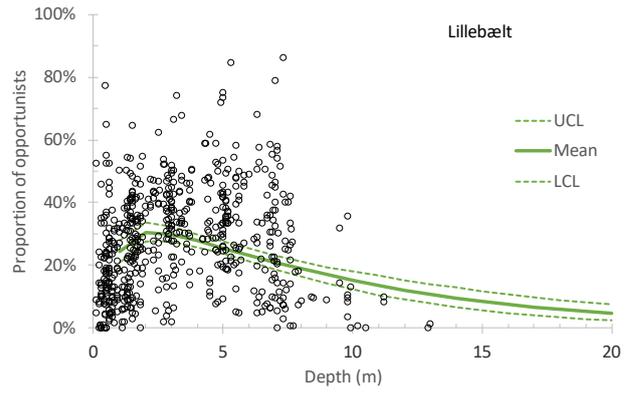
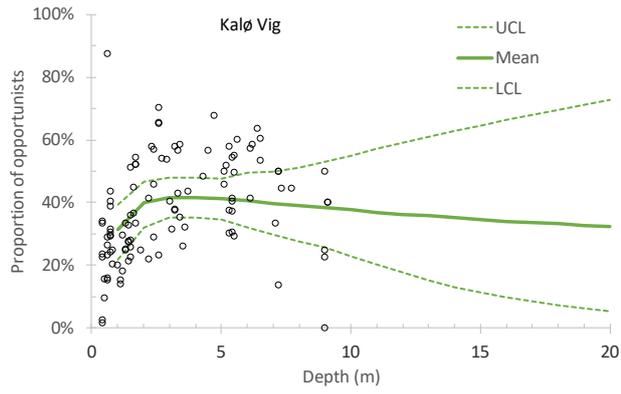
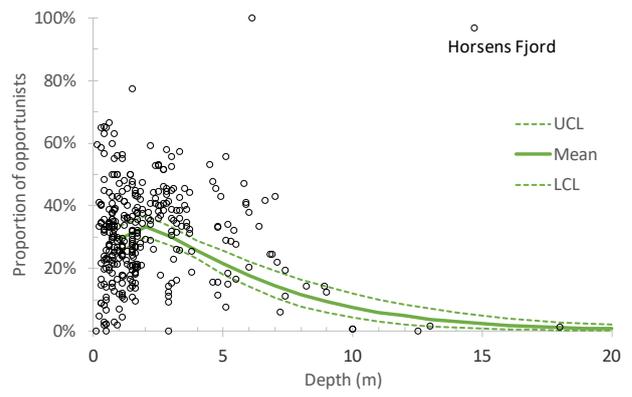
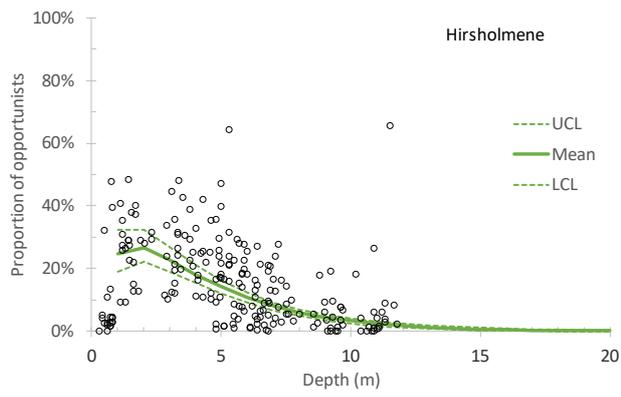


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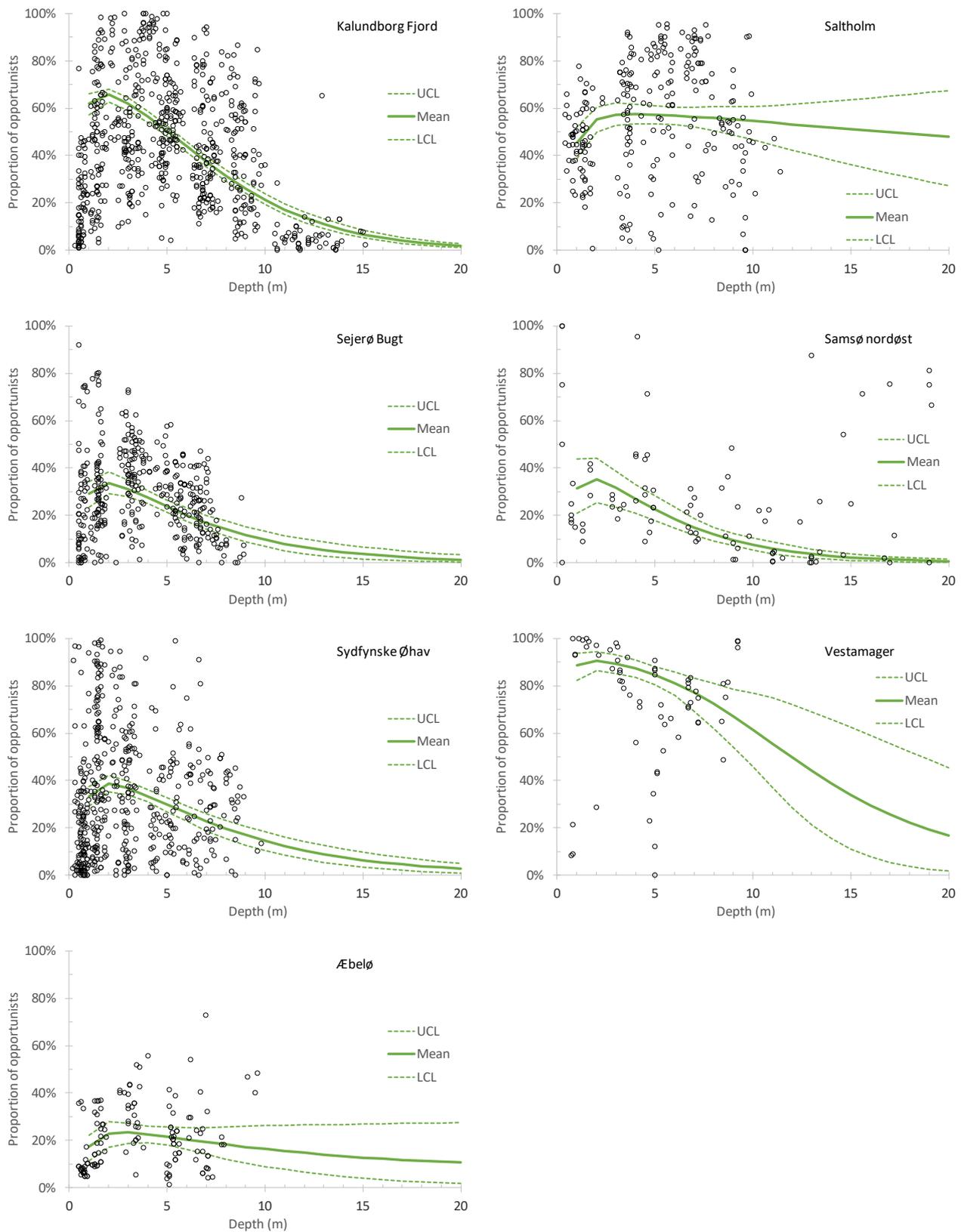


Figure 3.12. Proportion of opportunistic macroalgal species versus depth for the 21 investigated coastal habitats. For each coastal habitat, the estimated depth relationship from the non-linear model (Eq. 2.12) is shown with the 95 % confidence interval of the model. The estimated model and confidence interval represent the geometric mean, corresponding to the median distribution.

The estimated effect of physical exposure was a reduced proportion of opportunists in the top 2-3 m, indicating that perennial species are better adapted than opportunistic species to the stronger wave action in shallow waters. This

estimated relationship was quite similar to that obtained for stone reefs and that reported in earlier analyses of macroalgal data from Danish waters (Carstensen et al. 2014).

3.3.3 Number of perennial species

The model for the number of perennial species explained 62 % of the observed variation, but there was still considerable variation (± 44 %) around the regression lines (Fig. 3.13). The highest number of perennial species was found at Hirsholmene ($n = 22$), whereas in Roskilde Fjord 5 perennial species were recorded at most. There were significant differences in the maximum number of perennial species (C_{max}) among coastal habitats ($P < 0.0001$), ranging from 2.33 in Roskilde Fjord to 14.5 at Samsø nordøst. The number of perennial species did not display decreasing tendencies with depth at Gilleleje, Karrebæksminde and Vestamager, yielding negative estimates for k_{bio} , as indicated by the flat model at deeper depths. Disregarding k_{bio} estimates for these three coastal habitats, a significant difference in k_{bio} -values ($P < 0.0001$) was found with the slowest attenuation in the number of perennial species with depth for Hesselø ($k_{bio} = 0.091$) and the fastest attenuation in Roskilde Fjord ($k_{bio} = 0.846$).

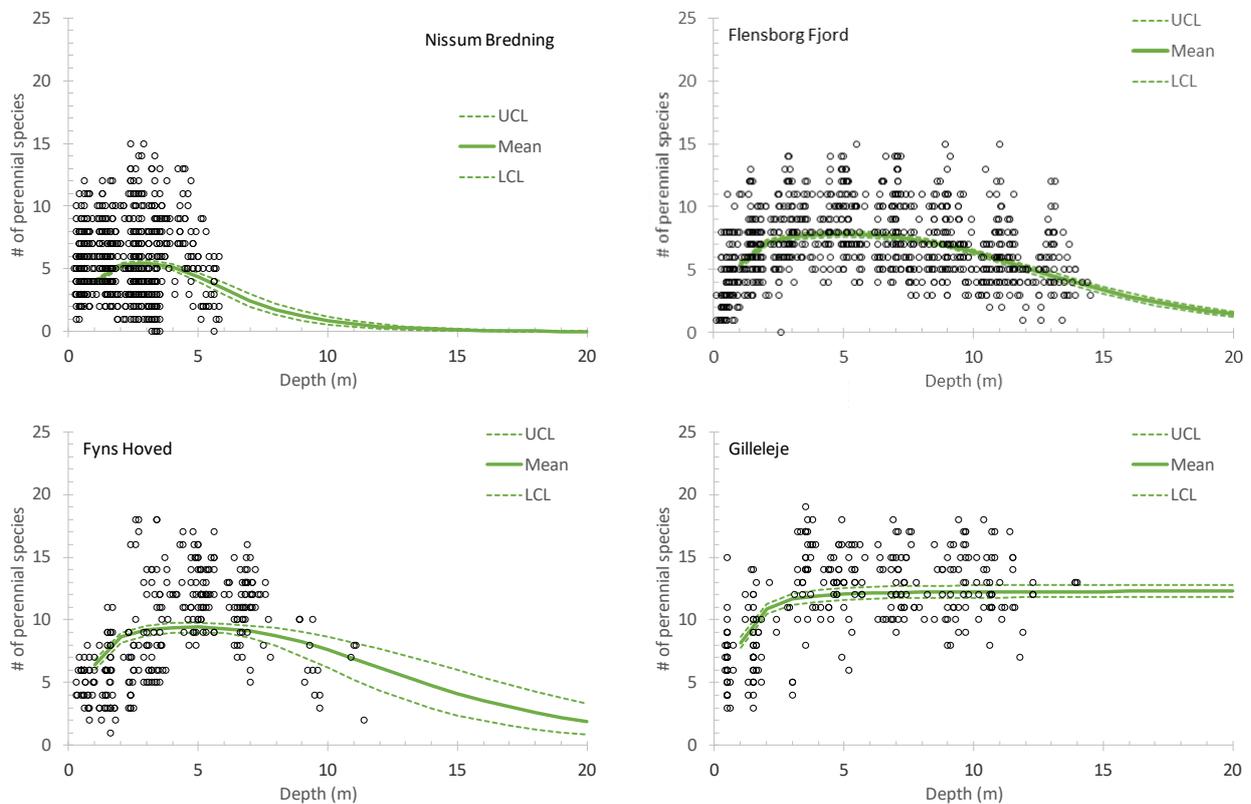


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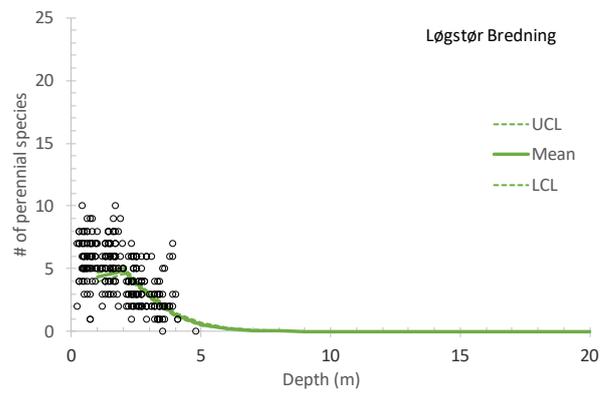
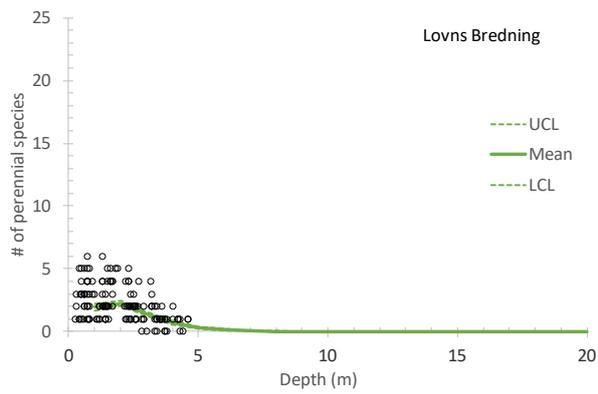
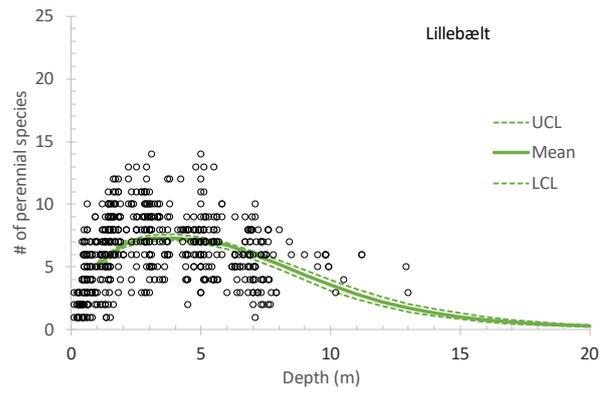
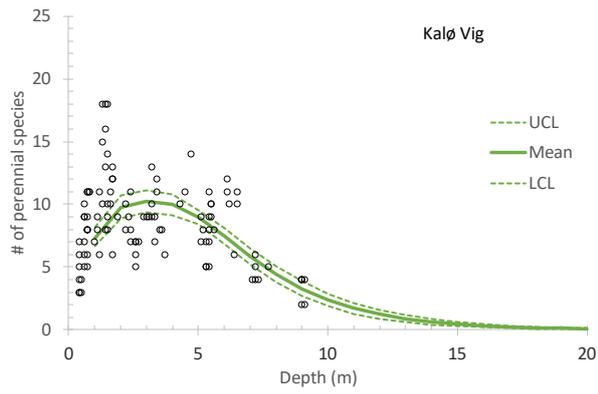
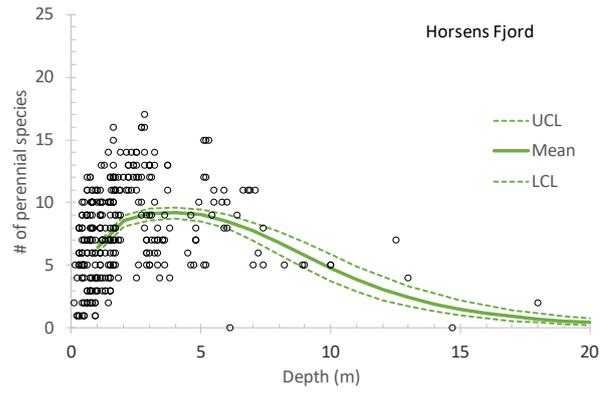
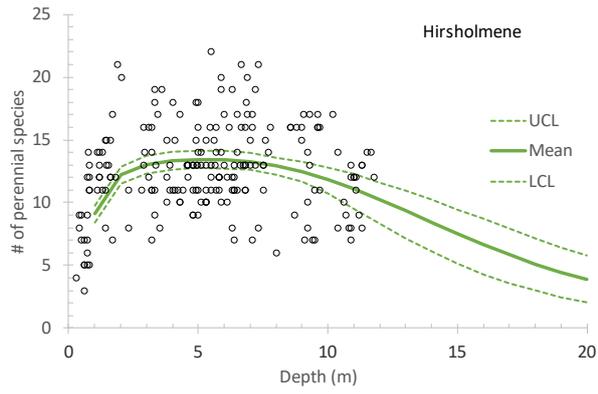
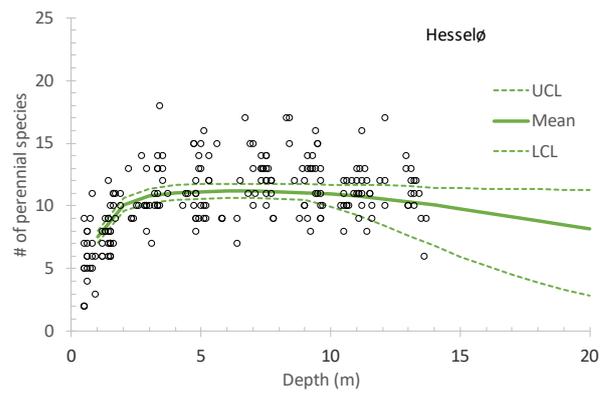
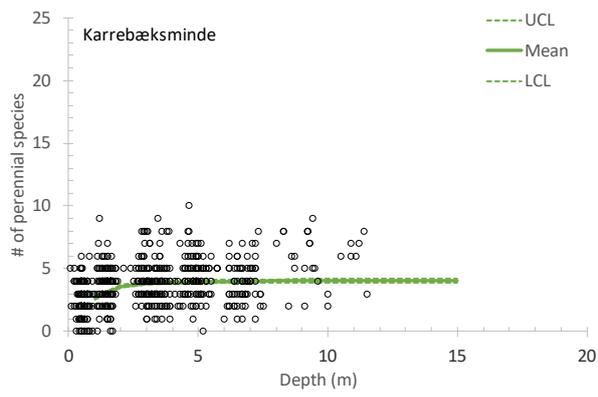


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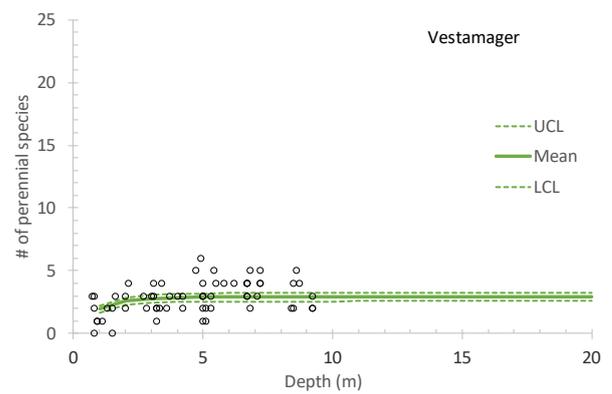
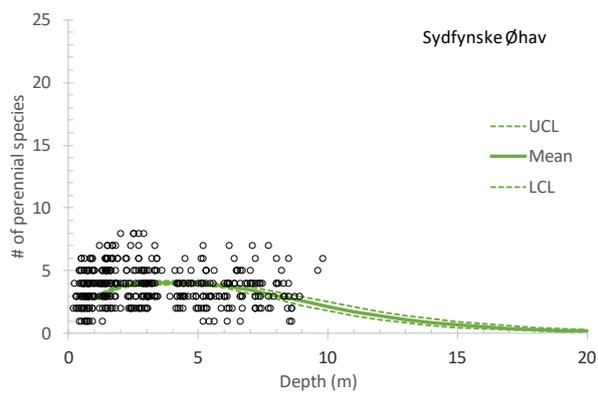
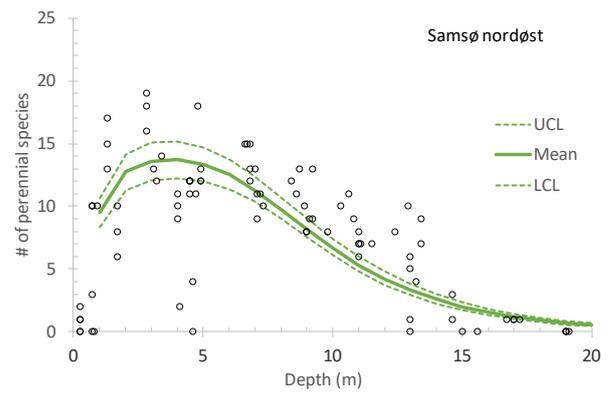
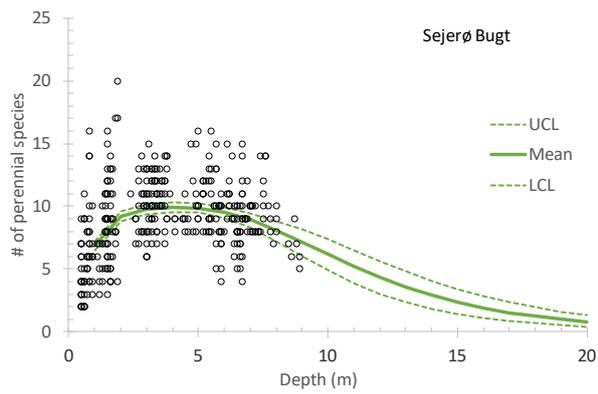
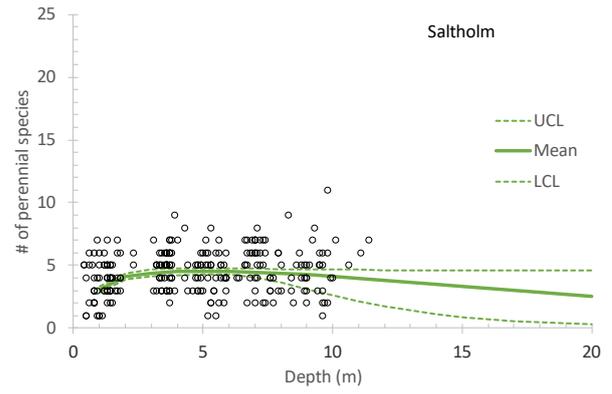
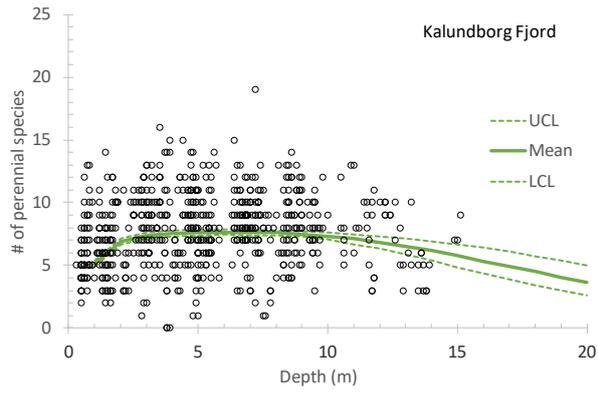
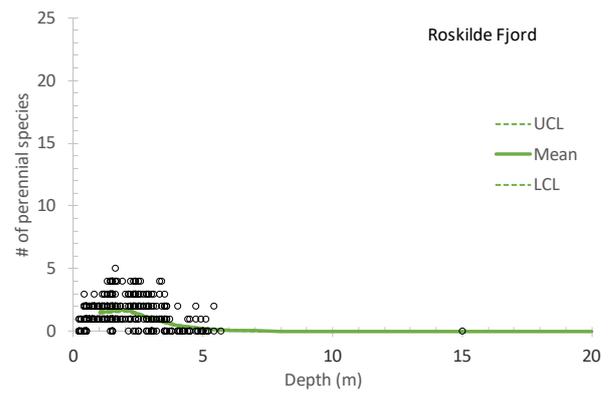
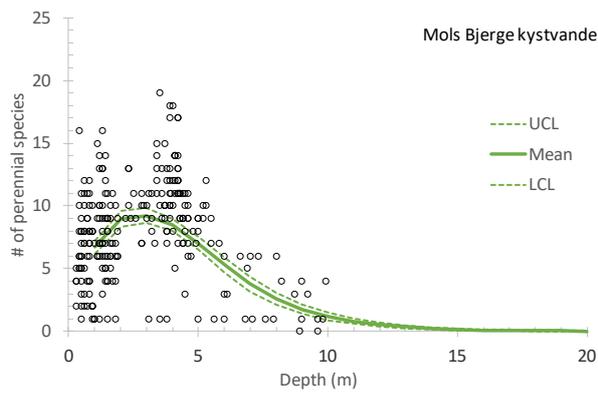


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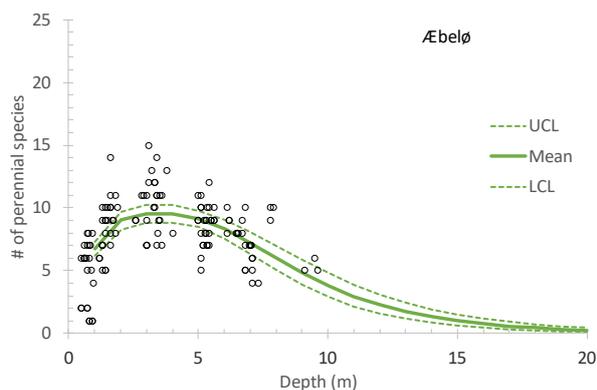


Figure 3.13. Number of perennial species versus depth for the 21 investigated coastal habitats. For each coastal habitat, the estimated depth relationship from the non-linear model (Eq. 2.11) is shown with the 95 % confidence interval of the model. The estimated model and confidence interval represent the geometric mean, corresponding to the median distribution.

The number of perennial species was significantly reduced by physical exposure ($P < 0.0001$) at depths shallower than 2 m (Fig. 3.11); a depth relationship that was similar to that for the cumulative cover and the proportion of opportunists. The depth relationship for coastal habitats associated with physical exposure was not as steep as for reefs in open water (Fig. 3.14b). However, the depth relationship for coastal habitats was based on many different sites, whereas the depth relationship for reefs in open waters was based on observations from Nordre Rønner only. It is possible that physical exposure has the same depth relationship for all three macroalgal indicators, as suggested for the coastal habitats (Fig. 3.11) and cumulative cover and proportion of opportunists for reefs (Fig. 3.4b), and that $f_{exposure}$ for the number of perennial species at reefs deviates from this generic pattern because it is based on data from a single site only. This would suggest an overall strong effect of physical exposure at depth shallower than 2 m, common to all three macroalgal indicators.

3.3.4 Variations in site-specific parameters

The spatial variation in the parameter estimates for cumulative cover (C_{max} and k_{bio}), proportion of opportunists (P_{site} and k_{bio}) and number of perennial species (C_{max} and k_{bio}) were related to salinity and Secchi depth through Eqs. 2.11 and 2.12. However, some parameter estimates were excluded, because reliable estimates were not obtained in the regression analysis.

Estimated maximum cumulative cover was significantly related to salinity (Fig. 3.14a), as suggested in the model for cumulative cover at reefs in open waters (Eq. 2.7). This confirms that macroalgae build fewer layers in low-salinity waters, most likely due to a reduced species richness (see below). In this relationship, it was also obvious that three sites in Limfjorden and Samsø nordøst deviated from the overall pattern. At least part of the Limfjorden has been reported to have a high density of sea urchins although data to support this are not available for this study. This can significantly reduce the macroalgal cumulative cover through grazing (Fig. 3.4a) and explain the much lower cumulative cover at these sites. The estimate of C_{max} at Samsø nordøst was more uncertain compared to the other estimates, and the combination of data from the shallow Stavns Fjord and the deeper reef area off Vejrø in the same Natura 2000 site could imply that this site did not constitute a homogenous entity, explaining the deviations in C_{max} . Severe sea urchin grazing on deeper waters is also reported for Vejrø, but neglected in this model dealing with coastal sites.

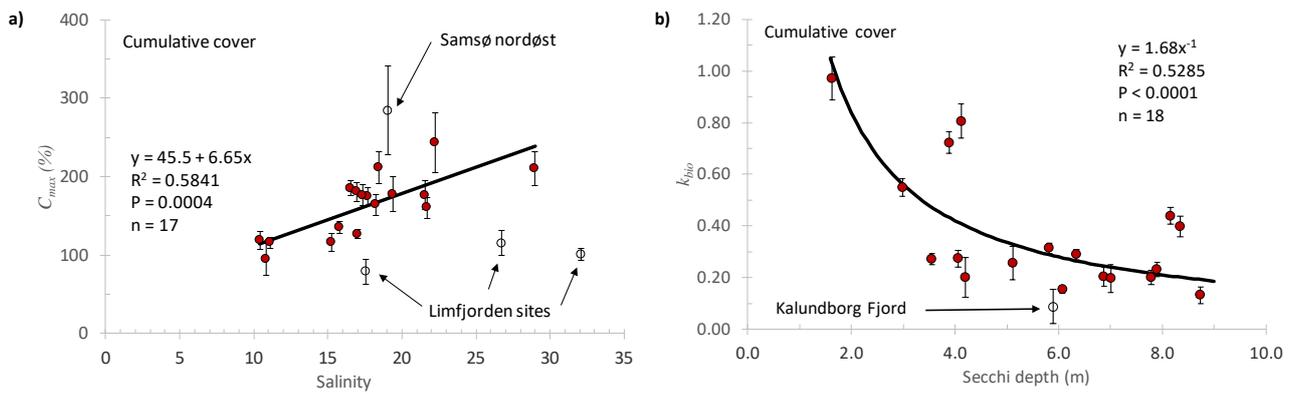


Figure 3.14. Site-specific estimates for the maximum cumulative cover parameter (C_{max}) versus salinity (a) and depth attenuation parameter (k_{bio}) versus Secchi depth (b). Note that Samsø nordøst, Nissum Bredning, Løgstør Bredning and Lovns Bredning were not included in the regression for C_{max} , and that Gilleleje, Kalundborg Fjord and Sydfynske Øhav were not included in the regression for k_{bio} (only Kalundborg shown), because observations of cumulative cover never reached the declining phase allowing k_{bio} to be estimated.

An inverse relationship between k_{bio} and Secchi depth was found for cumulative cover (Fig. 3.14b), demonstrating that sites with poor light conditions experienced a steeper decline in cumulative cover than sites with clearer waters. Three sites were not included, because the declining phase for cumulative cover was not well captured in the monitoring data (Fig. 3.3).

The scale parameter for the proportion of opportunists (P_{site}) had a non-linear relationship with salinity, decreasing with salinity below 22.6 and remaining constant thereafter (Fig. 3.15a). This relationship was similar to that estimated for reefs in open waters, indicating that the macroalgal community is increasingly dominated by opportunistic species for salinities lower than ~20. The depth relationship for the proportion of opportunists, however, was not related to Secchi depth (Fig. 3.15b).

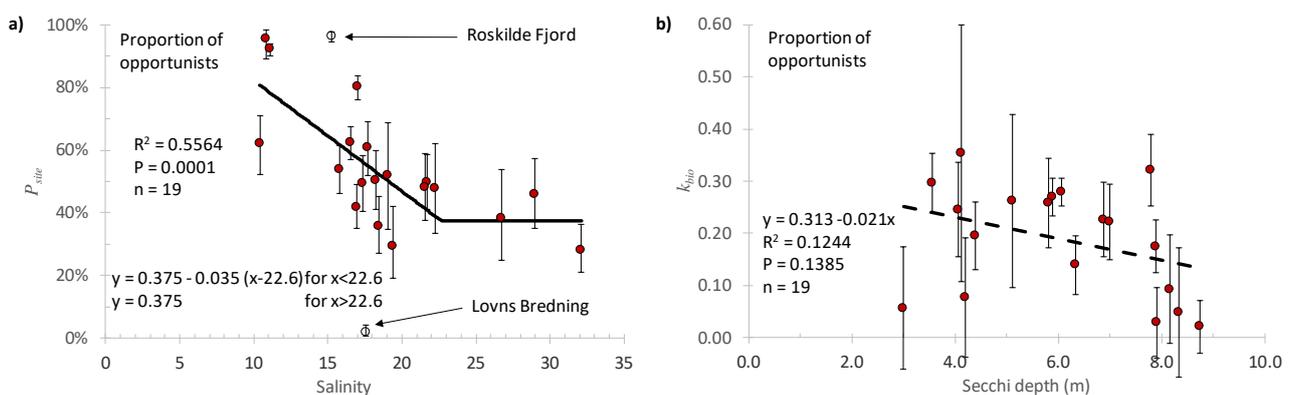


Figure 3.15. Site-specific estimates for the scale parameter (P_{site}) versus salinity (a) and depth attenuation parameter (k_{bio}) versus Secchi depth (b) for the proportion of opportunists. Note that Roskilde Fjord and Lovns Bredning were not included, as these sites exhibited an increasing proportion of opportunists with depth in contrast to all other sites.

The maximum number of perennial species estimated on a site basis was significantly related to salinity (Fig. 3.16a), a relationship similar to that found for reefs in open waters. This confirms that the species richness of perennial species is reduced by more than 50 % from the high salinity areas in Kattegat to the brackish coastal habitats towards the Baltic Sea. Similar to cumulative cover, the perennial species community was poorer in Limfjorden. This might

be caused by grazing from sea urchins. These low site-specific estimates, together with the modelled grazing effect from the stone reefs (Fig. 3.4a), strongly indicate that the presence of sea urchins reduces the diversity of the perennial macroalgal community. Hence, the three sites in Limfjorden were not included in the regression together with the parameter estimate from Samsø nordøst that was associated with larger uncertainty and represented a less homogenous coastal habitat (see above).

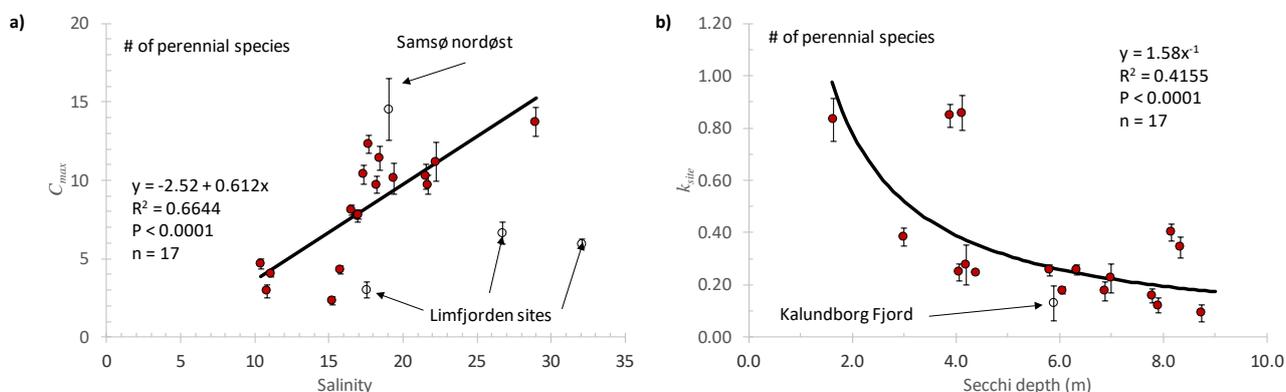


Figure 3.16. Site-specific estimates for the maximum perennial species parameter (C_{max}) versus salinity (a) and depth attenuation parameter (k_{bio}) versus Secchi depth (b). Note that Samsø nordøst, Nissum Bredning, Løgstør Bredning and Lovns Bredning were not included in the regression for C_{max} , and that Gilleleje, Kalundborg Fjord, Karrebæksminde and Sydfynske Øhav were not included in the regression for k_{bio} (only Kalundborg shown), because observations of cumulative cover never reached the declining phase allowing k_{bio} to be estimated.

Similar to the case for the cumulative cover, an inverse relationship between k_{bio} and Secchi depth was found for the number of perennial species (Fig. 3.16b), demonstrating that sites with poor light conditions experienced a steeper decline in species richness than sites with clearer waters. Four sites were not included because the declining phase for the number of perennial species was not well captured in the monitoring data (Fig. 3.6).

3.3.5 Possible target setting for cumulative cover

Targets for the cumulative cover and the number of perennial species could in principle be determined with a similar approach as for reefs in open waters, provided that targets for Secchi depths are available for the coastal Natura 2000 sites. Such targets are currently being developed for WFD water bodies and may be applicable, where water bodies and Natura 2000 sites are expected to have similar Secchi depths. Consequently, it is possible to develop targets for good conservation status sensu the Habitats Directive for some Natura 2000 sites.

4. Conclusions

In this report, we have analysed three macroalgal indicators from 12 stone reefs and 21 coastal habitats that had sufficient data, with the aim of developing statistical models that could disentangle effects of physical exposure, light limitation, sea urchin grazing and salinity-dependent responses. Due to differences in data availability and homogeneity of monitoring across years, slightly different models were employed for stone reefs and coastal habitats. Based on the results from these models, we conclude:

- Physical exposure has a significant reducing effect on the cumulative cover, the proportion of opportunists and the number of perennial species in shallow waters, most pronounced for depths shallower than 2 m in coastal habitats and shallower than 4 m at stone reefs.
- Grazing by sea urchins significantly reduces cumulative cover and the number of perennial species, whereas it increases the proportion of opportunists. The latter could be due to opportunistic species better coping with high grazing rates because of their high growth rates. A strong effect of sea urchin grazing is observed for sea urchin cover of 1 % and above. The cover of sea urchins was only recorded for reefs in open waters, but sea urchins likely also play a role in coastal habitats.
- Light conditions regulate the cumulative cover and the number of perennial species at deeper depths, reducing the number of species and the cumulative cover exponentially with depth in a manner similar to the attenuation of light. The steepest declines in the number of perennial species and the cumulative cover were observed at locations with poor light conditions. Examining the interannual variations in the macroalgal indicators at reefs in open waters documented that year-to-year variations in Secchi depth could explain changes in cumulative cover over time. Hence, macroalgae grow deeper and denser at stone reefs in years with good light conditions.
- Salinity is an important factor for all three macroalgal indicators. The maximum cumulative cover and the number of perennial species increase with salinity, exhibiting more than a doubling over the salinity range. This implies that macroalgal communities are richer in saline waters, allowing multilayered species structures. The proportion of opportunists also changed with salinity, with brackish water hosting a larger proportion of opportunists than waters with salinity >20. The strong relationships with salinity highlight the importance of taking salinity into account when comparing macroalgal data across Danish Natura 2000 sites.
- The significant relationship between interannual variations in cumulative cover and Secchi depth at stone reefs documents that this indicator is suitable as an ecological indicator, as it responds to changes in environmental conditions on annual scales. Since neither historical data nor data for sites unaffected by human activities are available, we propose to translate environmental targets from the Baltic Sea Action Plan to ecological targets for cumulative cover for the stone reefs in the open waters.

5. Suggestions for future work

Some coastal Natura 2000 sites include both a very sheltered coastal bay and reef site that can be regarded as an offshore area. One example is the Natura 2000 site Stavns Fjord. Sites with such characteristics would need to be divided into separate sites for successful application of the presented approach.

Data on sea urchin presence are another topic for improving models for the coastal sites.

Site-specific Secchi depth targets from the WFD implementation can support target setting for the macroalgal indicators in the coastal sites for the HD implementation, ensuring consistency between the two directives.

6. References

Carstensen J, Krause-Jensen D, Dahl K, Henriksen P (2008) Macroalgae and phytoplankton as indicators of ecological status of Danish coastal waters. National Environmental Research Institute, University of Aarhus. 90 pp. - NERI Technical Report No. 683. <http://www.dmu.dk/Pub/FR683.pdf>

Carstensen J, Krause-Jensen D, Josefson A (2014) Development and testing of tools for intercalibration of phytoplankton, macrovegetation and benthic fauna in Danish coastal areas. Aarhus University, DCE – Danish Centre for Environment and Energy, 85 pp. Scientific Report from DCE – Danish Centre for Environment and Energy No. 93. <http://dce2.au.dk/pub/SR93.pdf>

Duarte CM (1991) Seagrass depth limits. *Aquatic Botany* 40:363-377.

Hansen JW (red.) (2018) Marine områder 2016. NOVANA. Aarhus Universitet, DCE – Nationalt Center for Miljø og Energi, 140 s. - Videnskabelig rapport fra DCE - Nationalt Center for Miljø og Energi nr. 253. <http://dce2.au.dk/pub/SR253.pdf>

HELCOM (2013) Approaches and methods for eutrophication target setting the Baltic Sea region. *Baltic Sea Environmental Proceedings* No. 133. <http://www.helcom.fi>.

Platt T, Jassby AD (1976) The relationship between photosynthesis and light for natural assemblages of coastal marine phytoplankton. *Journal of Phycology* 12:421-430.

MACROALGAL INDICATORS FOR DANISH NATURA 2000 HABITATS

This report investigates the potential for assessing good ecological potential according to the Habitats Directive for 12 stone reefs and 21 coastal habitats in Denmark. Three different macroalgal indicators were investigated and they were shown to be regulated by light availability, salinity, physical exposure and the presence of sea urchins. The indicators allow separation of anthropogenic influence from natural variations. Thresholds for good ecological potential at stone reefs are proposed and an approach for similar values in coastal habitats is proposed.