MACROALGAE INDICATORS FOR ASSESSING ECOLOGICAL STATUS IN DANISH WFD WATER BODIES

Technical Report from DCE – Danish Centre for Environment and Energy No. 170 2020

AARHUS UNIVERSITY
DCE – DANISH CENTRE FOR ENVIRONMENT AND ENERGY

MACROALGAE INDICATORS FOR ASSESSING ECOLOGICAL STATUS IN DANISH WFD WATER BODIES

Technical Report from DCE – Danish Centre for Environment and Energy No. 170 2020

Jacob Carstensen

Aarhus University, Department of Bioscience

AARHUS UNIVERSITY
DCE – DANISH CENTRE FOR ENVIRONMENT AND ENERGY

Data sheet

Series title and no.: Technical Report from DCE – Danish Centre for Environment and Energy No. 170

Title: Macroalgae indicators for assessing ecological status in Danish WFD water bodies
Author: Jacob Carstensen
Institution: Aarhus University, Department of Bioscience

Publisher: Aarhus University, DCE – Danish Centre for Environment and Energy ©
URL: http://dce.au.dk/en

Year of publication: May 2020
Editing completed: May 2020

Referee: Dorte Krause-Jensen, Department of Bioscience, Aarhus University
Quality assurance, DCE: Signe Jung-Madsen
Linguistic QA: Anne van Acker

External comments: The Danish Environmental Protection Agency (Miljøstyrelsen). The comments are available here: http://dce2.au.dk/pub/komm/TR170_komm.pdf

Financial support: The Danish Environmental Protection Agency (Miljøstyrelsen)

Please cite as: Carstensen J. 2020. 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

Abstract: This report investigates three macroalgae indices for assessing ecological status according to the Water Framework Directive using monitoring data from 58 water bodies in Denmark. The three indices are cumulative cover, perennial species richness and relative cover of opportunists. The different macroalgae indices were analysed with non-linear statistical models, describing regulation by light availability, salinity, grazing and physical exposure. These models separate anthropogenic disturbance from natural variations. The attenuation of cumulative cover and number of perennial species with depth constitute operational macroalgae indicators, which respond clearly to eutrophication status. Reference conditions and class boundaries, calculated from established values for light attenuation, as well as an aggregation scheme for combining macroalgae indicators with the indicator for eelgrass main depth limit are proposed. The macroalgae indicator based on the relative cover of opportunists was not operational, as it did not respond to any primary eutrophication variable.

Keywords: Water Framework Directive, eutrophication, sea urchins, physical exposure, salinity, light regulation, ecological status, indicator aggregation, macroalgae, eelgrass, angiosperms

Layout: Anne van Acker
Drawings: The author
Front page photo: Macroalgae observed during monitoring of transect. Photo Jens Deding

ISBN: 978-87-7156-490-7
ISSN (electronic): 2245-019X

Number of pages: 74

Internet version: The report is available in electronic format (pdf) at https://dce2.au.dk/pub/TR170.pdf

Contents

[Blank page]

Preface

This report is the outcome of a project investigating the applicability of using macroalgae indicators for assessing ecological status according to the European Water Framework Directive. The report also proposes a method for combining these indicators with the existing indicator for eelgrass main depth limits. Financial support was provided by the Danish Environmental Protection Agency (Miljøstyrelsen). The Danish Environmental Protection Agency (Miljøstyrelsen) has commented on a draft version of the report before publication. The report will be submitted to the European Commission as a supporting document for the WFD intercalibration.

Summary

The ecological status of macroalgae is an important component of the EU Water Framework Directive biological quality element 'Macroalgae and angiosperms'. In this report, a comprehensive macroalgae data set from the Danish monitoring programme has been compiled to produce three macroalgae indices (indices represent aggregated observations from the raw monitoring data): 1) cumulative cover of macroalgae, 2) number of perennial macroalgae species, and 3) relative cover of opportunistic macroalgae species. The data set spans 58 water bodies distributed over 23 different types with depth ranges down to 25 m. These data were combined with monitoring data describing environmental conditions in the analysed water bodies.

The three macroalgae indices were analysed using non-linear statistical models, which partitioned natural variations from the effects of human disturbance. Changes in the macroalgae indices with depth were described by physical exposure, grazing by sea urchins, salinity and light conditions. Cumulative cover and the number of perennial species typically exhibit three distinct phases over the depth gradient from regulation by physical exposure near the surface, maximum levels of these macroalgae indices at intermediate depths, and attenuation at deeper depths due to light limitation. Parameter estimates for the attenuation of cumulative cover and number of perennial species with depth are suitable macroalgae indicators, because they show clear responses to light attenuation and therefore constitute sentinels of eutrophication. On the other hand, the relative cover of opportunists was primarily controlled by salinity and did not express variations in response to changing light or nutrient conditions. Consequently, the relative cover of opportunists is not suitable as operational indicator (based on current monitoring data) for ecological status assessment in relation to the WFD, as it appears more driven by natural variations than eutrophication.

Reference conditions and class boundaries for the attenuation of cumulative cover and number of perennial species with depth can be computed using existing reference conditions and class boundaries for light attenuation (based on historical eelgrass depths), translating these by means of the established linear relationship between attenuation of macroalgae indicators and light attenuation. Reference conditions and class boundaries are proposed for all water bodies with light attenuation reference values, except for Limfjorden. Further analyses are required for developing a macroalgae assessment method for Limfjorden.

For assessing ecological status of 'Macroalgae and angiosperms', the two macroalgae indicators (attenuation of cumulative cover and number of perennial species) should be combined with the existing indicator for eelgrass main depth limit. A method for combining these indicators is proposed that first combines the two macroalgae indicators before combining these with the eelgrass indicator. For combining such indicators, it is important that they are first transformed into a common scale, which is proposed to be a standardized EQR scale obtained through a piecewise linear transformation. The two macroalgae indicators are combined by averaging their EQR standardised values before this average is combined with the EQR standardised value for eelgrass main depth limit by averaging. The combination method is illustrated step-

by-step with examples from two water bodies and two periods. It is recommended to use this approach for assessing the ecological status of 'Macroalgae and angiosperms' and to quantify the confidence in the classification.

Sammenfatning

Den økologiske status på makroalger udgør en vigtig komponent i EU's vandrammedirektiv som en del af det biologiske kvalitetselement 'Makroalger og blomsterplanter'. I denne rapport er et stort datasæt fra det danske overvågningsprogram blevet undersøgt og tre makroalgeindeks (indeks repræsenterer afledte observationer fra de rå overvågningsdata) er specifikt analyseret: 1) kumulativ dækning af makroalger, 2) antallet af flerårige makroalgearter, og 3) relative dækning af opportunistiske makroalger. Datasættet dækker 58 vandområder fordelt på 23 forskellige typer med observationer ned til 25 m's dybde. Disse makroalgeindeks er sammenstillet med andre overvågningsdata, som beskriver den generelle vandkvalitet i vandområderne.

De tre makroalgeindeks er blevet analyseret med en ikke-lineær statistisk model, som adskiller naturlige variationer fra menneskelig påvirkning. Ændringer i de tre makroalgeindeks med dybden er således beskrevet ud fra ændringer i fysisk eksponering, græsning fra søpindsvin, saltholdighed og lysforhold. Kumulativ dækning og antallet af flerårige arter ændrer sig typisk i et trefaset forløb med stor effekt af fysisk eksponering på lave vanddybder og maksimale makroalgeindeksværdier ved middeldybder efterfulgt af aftagende værdier som følge af lysbegrænsning. Parameterestimater, som beskriver hastigheden, hvormed kumulativ dækning og antallet af flerårige arter aftager med dybden, er egnede som indikatorer, da de relaterer sig til vandets klarhed og dermed graden af eutrofiering. Derimod var den relative dækning af opportunistiske makroalger kun relateret til saltholdigheden og udviste ingen korrelation til lysforhold eller næringsstofkoncentrationer. Derfor vurderes den relative dækning af opportunistiske makroalger ikke at være egnet som operationel indikator (baseret på nuværende overvågningsdata) for tilstandsvurdering i henhold til vandrammedirektivet, da denne parameter er styret mere af naturlige variationer end af eutrofiering.

Relationer mellem hastigheden, hvormed kumulativ dækning og antallet af flerårige arter aftager med dybden, og lyssvækkelse for de enkelte vandområder kan benyttes til at omsætte referenceværdier og klassegrænser for lyssvækkelse (baseret på historiske dybdegrænser for ålegræs) til tilsvarende værdier for de to makroalgeindikatorer. Referenceværdier og klassegrænser er foreslået for alle vandområder, hvor der fandtes referenceværdier for lyssvækkelse med undtagelse af Limfjorden. Yderligere analyser er påkrævet for Limfjorden for at kunne udvikle en mere specifik metode for tilstandsvurdering af makroalger i Limfjorden.

For at vurdere den økologiske status på 'Makroalger og blomsterplanter' skal de to makroalgeindikatorer (aftagende hastighed med dybden for kumulativ dækning og antallet af flerårige arter) kombineres med den eksisterende indikator for hovedudbredelsen af ålegræs. En metode er foreslået som først kombinerer de to makroalgeindikatorer til et indeks og derefter kombinerer dette med ålegræsindikatoren. For at kunne kombinere sådanne indikatorer er det nødvendigt at transformere indikatorerne til en fælles standardiseret EQR skala via en stykvis lineær transformation. Ved hjælp af denne transformation kan makroalgeindikatorer kombineres med ålegræs ved gennemsnitsberegninger. Metoden er eksemplificeret med eksempler fra to vandområder og to perioder. Det anbefales at benytte denne metodik til vurdering af økologisk

tilstand af 'Makroalger og blomsterplanter' og ligeledes bestemme konfidensen (sikkerheden) i denne tilstandsvurdering.

1 Introduction

The objective of this report is to demonstrate the applicability of indicators derived from three proposed macroalgae indices (*Fig. 1.1*) (Carstensen et al. 2014) for the European Water Framework Directive (WFD) to the water bodies in Denmark, where macroalgae are monitored. The three tested algal indicators cover the sub-elements composition and abundance, defined in Annex V of the Directive, for macroalgae, which together with angiosperms define the biological quality element 'Macroalgae and angiosperms'.

Denmark has defined 109 coastal water bodies (VandPlan 3), which are categorised into 38 different types, depending on surface salinity, tidal regime, water depths, residence time, freshwater input, stratification, sediment characteristics and geographic location (Erichsen et al. 2019). Macroalgae are monitored (NOVANA programme) in a subset of these 109 coastal water bodies by a diver, reporting the cover of macroalgae species relative to the availability of suitable hard substrate at discrete points along a depth gradient from nearshore to the deeper part of the water body. The NOVANA programme sets a minimum level of 10 % hard stable substrate for a sampling location for macroalgae vegetation.

More specifically, the objective of this report is to develop indicators for the ecological status of macroalgae and document the sensitivity of these to eutrophication (mainly light conditions), taking into account other sources of natural variability. Using established reference conditions and class boundaries for the attenuation of light, reference conditions and class boundaries for the macroalgae indicators are proposed for the different coastal water bodies. Finally, a method for combining the macroalgae indicators with the existing indicator for eelgrass main depth limit is proposed.

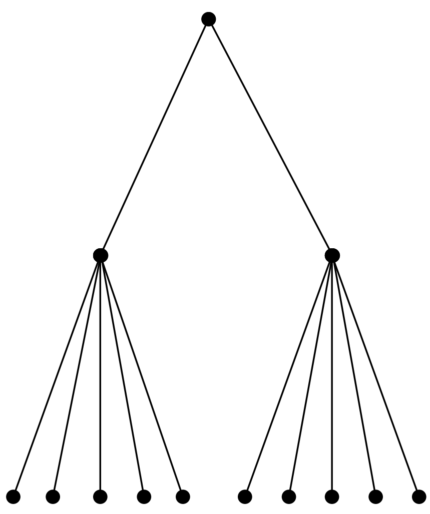


Figure 1.1. Overview of terms used in this report to clarify the distinction between indicators, indices and observations.

2 Macroalgae monitoring data and methods

Macroalgae monitoring data from the last 12 years (2007-2018) were extracted from the national database (ODA) and aggregated to three indices (Carstensen et al. 2014):

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

Indices represent observations with the same time and space sampling property as the monitoring data, but indices are compiled by aggregation of the raw species-specific observations (*Fig. 1.1*). More than 15,000 point samples were used for investigating the three macroalgae indices distributed over 58 water bodies, represented by 261 transects (*Fig. 2.1*, *Table 2.1*). In addition, more than 500 point observations from stone reefs outside the WFD area, defined as the baseline plus one nautical mile, were included to improve the model estimation, as these sites in open marine waters exhibit larger spans in depths and sea urchins have been monitored consistently since 1994.

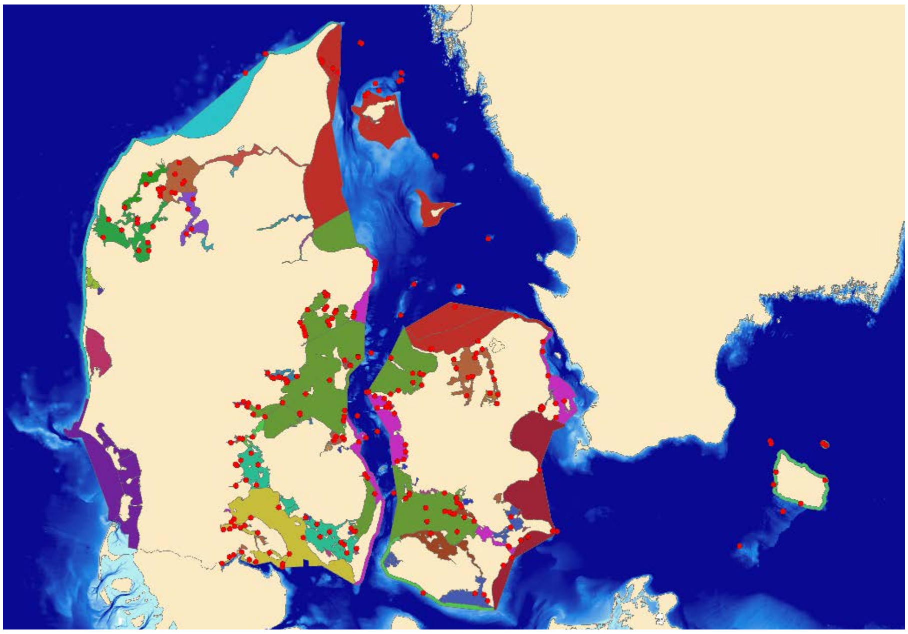


Figure 2.1. Macroalgae transects (red dots) investigated in the present study and the WFD water bodies coloured for different types (T.1-T.38; Erichsen et al. 2019). Note that transects from open-water stone reefs were also included to improve parameter estimates.

Pelagic monitoring stations were associated with the selected water bodies to provide information on environmental conditions representative for the macroalgae. Although these pelagic monitoring stations do not exactly represent the environmental conditions at the macroalgae transects, it is assumed that they give a reasonable local representation relative to the large-scale variation across water bodies. From these pelagic stations, the average salinity profile with depth was calculated and combined with the depth-specific indices for macroalgae. Furthermore, water body-specific and annual means for

Secchi depth, total nitrogen (TN) and total phosphorus (TP) were computed following the methodology in Hansen et al. (2018).

Table 2.1. Overview of data used for analysing macroalgae indices and indicators (cf *Fig. 1.1*). For each water body, the type, depth range, number of transects and number of point observations are listed.

Water body	Type	Depths (m)	# of transects	# of obs.
Østersøen, Bornholm	T.5	0.1-17.9	3	667
Østersøen, Christiansø	T.5	0.5-20.6	3	22
Femerbælt	T.6	8.2-8.2	1	1
Lillebælt, Snævringen	T.6	0.2-7.4	2	14
Nyborg Fjord	T.6	0.5-3.1	2	18
Faaborg Fjord	T.7	0.3-3.1	1	10
Lunkebugten	T.7	0.5-5.1	2	18
Avnø Fjord	T.9	0.4-5.2	1	33
Smålandsfarvandet, syd	T.9	0.3-5.3	1	75
Horsens Fjord, indre	T.10	0.2-5.0	5	168
Horsens Fjord, ydre	T.10	0.3-5.2	5	196
Lillestrand	T.11	0.6-3.0	1	9
Hjelm Bugt	T.12	0.3-19.8	7	156
Køge Bugt	T.12	5.1-14.6	1	5
Det sydfynske Øhav	T.13	0.2-9.8	8	581
Lillebælt, Bredningen	T.13	0.2-17.0	4	475
Rødsand og Bredningen	T.14	0.0-8.0	5	270
Stege Bugt	T.14	0.5-12.5	2	8
Lindelse Nor	T.16	0.6-1.8	1	18
Isefjord, indre	T.17	0.3-5.3	1	92
Isefjord, ydre	T.17	0.3-5.5	7	214
Kolding Fjord, ydre	T.17	0.1-2.7	1	15
Løgstør Bredning	T.17	0.1-5.7	13	649
Roskilde Fjord, ydre	T.17	0.4-5.2	4	124
Kås Bredning og Venø Bugt	T.19	0.1-4.9	7	631
Nissum Bredning	T.19	0.2-5.8	4	452
Djursland Øst	T.20	0.3-11.9	8	598
Grønsund	T.20	0.5-9.2	2	28
Jammerland Bugt og Musholm Bugt	T.20	0.1-19.0	6	448
Kalundborg Fjord	T.20	0.4-10.0	6	319
Nordlige Øresund	T.20	0.3-9.9	17	415
Storebælt, NV	T.20	0.3-11.4	3	223
Kattegat, Læsø	T.21	0.4-14.6	3	518
Kattegat, Nordsjælland	T.21	0.4-15.1	4	337
Kattegat, Nordsjælland > 20 m	T.21	0.5-13.7	1	151
Nordlige Kattegat, Ålbæk Bugt	T.21	0.3-12.6	5	336
Ebeltoft Vig	T.22	0.3-5.3	3	193
Kalø Vig	T.22	0.1-7.0	4	198
Knebel Vig	T.22	0.5-1.4	2	12
Langelandssund	T.22	0.4-7.3	2	218
Sejerø Bugt	T.22	0.5-15.1	10	580
Smålandsfarvandet, åbne del	T.22	0.1-20.2	18	898
Vejle Fjord, ydre	T.22	0.0-11.5	5	420
Århus Bugt og Begtrup Vig	T.22	0.2-8.0	10	453
Århus Bugt syd, Samsø og Nordlige Bælthav	T.22	0.3-21.0	14	596
Als Fjord	T.23	0.2-9.3	3	228
Flensborg Fjord, indre	T.23	0.1-5.7	3	188
Flensborg Fjord, ydre	T.23	0.2-13.6	8	574
Lillebælt, syd	T.23	0.1-13.9	4	534

Water body	Type	Depths (m)	# of transects	# of obs.
Åbenrå Fjord	T.23	0.1-7.7	5	407
Roskilde Fjord, indre	T.27	0.2-3.9	3	175
Kolding Fjord, indre	T.28	0.1-1.3	1	10
Vejle Fjord, indre	T.28	0.1-3.9	2	139
Odense Fjord, ydre	T.31	0.1-3.7	4	312
Skive Fjord mm.	T.32	0.2-5.9	4	454
Thisted Bredning	T.34	0.3-6.3	3	380
Hejlsminde Nor	T.35	0.5-1.5	3	8
Haderslev Fjord	T.36	0.3-3.1	3	45
Additional areas:				
Kattegat		3.8-25.0	8	226
Nordlige Kattegat		6.0-20.0	7	139
Skagerrak		7.7-20.9	4	153
Storebælt, nord		4.7-19.4	2	75
Storebælt, syd		9.0-17.2	1	44
Østersøen		5.0-23.5	8	84

2.1 Statistical models for macroalgae indices

The objective of the statistical analyses was to model variations in the three macroalgae indices as functions of location and depth, year, salinity, and cover of sea urchins (set to 0.04 % when missing). The three models are based on the approach in Carstensen & Dahl (2019), where the three macroalgae indices were analysed for stone reefs and coastal habitats.

2.1.1 Model for cumulative cover

Variations in macroalgae cumulative cover with depth are not always well-described using linear models. Macroalgae growth, and consequently macroalgae cover, depends on light availability, which decreases with depth. Similarly, physical exposure from waves can reduce macroalgae cover, but the physical exposure also decreases with depth. Cumulative cover 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).

$$I = I_0 \cdot \exp(-k_d \cdot d) \qquad \text{(Eq. 2.1)}$$

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

$$C_{pot} = C_{max} \cdot \tanh(\frac{I_0}{I_{sat}} \exp(-k_{bio} \cdot d)) \qquad \text{(Eq. 2.2)}$$

where C_{max} describes the maximum cover at irradiance levels sustaining maximum growth, k_{bio} describes the attenuation of macroalgae indices towards deeper waters (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 macroalgae cover potential may not be fully exploited due to physical exposure and grazing by sea urchins.

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

$$f_{exposure} = \frac{1}{1 + k_{exposure} \cdot \mathrm{d}^{-2}} \qquad \text{(Eq. 2.3)}$$

where $f_{exposure}$ is a scaling factor for the depth-specific physical exposure on macroalgae cover (approaching 1 at deeper depths) and $k_{exposure}$ is a parameter describing how fast the physical exposure decreases with depth.

Sea urchins also have an important negative effect on the cumulative cover through grazing, and this effect increases with the abundance of sea urchins. Hence, the grazing effect from sea urchins can also be modelled using Michaelis-Menten kinetics for the cover of sea urchins (log-transformed to describe the attenuating effect of sea urchins with high abundances):

$$f_{grazing} = \frac{1}{1 + k_{seaurchin} \cdot \log(C_{seaurchin} + 0.01)} \qquad \text{(Eq. 2.4)}$$

where $1/k_{seaurchin}$ describes the sea urchin cover (log-transformed), where grazing reduces macroalgae cover by 50 % (i.e. $k_{seaurchin} \cdot \log(C_{seaurchin} + 0.01) = 1$).

Combining the potential macroalgae cover with the limitations imposed by physical exposure and sea urchin grazing yields the following equation for the cumulative macroalgae cover (C):

$$C = C_{max} \cdot \tanh\left(\frac{I_0}{I_{sat}} \exp(-k_{bio} \cdot d)\right) \cdot \frac{1}{1 + k_{exposure} \cdot \mathrm{d}^{-2}} \cdot \frac{1}{1 + k_{seaurchin} \cdot \log(C_{seaurchin} + 0.01)} \qquad \text{(Eq. 2.5)}$$

This model (*Eq. 2.5*) was estimated based on observations of the cumulative cover of macroalgae using non-linear regression with a least squares criterion (PROC MODEL in SAS version 9.3). Since the cover of sea urchins was not reported for the majority of macroalgae monitoring transects (sea urchin cover was reported for 2.5 % of the water body observations, but this does not include zero values, which are not reported), the cover of sea urchins was set to 0.04 % in the absence of this information and the parameter in *Eq. 2.4* was fixed to the value ($k_{seaurchin} = 0.5436$) estimated in Carstensen & Dahl (2019) from stone reefs, where data on sea urchin cover are complete. The value of 0.04 % replacing missing observations of sea urchin cover was determined as a sufficiently low observation, but it is recommended to ensure that sea urchin cover is assessed and reported consistently as part of the monitoring programme (see also Carstensen & Dahl 2019). It should be noted that sea urchins are predominantly marine species and not found in brackish waters. The monitoring data suggest that only water bodies with salinity above 15 are potentially affected by sea urchin grazing.

The two terms for the effect of exposure and sea urchins were assumed generic for all macroalgae data, whereas the parameters C_{max} and k_{bio} were estimated for each water body. Furthermore, to account for potential changes over time, a yearly factor (k_{bio}) was added to k_{bio}, i.e. $k_{bio} = k_{site} + k_{year}$; thus, the attenuation of cumulative cover or number of perennial species varied both spatially and temporally.

It was assumed that the maximum macroalgae cumulative cover would depend on salinity, because the number of macroalgae species increases with salinity with multi-layered structures, resulting in generally higher cumulative cover. Therefore, the parameter estimates for C_{max} were investigated in relation to the average salinity of the water body. Similarly, the attenuation of macroalgae cover with depth (k_{bio}) was assumed to depend on the light attenuation and, therefore, the parameter estimates were examined in relation to the light attenuation coefficient k_d.

2.1.2 Model for perennial species richness

Variations in the number of perennial species were described with a model identical to that for cumulative cover (*Eq. 2.5*), assuming that the perennial species richness is low near the surface where only few species are capable of surviving the strong physical exposure, reaching a plateau at intermediate depths before decreasing towards deeper waters where light becomes an increasingly limiting resource. The grazing effect of sea urchins on the number of perennial species was modelled functionally similar to the effect on cumulative cover, but with a different parameter estimate ($k_{seaurchin}$ = 0.2701) obtained from Carstensen & Dahl (2019), who showed that the presence of sea urchins significantly reduces the number of perennial species. Missing observations of sea urchin cover were replaced with 0.04 % cover.

The two terms for the effect of exposure and sea urchins were assumed generic for all macroalgae data, whereas the parameters C_{max} and k_{bio} were estimated for each water body. In this case, C_{max} represented the maximum number of perennial species and k_{bio} described how the perennial species richness decreased towards deeper waters. Similar to cumulative cover, these estimates were investigated in relation to salinity and light attenuation.

2.1.3 Model for relative cover of opportunists

Variations in the relative cover of opportunists were modelled differently from macroalgae cumulative cover and perennial species richness, because this index showed no uniform depth gradient. This means that for some water bodies, the relative cover of opportunists increased with depth and for other water bodies it decreased with depth. However, for deeper transects there was a clear tendency for the relative cover of opportunists to decline around the position of the halocline, indicating that salinity constitutes an important control for the relative abundance of opportunists. Therefore, for describing the potential effect of changing salinity with depth, a salinity-dependent and site-specific model was proposed, similar to Carstensen & Dahl (2019).

$$P_S = \begin{cases} b_S \cdot (S - S_T) + P_{site} & S < S_T \\ P_{site} & S \geq S_T \end{cases} \qquad \text{(Eq. 2.6)}$$

This model suggests that the relative cover of opportunists reaches a site-specific value (P_{site}) when salinity exceeds the threshold S_T, and that relative cover of opportunists increases as salinity decreases below the threshold (described with the parameter b_S).

In addition, the effect of physical exposure and sea urchin grazing was modelled the same way as for cumulative cover and number of perennial species, although with a different parameter estimate ($k_{seaurchin} = -0.0212$) obtained from Carstensen & Dahl (2019). Furthermore, to account for potential changes over time, a yearly factor (k_{year}) was also included. Thus, the model for the proportion of opportunists (P), using the logit transformation, was (shown prior to taking the log):

$$\frac{P}{1-P} = P_S \cdot \frac{1}{1 + k_{exposure} \cdot \mathrm{d}^{-2}} \cdot \frac{1}{1 + k_{seaurchin} \cdot \log(C_{seaurchin} + 0.01)} \cdot k_{year} \qquad \text{(Eq. 2.7)}$$

The effects of grazing and physical exposure were generic for all macroalgae data as well as the b_S parameter, whereas the P_{site} parameter was estimated for each water body separately. This site-specific parameter, describing the proportion of opportunists at salinities above the threshold S_T, was investigated in relation to salinity and Secchi depth.

2.2 Indicator estimation and status assessment

For assessing the ecological status of macroalgae, there is a need to maximize the sensitivity of the indices by accounting for the natural variation. This can be done on the basis of the developed models. From the description above, the macroalgae models for cumulative cover, number of perennial species and relative cover of opportunists separate variations in the indices into natural and human-induced variations. This implies that the anthropogenic signal is primarily contained in the parameters k_{bio} (cumulative cover and number of perennial species) and P_{site} (relative cover of opportunists). Thus, these parameters constitute macroalgae indicators that are sentinels of human disturbance and applicable for assessing ecological status in relation to the WFD.

The statistical models (*Eq. 2.5* and *2.7*) describe variations in a large data set encompassing many water bodies, which allow for estimation of generic parameters ($k_{exposure}$, $k_{seaurchin}$, k_{year}, $\frac{I_0}{I_{sat}}$, b_S, and S_T), i.e. common to all water bodies. Due to the complexity of the model, these parameters cannot be estimated using data from a single water body alone, and they were estimated from the entire data set (*Table 2.1*). Therefore, these parameters are fixed for estimating the macroalgae indicators for ecological status assessment. However, for a 6-year status assessment it is not necessary to describe the interannual variation within the six years and consequently, the parameter k_{year} should not be included. This implies that the parameters k_{bio} and C_{max} in *Eq. 2.5* and P_{site} in *Eq. 2.7* are estimated using data from a single water body and assessment period, and the three parameter estimates of k_{bio} (two macroalgae indices – cumulative cover and number of perennial species) and P_{site} (relative cover of opportunists) constitute macroalgae indicators that should be evaluated against a set of ecological class boundaries.

2.3 Deriving ecological class boundaries

Reference conditions can be established following the WFD CIS Guidance Document No. 5 (2003), where four principles are laid out using values from:

1) existing undisturbed sites, 2) historical data, 3) models, or 4) expert judgement. Expert judgement should be employed in combination with the other approaches and not alone, unless the three other approaches fail.

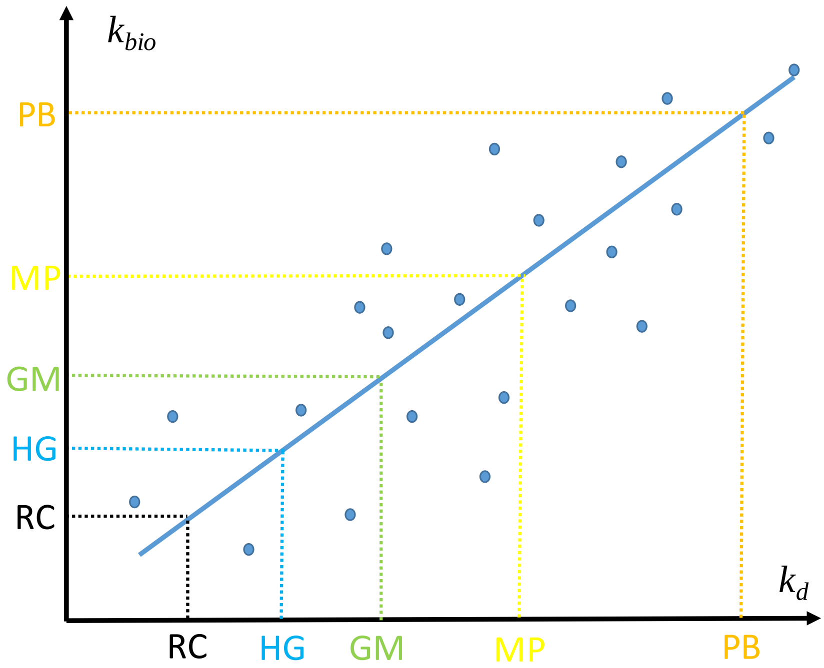
The first principle assumes that there are water bodies belonging to the main types (*Table 2.1*), which are unaffected or having minimal disturbance from human activities. In Danish coastal waters, the main pressure is nutrient enrichment and it is assessed that all coastal water bodies are affected by eutrophication to a varying degree. This assertion is also true for water bodies in Germany and Sweden sharing the same intercalibration types with Denmark. Moreover, the macroalgae monitoring data in these two countries are not strictly comparable to the Danish data, which prevents the use of potential reference values from other countries, if they existed.

The second principle relies on the presence of historical data representing a period with minor disturbance from human activities. Historical macroalgae data from many coastal areas in Denmark are available, dating back to around 1900 (Høgslund et al. 2018). However, these data were mostly of qualitative nature and could not be used for establishing quantitative reference conditions for the macroalgae indicators (Høgslund et al. 2018).

The third principle is typically based on coupled hydrodynamic, biogeochemical and ecological models, although statistical models have also been employed, to describe indicator distribution in different water bodies from a reference scenario of nutrient inputs. At present, however, models that describe the three macroalgae indicators from environmental conditions are not well developed and validated.

Apparently, none of the standard approaches described in the WFD CIS Guidance Document No. 5 (2003) can be employed to establish reference conditions for the three macroalgae indicators. However, revised reference conditions and class boundaries for eelgrass main depth limits have been established for Danish coastal water bodies (ongoing project), which have been converted to light attenuation coefficients (k_d) under the assumption that the eelgrass main depth limit corresponds to 16 % surface irradiance (Timmermann et al. 2020). These k_d reference conditions and class boundaries can be translated into values for k_{bio} for cumulative cover and number of perennial species and P_{site} for the relative cover of opportunists, provided that relationships between these macroalgae model parameters and k_d can be established (*Fig. 2.2*). Reference conditions and class boundaries for k_d for a selection of Danish water bodies were obtained from Timmermann et al. (2020).

Figure 2.2. Principle of translating reference conditions and class boundaries from an existing set (k_d) to the new developed macroalgae indicator (k_{bio}) using an empirical linear relationship. The blue line shows an established empirical relationship between k_d and k_{bio}, and through this relationship established reference conditions (RC) and class boundaries (HG = High-Good, GM = Good-Moderate, MP = Moderate-Poor and PB = Poor-Bad) for k_d are translated into similar values for k_{bio}.



2.4 Combining indicators for status assessment

The ecological status of coastal waters should be assessed from the status of phytoplankton, macroalgae and angiosperms, and benthic fauna, using the one-out-all-out principle (*Fig. 2.3*). For each of these biological quality elements (BQEs), the status should be assessed based on a set of different parameters expressing different aspects of the given BQE. For macroalgae and angiosperms, these parameters include information on composition, abundance and diversity. Finally, at the base of the hierarchical assessment structure are the indicators, which are estimated from monitoring data. Importantly, the confidence of a status classification can also be calculated based on the uncertainty of the indicators used, and uncertainty assessment can be made at all levels of the hierarchy.

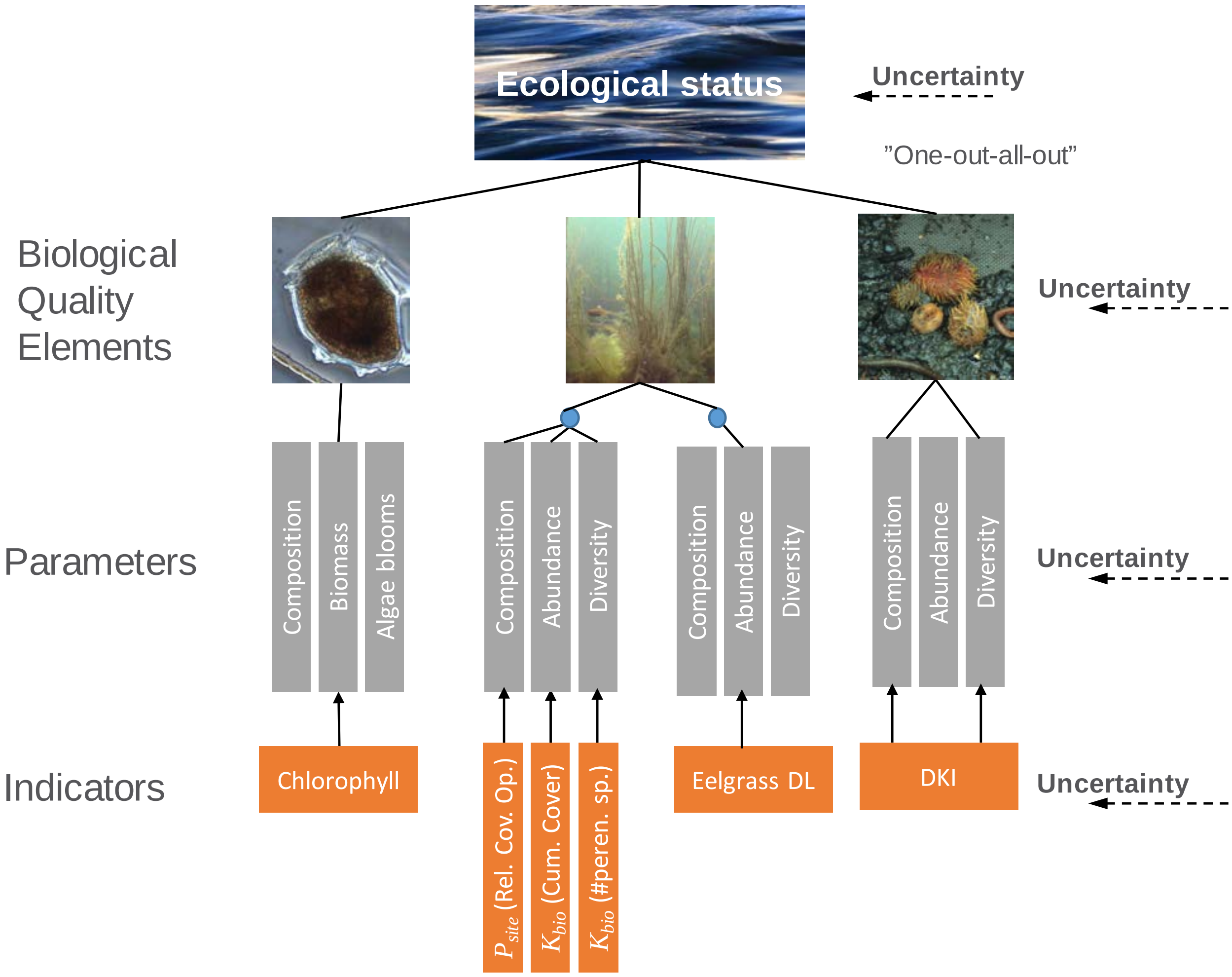


Figure 2.3. Conceptual figure illustrating the nesting of the proposed macroalgae indicators at the base representing different BQE parameters and the combination with existing indicators at the BQE level (from left to right: phytoplankton, macroalgae and angiosperms, benthic fauna) to obtain the final ecological status assessment. Uncertainty derived from estimating the indicators penetrate throughout all levels of the assessment and can be quantified, provided that uncertainties of the indicators are quantified.

The WFD CIS Guidance Document No. 13 (2005) provides directions for aggregating parameters and indicators. Particularly, the guidance document recommends that parameters relevant to assess the effect of particular pressures can be combined to reduce the risk of misclassification and improve confidence in the assessment. However, it is also stated that parameters responding to different pressures should not be combined as this may conceal failures to meet ecological criteria. Aggregation methods are not specified, but the guidelines mention arithmetic averages and weighed averages as examples (WFD CIS Guidance Document No. 13, 2005).

The biological quality element (BQE) 'Macroalgae and angiosperms' includes the sub-elements: macroalgae and angiosperms. At present, the sub-element angiosperms is represented by the eelgrass main depth limit, but it has been proposed to modify this indicator to also include the depth limit of other angiosperm species in order to better cover low-salinity water bodies (Carstensen & Krause-Jensen 2018; Krause-Jensen & Carstensen 2018). However, for illustrating the aggregation principles we will consider eelgrass main depth limit only in this report.

For Danish coastal waters, it is well-established that both eelgrass and macroalgae respond to eutrophication as a main pressure through reduced light conditions. Consequently, these two sub-elements should be aggregated, provided that eutrophication is the only anthropogenic pressure. However, if the pressures acting on macroalgae and angiosperms are different, other aggregation methods, e.g. one-out-all-out, should be employed to maintain the sensitivity of the sub-elements to the different pressures. An ongoing project is aiming to map the pressures and their importance on different components of the marine ecosystem.

In this report, three indicators are tested for macroalgae and these should be combined with the existing indicator for angiosperms, i.e. eelgrass main depth limit. However, in order to give equal weight to the two sub-elements of the BQE, macroalgae indicators should be aggregated before combined with the indicator for angiosperms - eelgrass main depth limit.

The WFD CIS Guidance Document No. 13 (2005) does not give any recommendations to whether arithmetic averages or weighted averages should be used for aggregating indicators and sub-elements, if averaging is used for aggregation, and it does not give any recommendations on how to determine weights in the case of weighted averages. Therefore, unless there are good arguments to assign different weights to sub-elements and indicators, then arithmetic averaging using equal weights should be used as the default for aggregation. However, there will be cases where specific indicators have to be discarded, because they are not representative for the water body. For example, macroalgae indicators may not be relevant for water bodies with no or little hard substrate and in such cases the ecological status assessment should be based on eelgrass depth limit only. Moreover, indicators can also be discarded because their estimation is poor due to ill-conditioned data, e.g. too few data or lack of depth gradient.

The different indicators cannot be directly compared, since they have different scales and ranges. Hence, indicator aggregation is only possible if all indicators are operating on the same assessment scale. For this, all indicators must be normalised to a scale from 0.0 to 1.0, equivalent to the ecological quality ratio (EQR). The indicator scale is transformed to the normalised scale by continuous piecewise linear transformation (*Fig. 2.4*). To ease the subsequent aggregation of indicators, the normalised EQR scale employs equidistant classes, i.e. 0.2 units for each class such that 0.8 is always the boundary between high and good, 0.6 is always the boundary between good and moderate, and so forth. The EQR values of 0.0 and 1.0 correspond to the ultimate range that can be expected in the measured indicator values. Using this transformation to a normalised EQR scale, implies that indicator values can be directly compared and aggregated, since they represent the same "currency". It should also be noted that this transformation is unambiguous (back-transformation is possible), meaning that it is always possible to calculate the indicator values from the EQR scale.

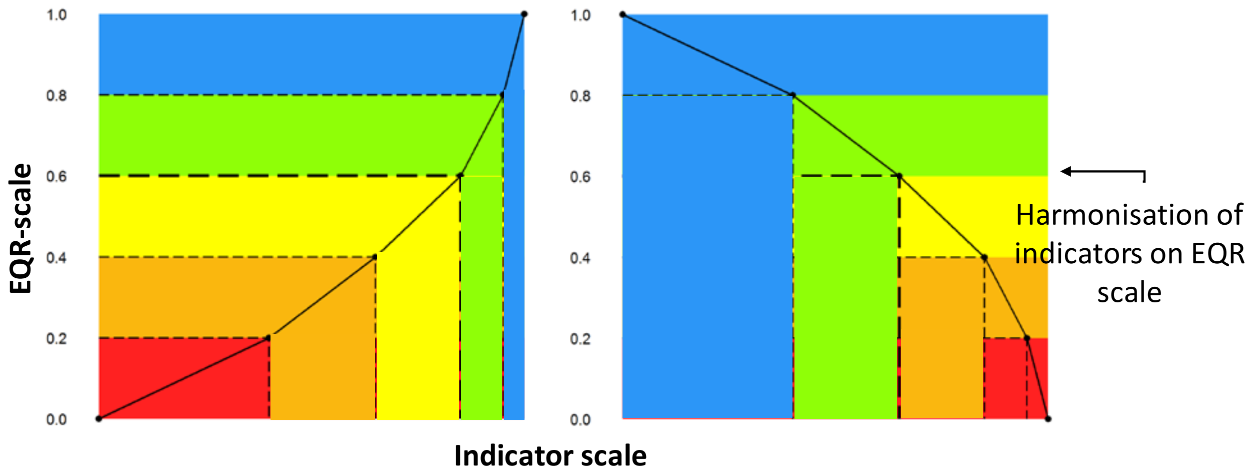


Figure 2.4. The piecewise linear transformation of indicators to an EQR scale with equidistant class boundaries, employed for an indicator with increasing quality (left) and decreasing quality (right). The breakpoints are defined using the boundaries between status classes at the indicator scale.

Importantly, all indicators in the aggregation scheme (*Fig. 2.3*) should be given by their distributions. The distributions of aggregated indicators are determined though Monte Carlo simulations. Monte Carlo simulation is a general approach to assess the resulting distribution of a functional transformation, when the input distributions are known. In practice, the distributions of the estimated indicators, which are approximately normal, are simulated n times (typically, n = 1000), and for each of these n simulations, the result of the aggregation scheme is calculated. After many Monte Carlo simulations, the output distribution can be assessed. Due to the non-linear transformations involved (*Fig. 2.4*), the resulting distributions of aggregated indices can be strongly skewed and twisted. Nevertheless, these irregular distributions still provide exact measures of the probabilities of the different status classes. The status class derived from the distributions depends on the chosen confidence level, which for the face value approach (WFD CIS Guidance Document No. 7, 2003) implies the median. Similarly, percentiles of the distribution corresponding to the benefit-of-doubt and fail-safe approaches can be used for classification.

3 Results

In this section, the performances of the statistical models for cumulative cover and perennial species richness are presented together, because the models are similar and the only major difference is the parameter estimates used to describe changes in these two macroalgae indicators with depth. Next, the results from the statistical model for the relative cover of opportunistic species are presented.

After presenting the overall performance of the statistical models, parameter estimates describing the water body-specific behaviour of the models are related to the environmental conditions. Finally, these regressions are used to propose reference and boundary values for the macroalgae indicators.

3.1 Cumulative cover and perennial species richness

The model for cumulative cover explained 59 % of the observed total variation, although considerable variation (relative standard deviation: ±76 % of individual observations) around the regression lines remained (*Figs. 3.1-3.3*), highlighting the inherent variability in the data. Similarly, the model for perennial species richness explained 64 % of the observed variation with residual variation of individual observation varying by ±44 % (relative standard deviation).

Overall, the regression model described well the three phases: 1) reduced cumulative cover and number of species at shallow depths due to physical exposure, 2) the plateau of maximum cover and richness at intermediate depths where neither physical exposure nor light limitation was important, and 3) the gradual reduction at deeper depths with light becoming limiting for cumulative cover and number of perennial species.

However, for several water bodies, it was not possible to estimate the attenuating phase for cumulative cover and/or perennial species richness (k_{bio} parameter), because there were too few observations at depths where light conditions affected these macroalgae indicators. In such cases, an indicator value could not be computed. Furthermore, it should be stressed that there were large differences in the number of observations available to estimate the two site-specific parameters (k_{bio} and C_{max}).

3.1.1 Estuarine types

The cumulative cover varied broadly across water bodies and with depth, displaying an exponential decline towards deeper depth due to light limitation (*Fig. 3.1*). The cumulative cover typically peaked at depths around 2-3 m, which appeared to be well captured by the regression model (*Eq. 2.5*). Importantly, the peak cumulative cover varied substantially with lower values in brackish areas such as Roskilde Fjord indre and higher values in more saline water with the exception of water bodies belonging to Limfjorden (Løgstør Bredning, Kås Bredning and Venø Bugt, Nissum Bredning, Skive Fjord, and Thisted Bredning). However, not all water bodies had sufficient deep data to represent the entire depth gradient from peak cover to disappearance. Actually, only a few water bodies had cumulative cover observations approaching zero with depth. The two parameters, k_{bio} and C_{max}, could be estimated for 16 out of 20 water bodies.

The number of perennial species varied markedly among water bodies with the highest species richness found in Horsens Fjord and Nissum Bredning and the lowest species richness found in Hejlsminde Nor (*Fig. 3.1*). Similar to cumulative cover, the perennial species richness peaked around 2-3 m and then decreased exponentially with depth. The two parameters, k_{bio} and C_{max}, could be estimated for 14 out of 20 water bodies.

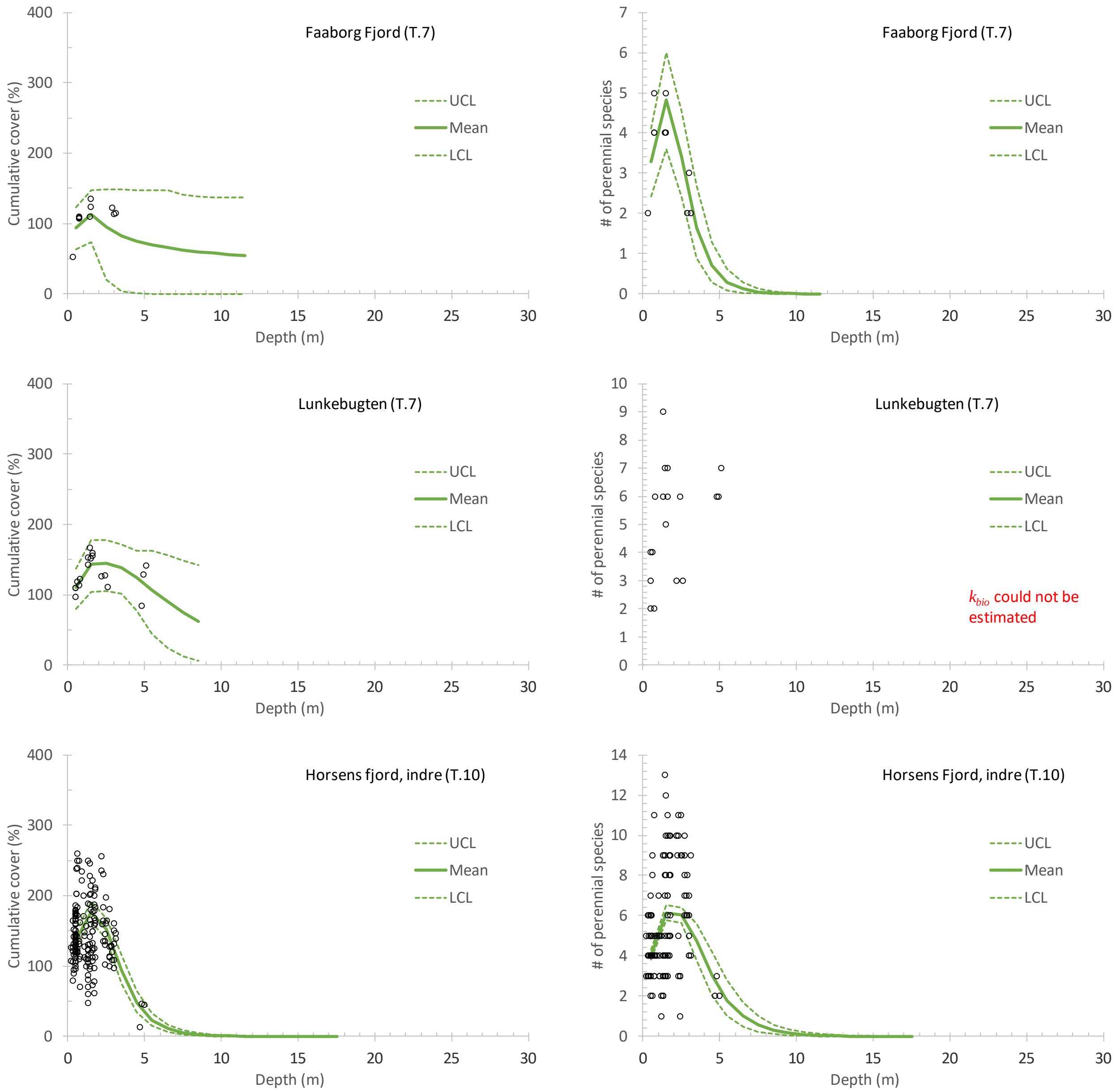


Figure 3.1 continues on next page.

Figure 3.1 continued.

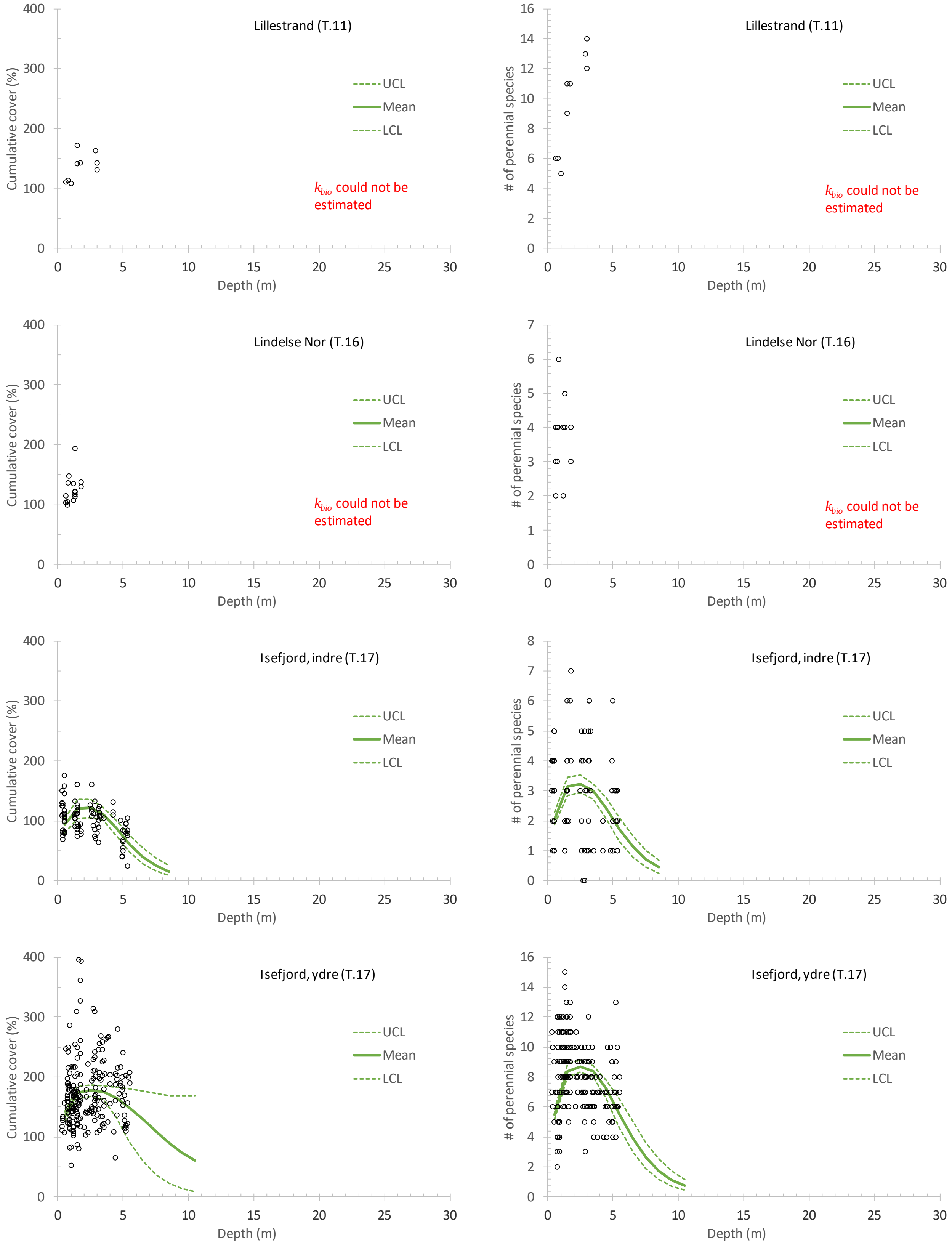


Figure 3.1 continues on next page.

Figure 3.1 continued.

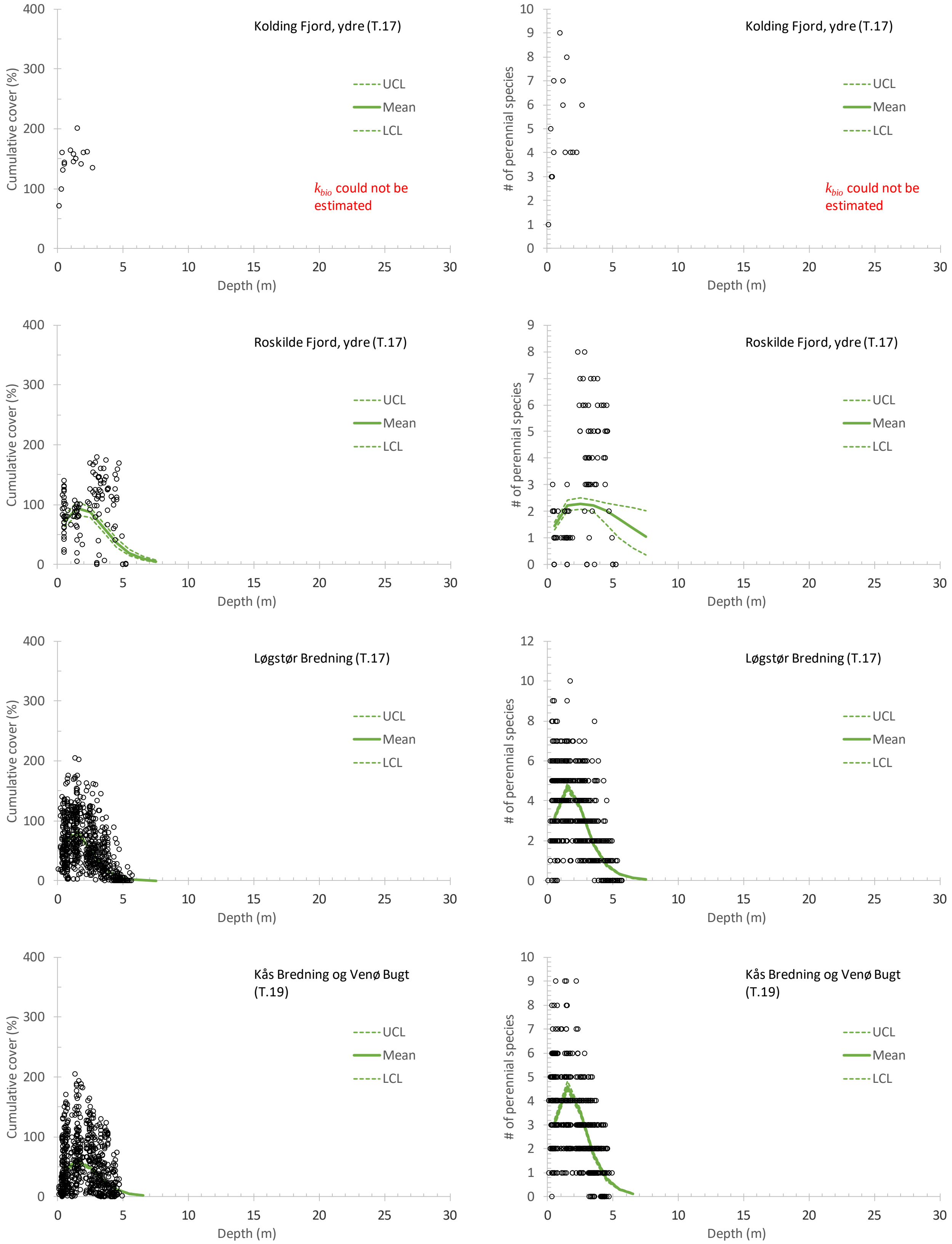


Figure 3.1 continues on next page.

Figure 3.1 continued.

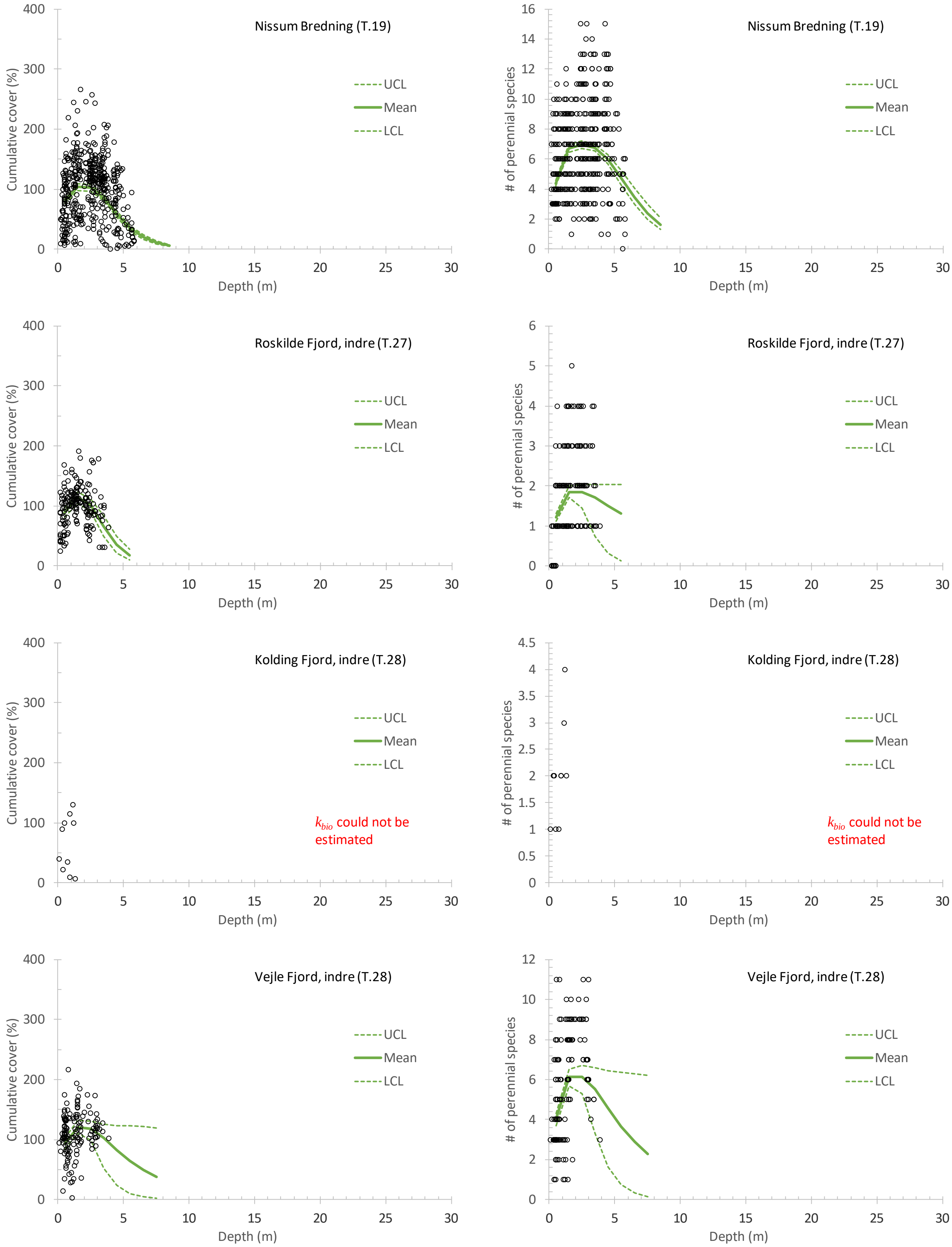


Figure 3.1 continues on next page.

Figure 3.1 continued.

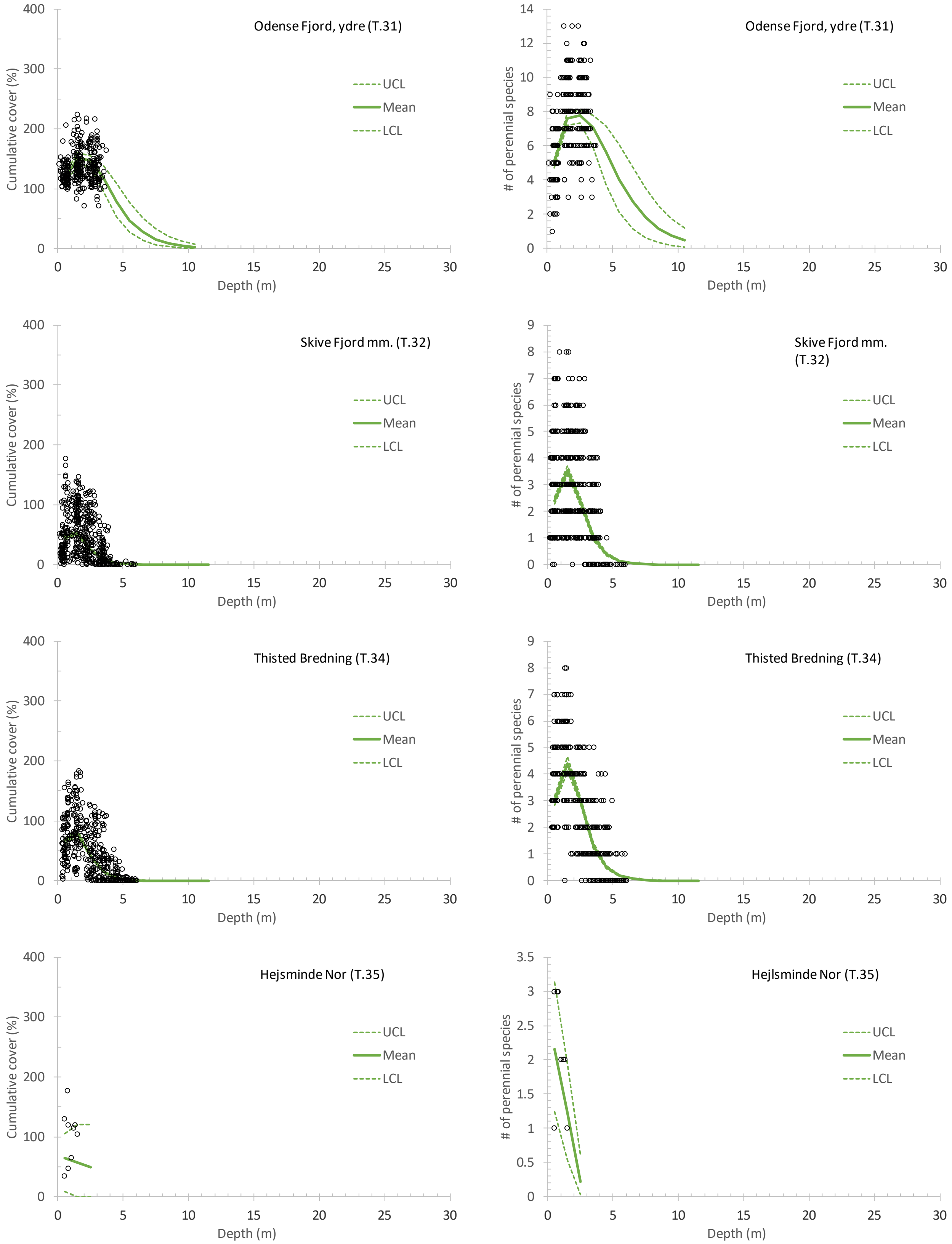


Figure 3.1 continues on next page.

Figure 3.1 continued.

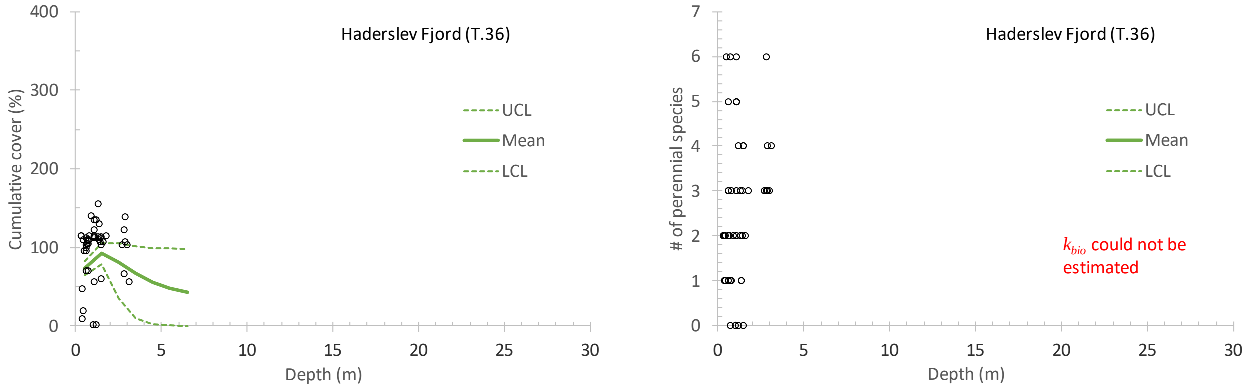


Figure 3.1. Observed cumulative cover (left panel) and perennial species richness (right panel) versus depth for the 20 investigated water bodies belonging to estuarine types (Kystvandstype = Fjord(Fj)). The water bodies are shown with ascending type number. For each water body the estimated depth relationship from the non-linear model (*Eq. 2.5*) without sea urchins ($cover_{seaurchin}$ = 0) is shown (solid green line) with the 95 % confidence interval of the model (dashed lines). The estimated model and confidence interval represent the geometric mean, corresponding to the median distribution. Note that the depth relationship is predicted for the depth of the water column at the corresponding hydrochemistry station, i.e. for a few water bodies the model predictions do not extend to the deepest depths with macroalgae data.

3.1.2 Coastal types

The cumulative cover for the coastal water bodies also expressed the three phases from lower values at shallow depth, a maximum at intermediate depths and an exponential decrease at deeper depths (*Fig. 3.2*). However, there was considerable variation among water bodies in the maximum cumulative cover attained, typically ranging from 100 % to 200 %, and the decrease in cumulative cover at deeper depths. For some coastal water bodies, macroalgae disappeared at depths around 10 m, whereas macroalgae would grow deeper than 20 m for other coastal water bodies. In fact, macroalgae in many water bodies did not enter the light-regulated phase at shallow depths than 10 m. The two parameters, k_{bio} and C_{max}, could be estimated for 30 out of 37 water bodies.

The number of perennial species varied markedly among water bodies with the highest species richness found in Kattegat, Læsø and Nordlige Kattegat, Ålbæk Bugt, and the lowest species richness was found in Østersøen, Christiansø (*Fig. 3.2*). Similar to cumulative cover, the perennial species richness peaked somewhere between 3 and 10 m, followed by an exponential decline at deeper depths. The two parameters, k_{bio} and C_{max}, could be estimated for 24 out of 37 water bodies.

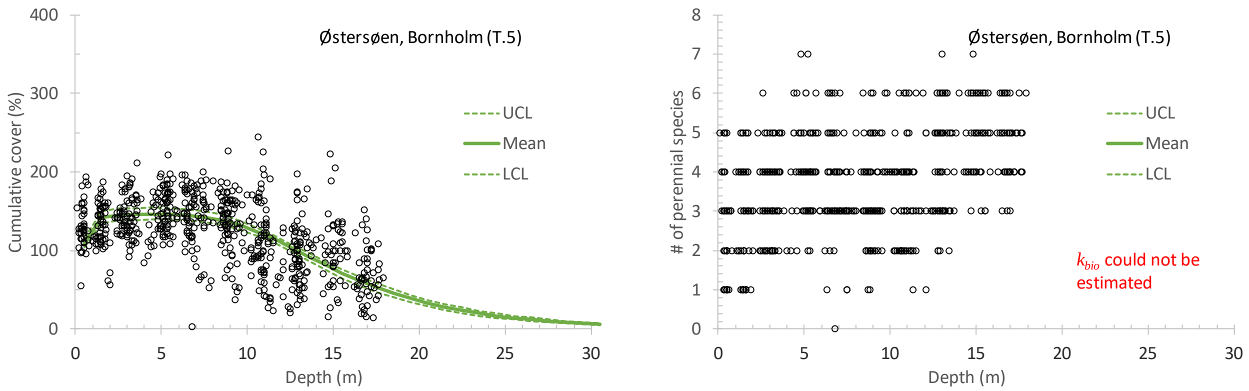


Figure 3.2 continues on next page.

Figure 3.2 continued.

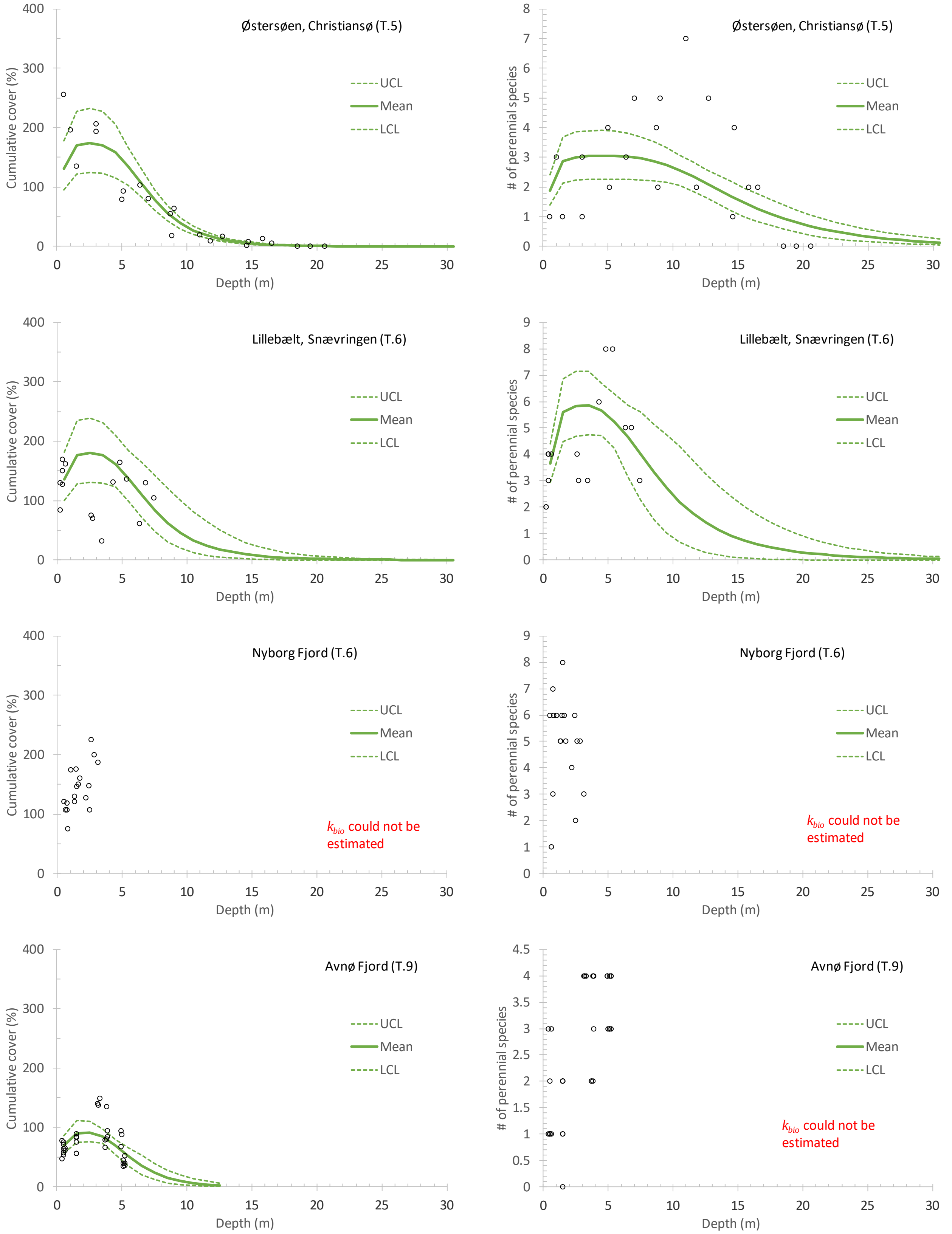


Figure 3.2 continues on next page.

Figure 3.2 continued.

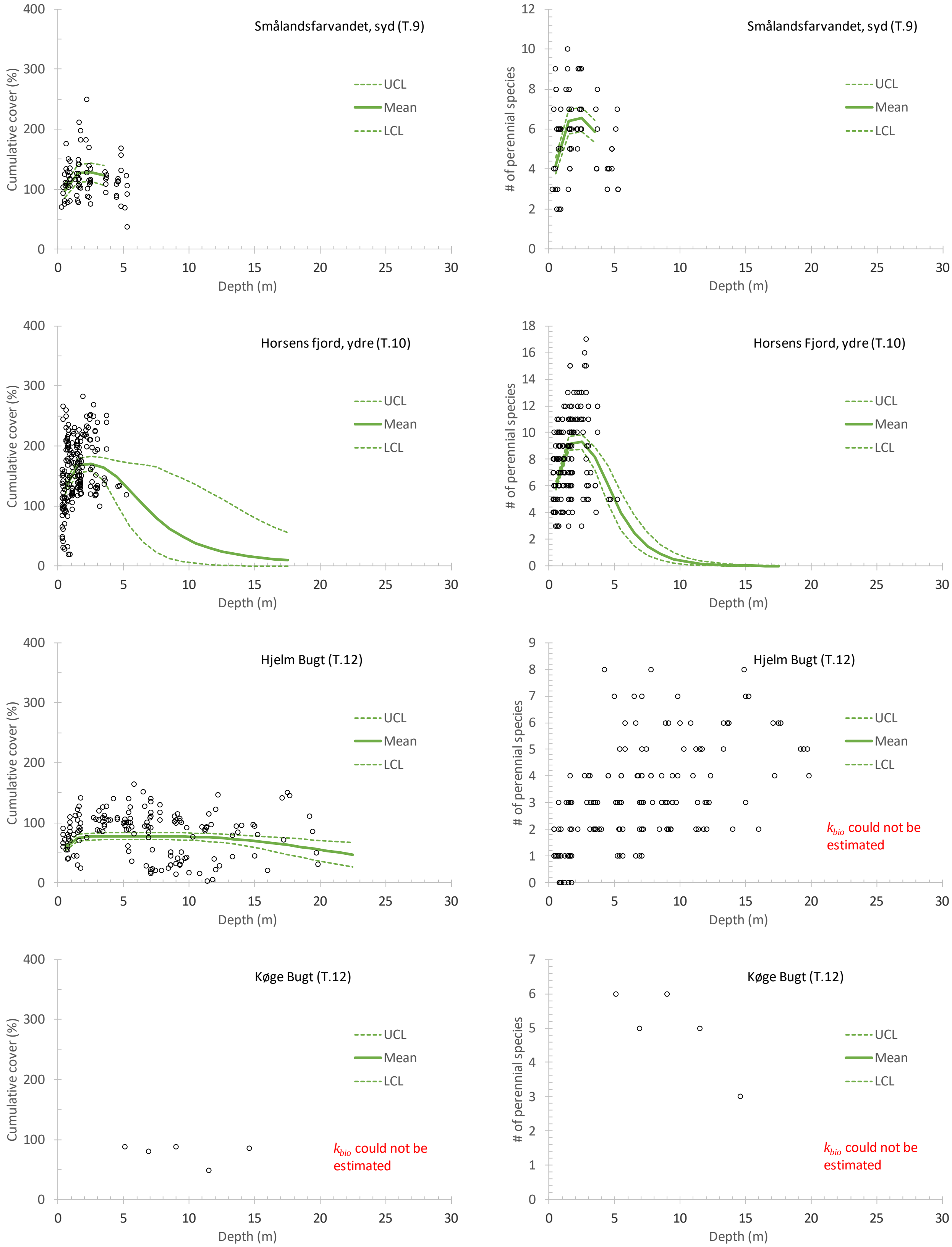


Figure 3.2 continues on next page.

Figure 3.2 continued.

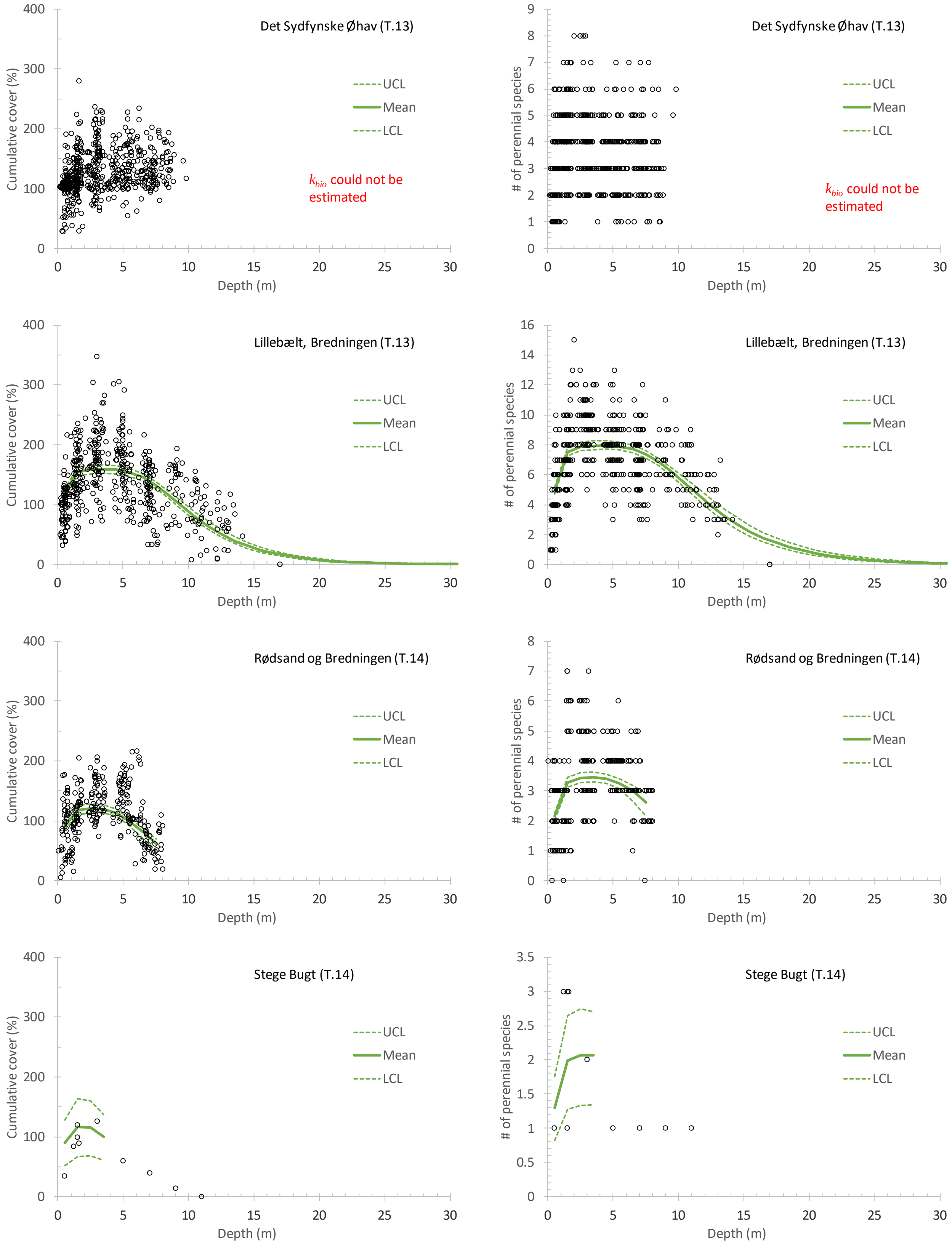


Figure 3.2 continues on next page.

Figure 3.2 continued.

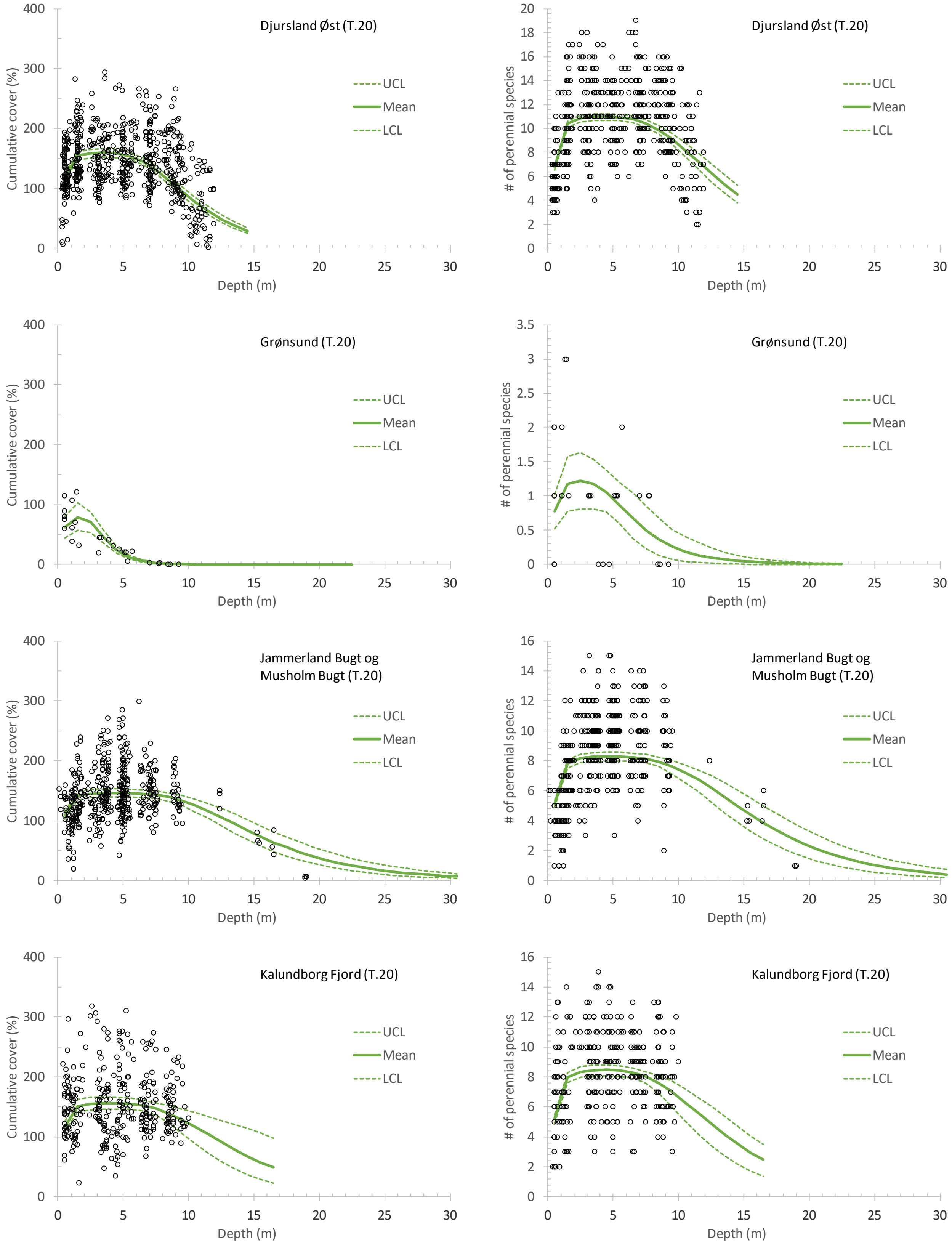


Figure 3.2 continues on next page.

Figure 3.2 continued.

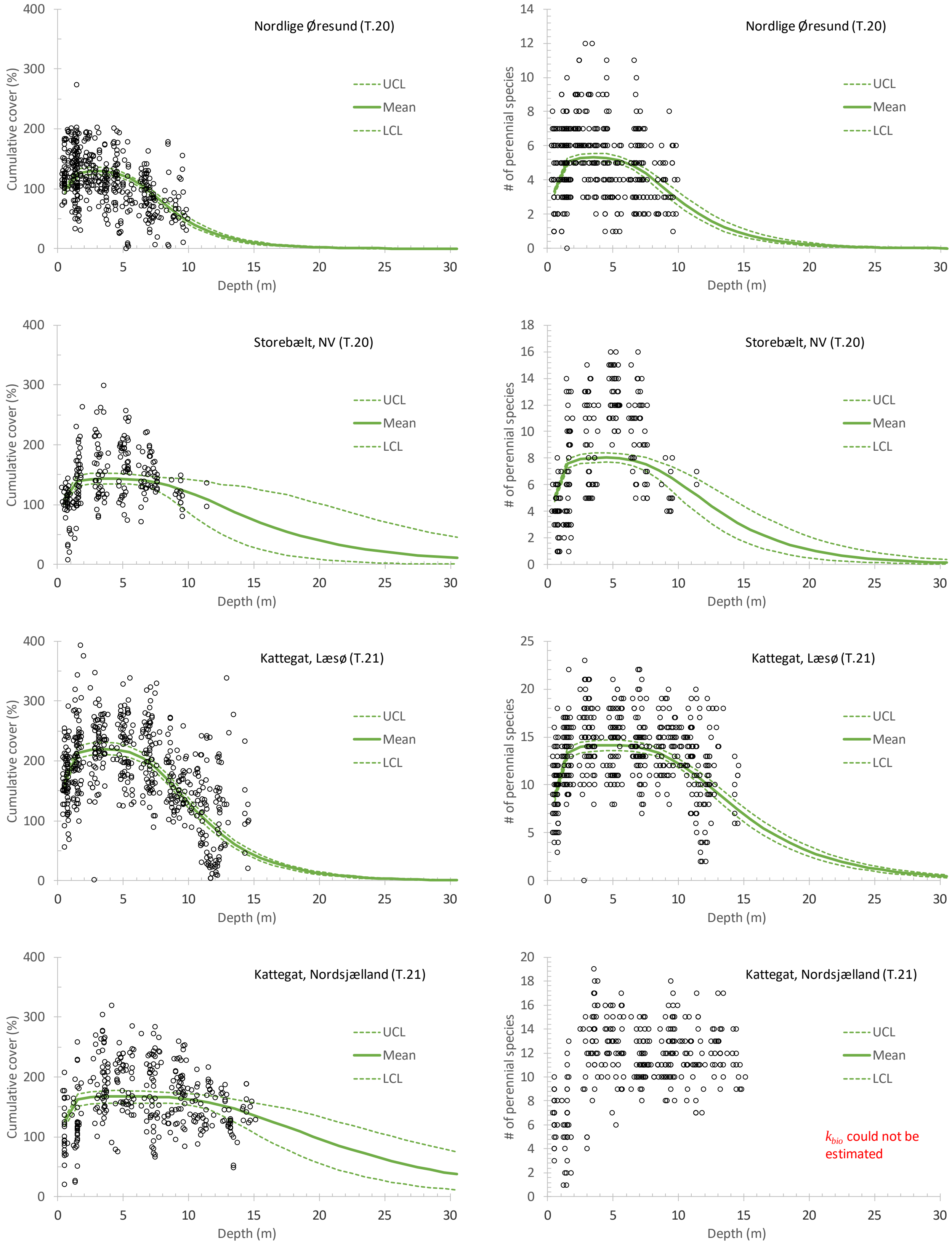


Figure 3.2 continues on next page.

Figure 3.2 continued.

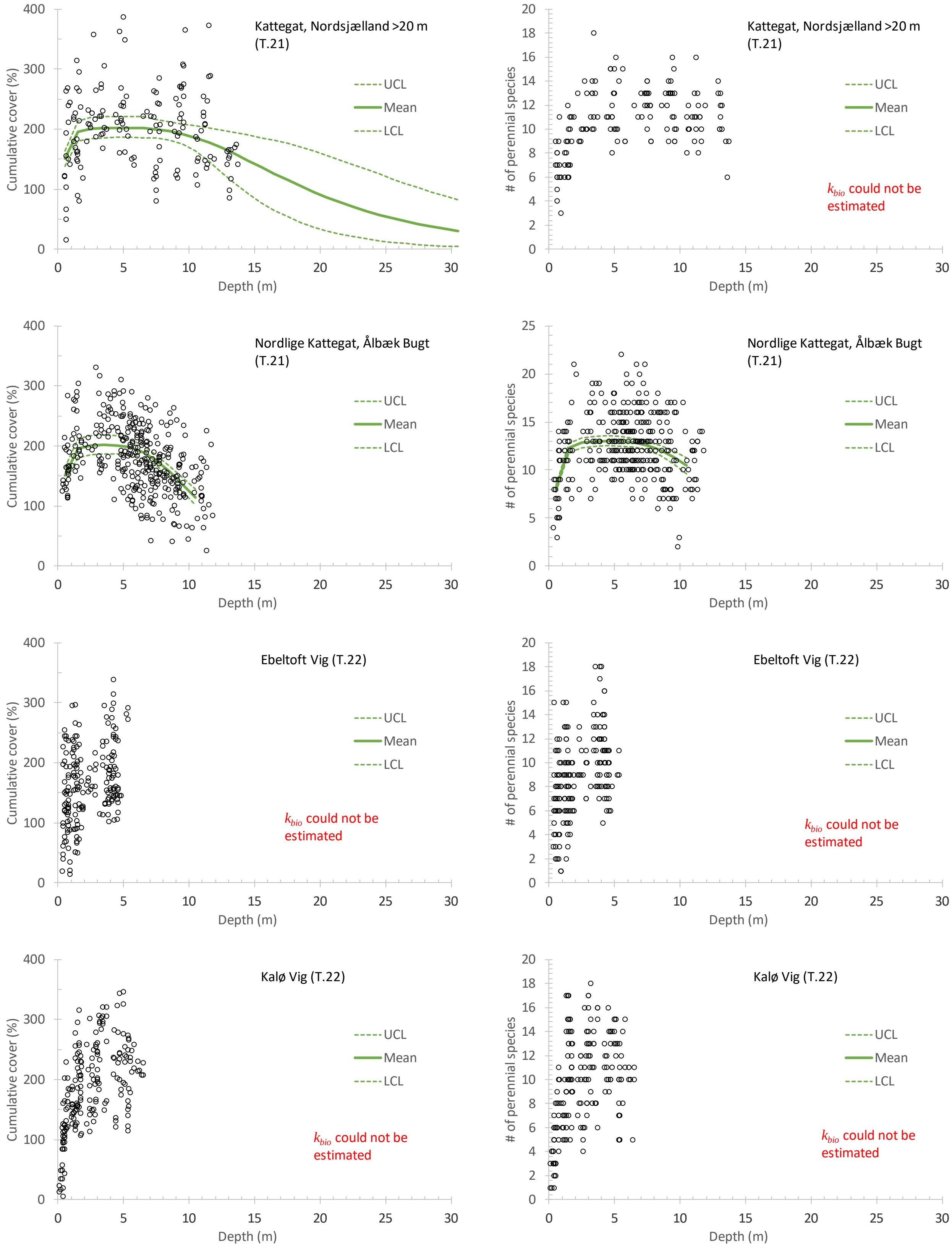


Figure 3.2 continues on next page.

Figure 3.2 continued.

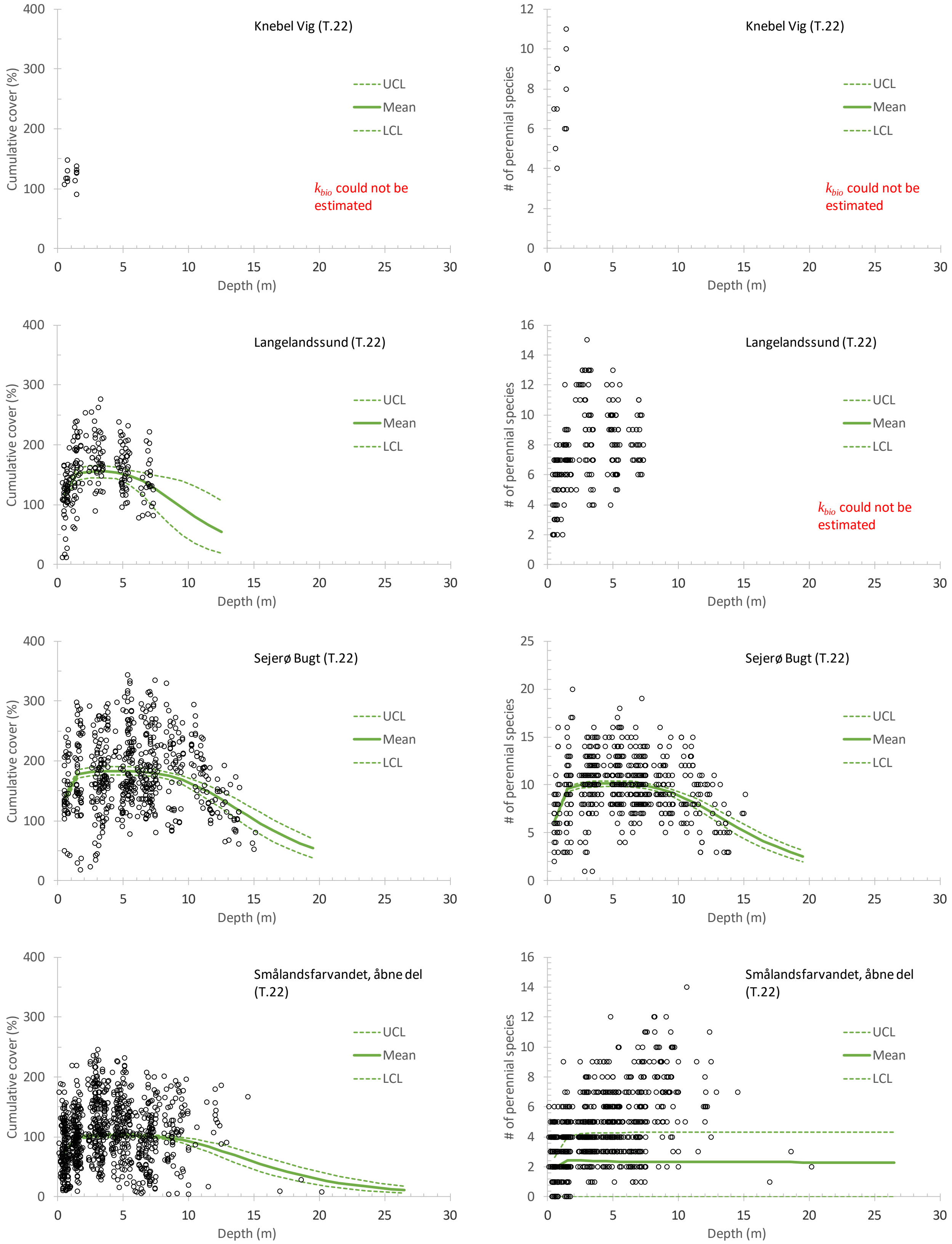


Figure 3.2 continues on next page.

Figure 3.2 continued.

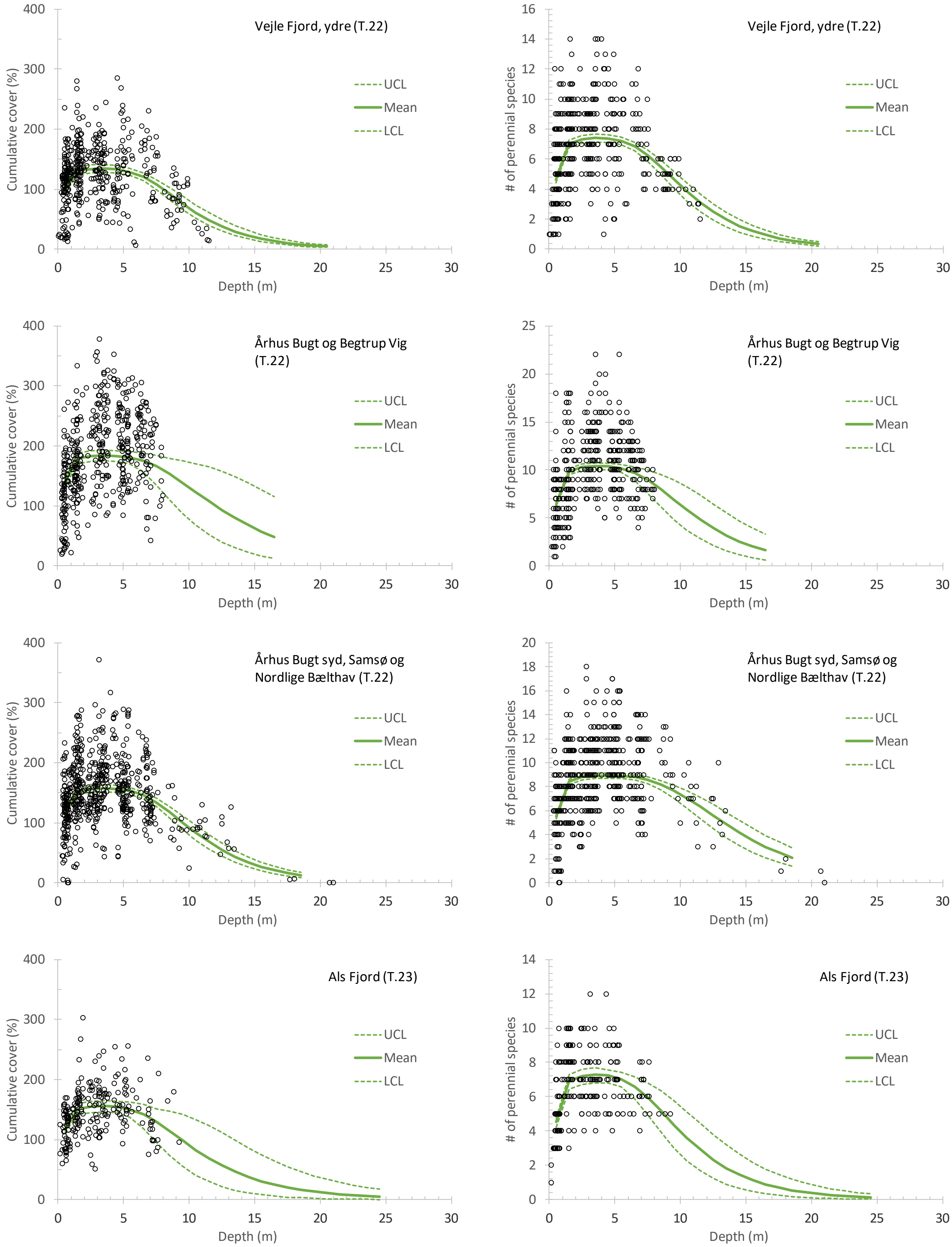


Figure 3.2 continues on next page.

Figure 3.2 continued.

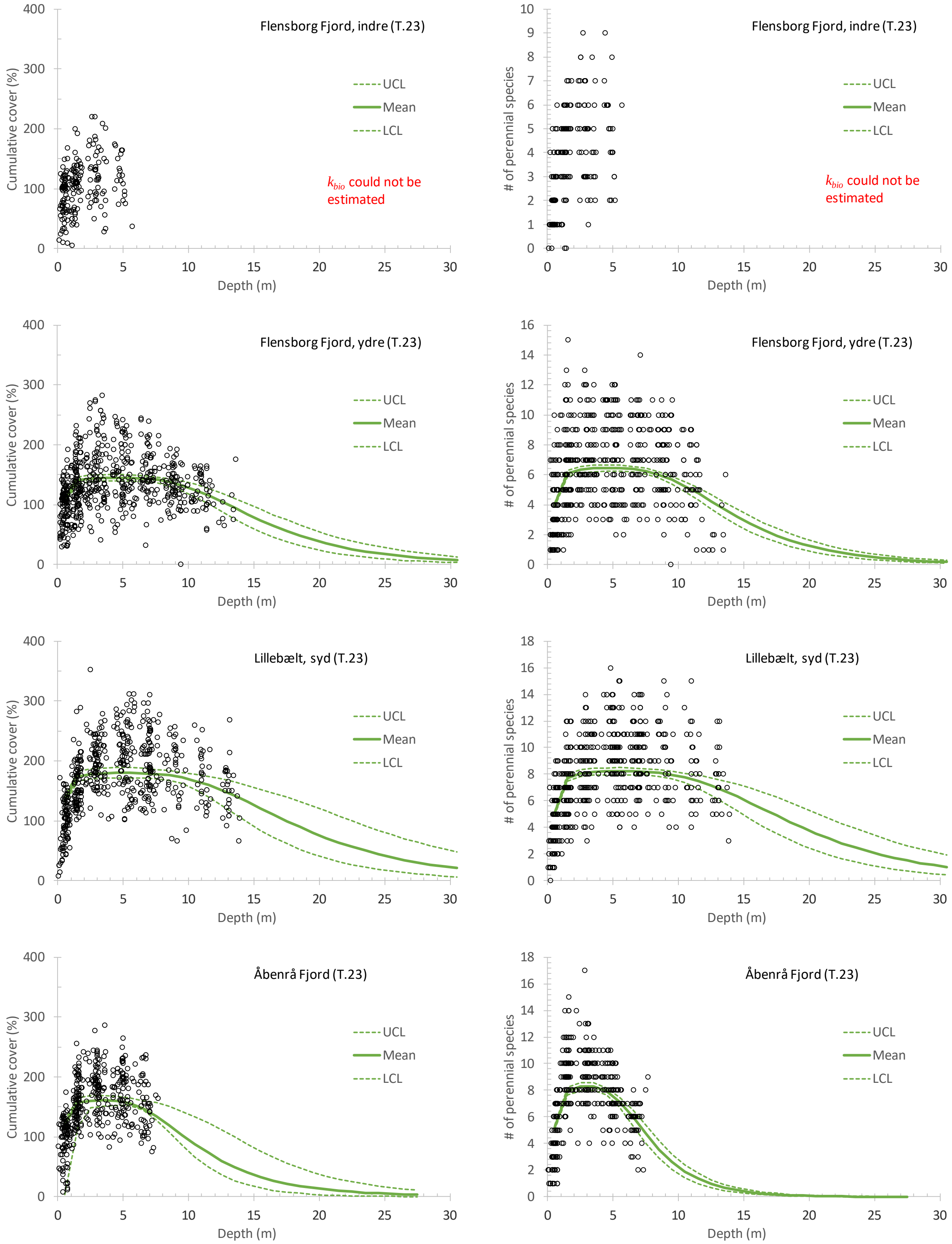


Figure 3.2. Observed cumulative cover (left panel) and perennial species richness (right panel) versus depth for the 37 investigated water bodies in the coastal types (kystvandstype = Bælthav, Kattegat, Østersø). The water bodies are shown with ascending type number. For each water body, the estimated depth relationship from the non-linear model (*Eq. 2.5*) without sea urchins ($cover_{seaurchin} = 0$) is shown (solid green line) with the 95 % confidence interval of the model (dashed lines). The estimated model and confidence interval represent the geometric mean, corresponding to the median distribution. Note that the depth relationship is predicted for the depth of the water column at the corresponding hydrochemistry station, i.e. for a few water bodies the model predictions do not extend to the deepest depths with macroalgae data.

3.1.3 Additional open-water areas

The selected additional areas represent a distinctive open-water gradient from the brackish Baltic Sea towards the saline Skagerrak. Data from these areas are from stone reefs and therefore have almost no data from depths shallower than 5 m. All areas displayed an almost constant level at intermediate depths for cumulative cover and perennial species richness, followed by a decline at depths > 10 m, with the exception of Skagerrak where the decline began at 5 m according to the model predictions (*Fig. 3.3*). For the Skagerrak area, the model implies that cumulative cover round 200-350 % would have been observed, if there would have been monitoring data at 3-4 m depth.

It is noteworthy, that cumulative cover and perennial species richness in Østersøen were generally low, and that the decline with depth was not as well defined as for the other areas. Cumulative cover and species richness were highest in Kattegat with expected means of 200 % and 10 for the two indicators, respectively.

Overall, the regression model performed well in mimicking observational data, although there was considerable scatter around the regression lines.

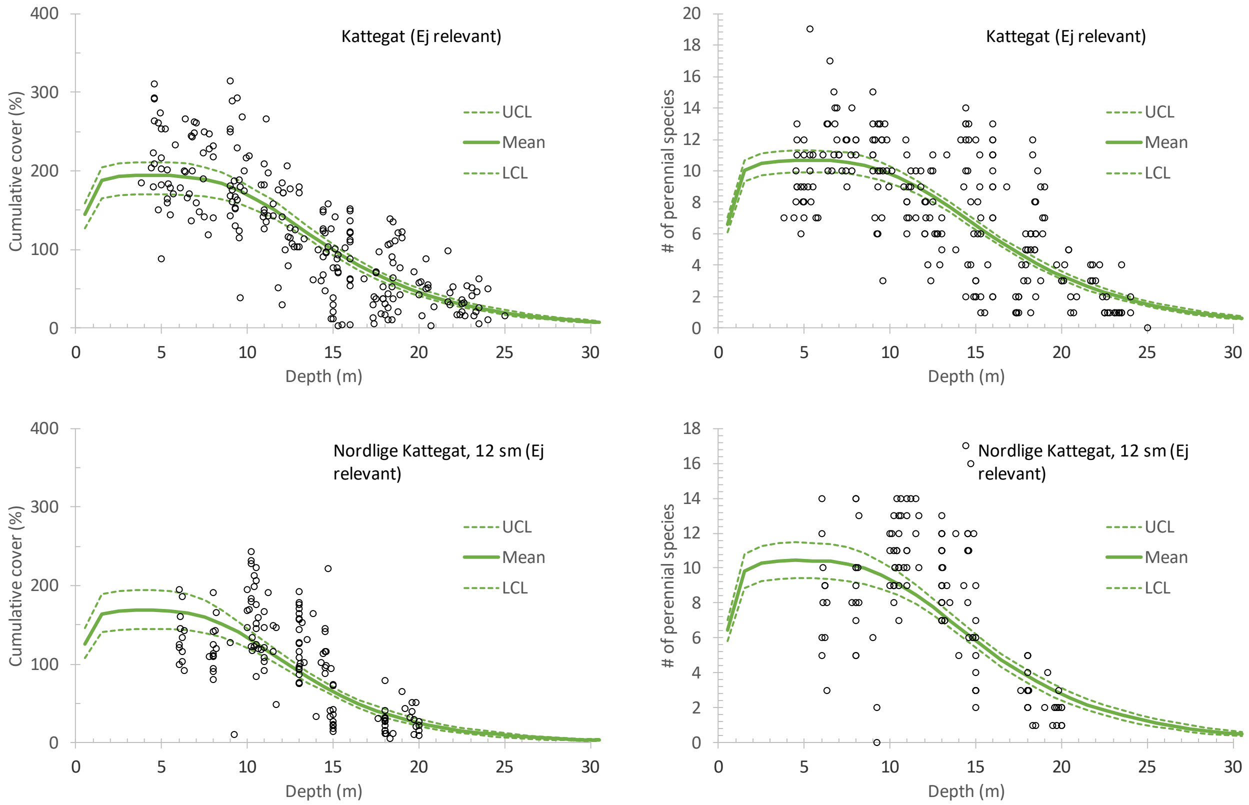


Figure 3.3 continues on next page.

Figure 3.3 continued.

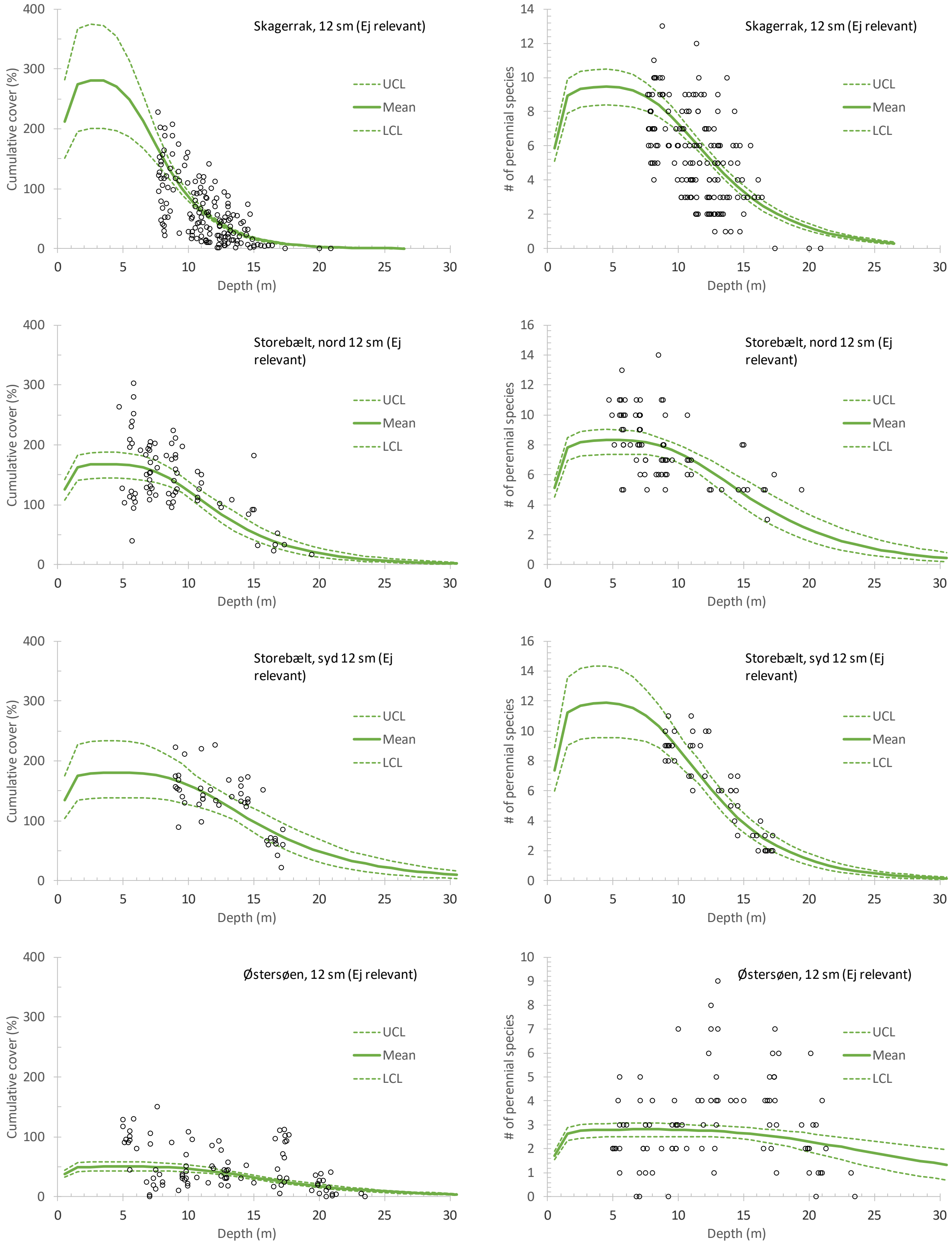


Figure 3.3. Observed cumulative cover (left panel) and perennial species richness (right panel) versus depth for the 6 additional areas outside the WFD baseline (these water bodies are denoted ‘Ej relevant’ in the Danish implementation of the WFD). For each water body the estimated depth relationship from the non-linear model (*Eq. 2.5*) without sea urchins ($cover_{seaurchin} = 0$) is shown (green solid line) with the 95 % confidence interval of the model (dashed lines). The estimated model and confidence interval represent the geometric mean, corresponding to the median distribution. Note that the depth relationship is predicted for the depth of the water column at the corresponding hydrochemistry station, i.e. for a few water bodies the model predictions do not extend to the deepest depths with macroalgae data.

3.2 Relative cover of opportunists

The model for relative cover of opportunists explained 37 % of the observed variation, although considerable variation around the regression lines remained (*Figs. 3.4-3.6*), highlighting the inherent variability in the data. In fact, at some water bodies the relative cover of opportunists could vary from 0 % to 100 % at similar depths. Since the model for the relative cover of opportunists did not include a depth gradient, the parameters could be estimated for all water bodies (*Table 2.1*). However, it should be stressed that there were large differences in the number of observations available for estimating the regression model (*Eq. 2.7*) among water bodies.

3.2.1 Estuarine types

The relative cover of opportunists varied broadly among the estuarine water bodies (*Fig. 3.4*), from almost complete dominance of opportunists in Roskilde Fjord and Stege Bugt to low relative cover in Faaborg Fjord, Hejlsminde Nor, Lindelse Nor, and Lunkebugten. Some sites exhibited decreasing relative cover of opportunists with depth, but this tendency was not general across all sites. Differences in depth gradients could only partially be explained by changing salinity with depth in the model (*Eq. 2.7*).

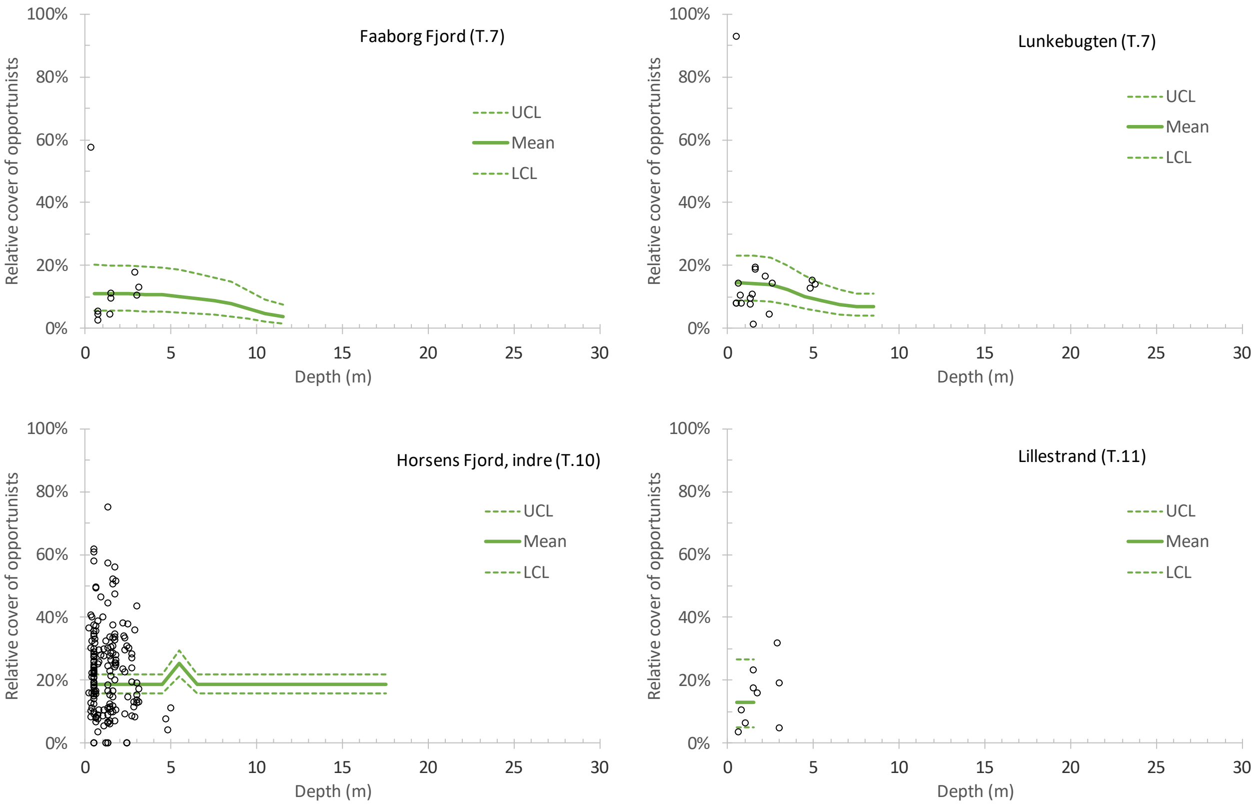


Figure 3.4 continues on next page.

Figure 3.4 continued.

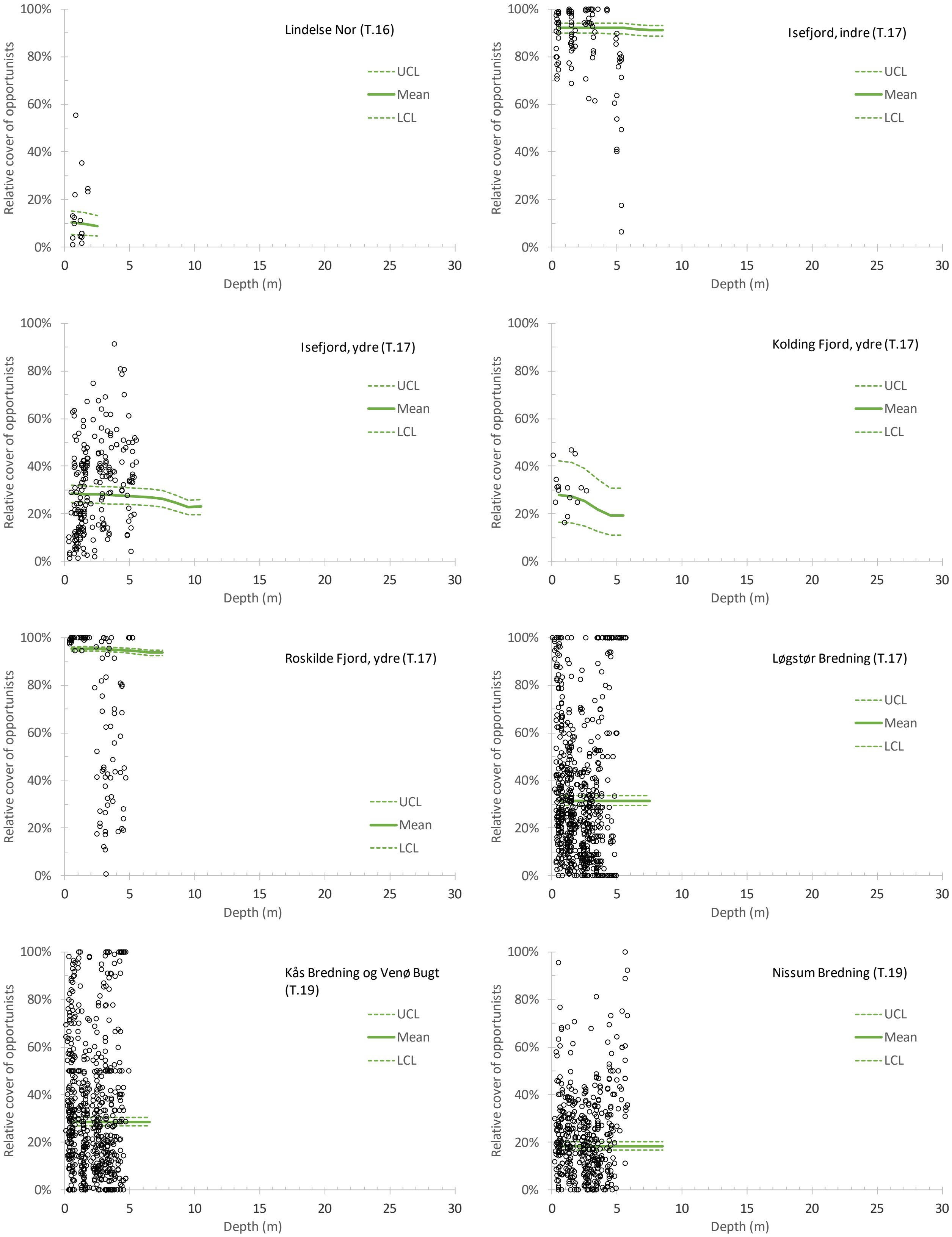


Figure 3.4 continues on next page.

Figure 3.4 continued.

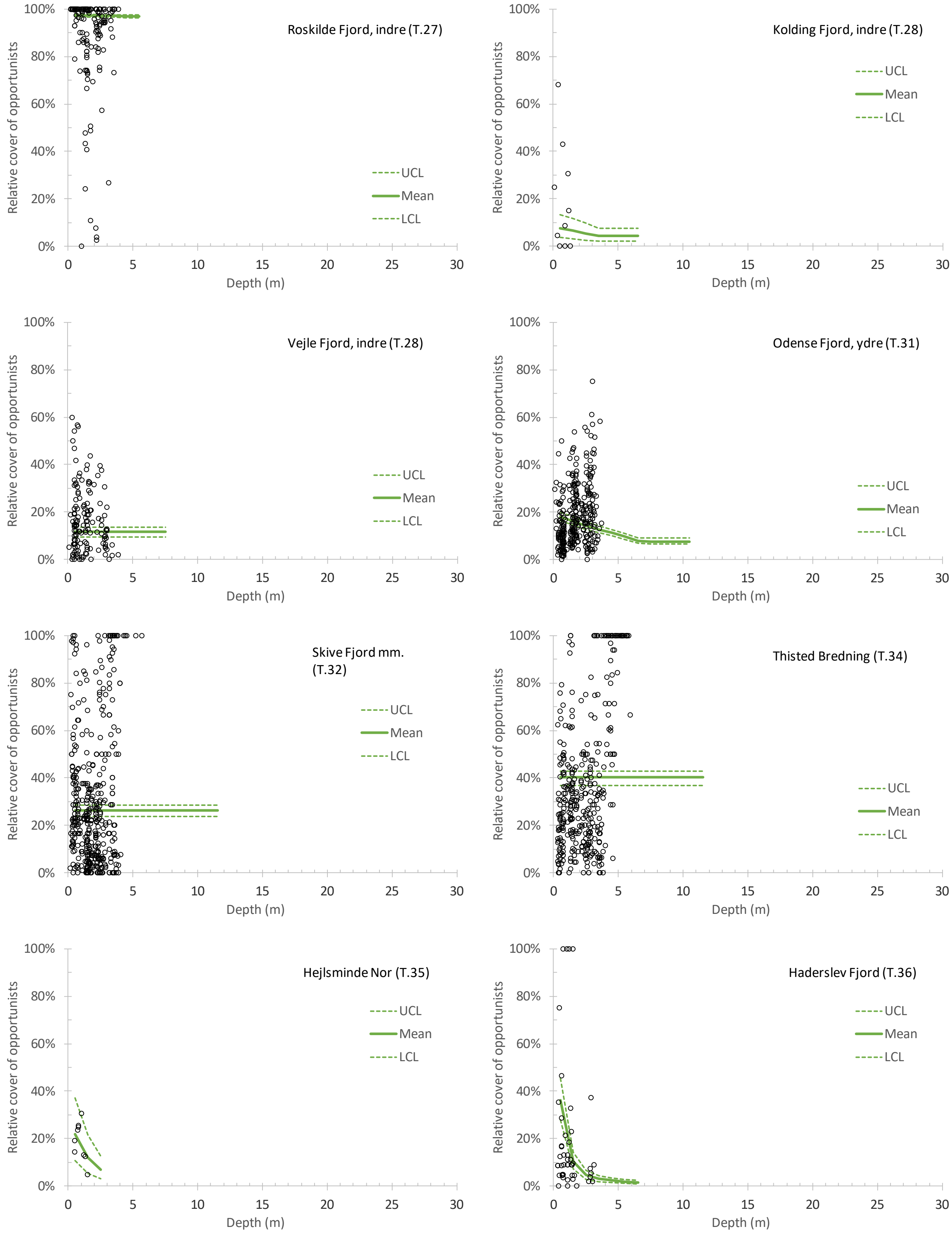


Figure 3.4. Observed relative cover of opportunists versus depth for the 20 investigated water bodies belonging to estuarine types (Kystvandstype = Fjord(Fj)). The water bodies are shown with ascending type number. For each water body the estimated depth (i.e. salinity) relationship from the non-linear model (*Eq. 2.7*) without sea urchins ($cover_{seaurchin} = 0$) is shown (green solid line) with the 95 % confidence interval of the model (dashed lines). The estimated model and confidence interval represent the geometric mean, corresponding to the median distribution. Note that the depth relationship is predicted for the depth of the water column at the corresponding hydrochemistry station, i.e. for a few water bodies the model predictions do not extend to the deepest depths with macroalgae data.

3.2.2 Coastal types

The coastal water bodies also displayed broad ranges of variation among all sites (*Fig. 3.5*). A few sites had pronounced depth gradients with maximum values at intermediate depths (e.g. Lillebælt, Bredningen and Kattegat, Læsø), but this pattern was not found consistently across all sites. The relative cover of opportunists decreased with depth in water bodies with deeper macroalgae populations, mainly due to increasing salinity (*Eq. 2.7*). Such gradients were apparent in Als Fjord, Djursland Øst, Flensborg Fjord ydre, Jammerland Bugt og Musholm Bugt, Kalundborg Fjord, Kattegat, Nordsjælland, Lillebælt, Bredningen, Lillebælt, syd, Sejerø Bugt, Smålandsfarvandet, åbne del and Århus Bugt syd, Samsø og Nordlige Bælthav. Nevertheless, the variability in this macroalgae indicator within water bodies was indeed large, ranging over the entire span from 0 to 100 % for most of the water bodies with many observations.

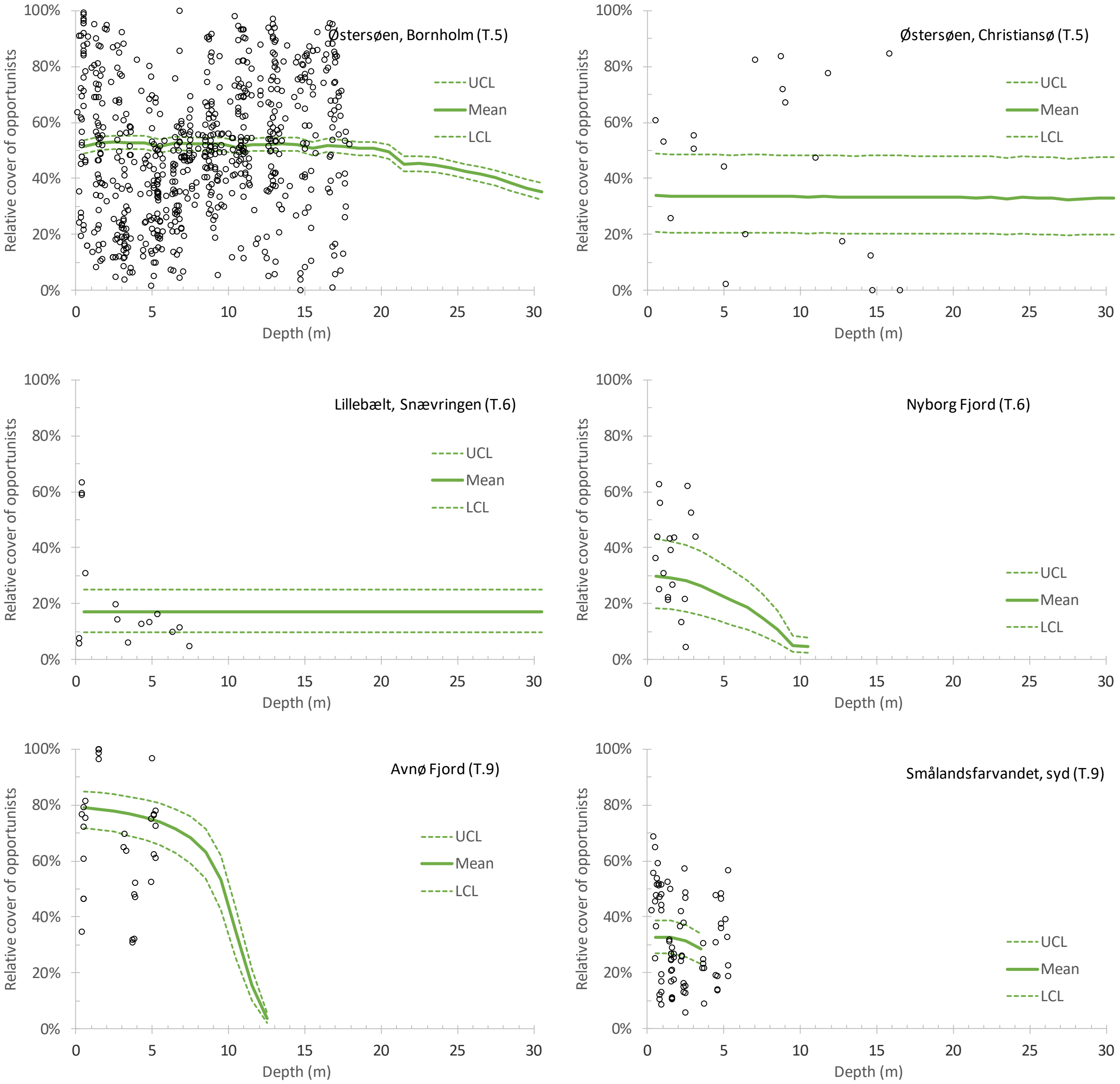


Figure 3.5 continues on next page.

Figure 3.5 continued.

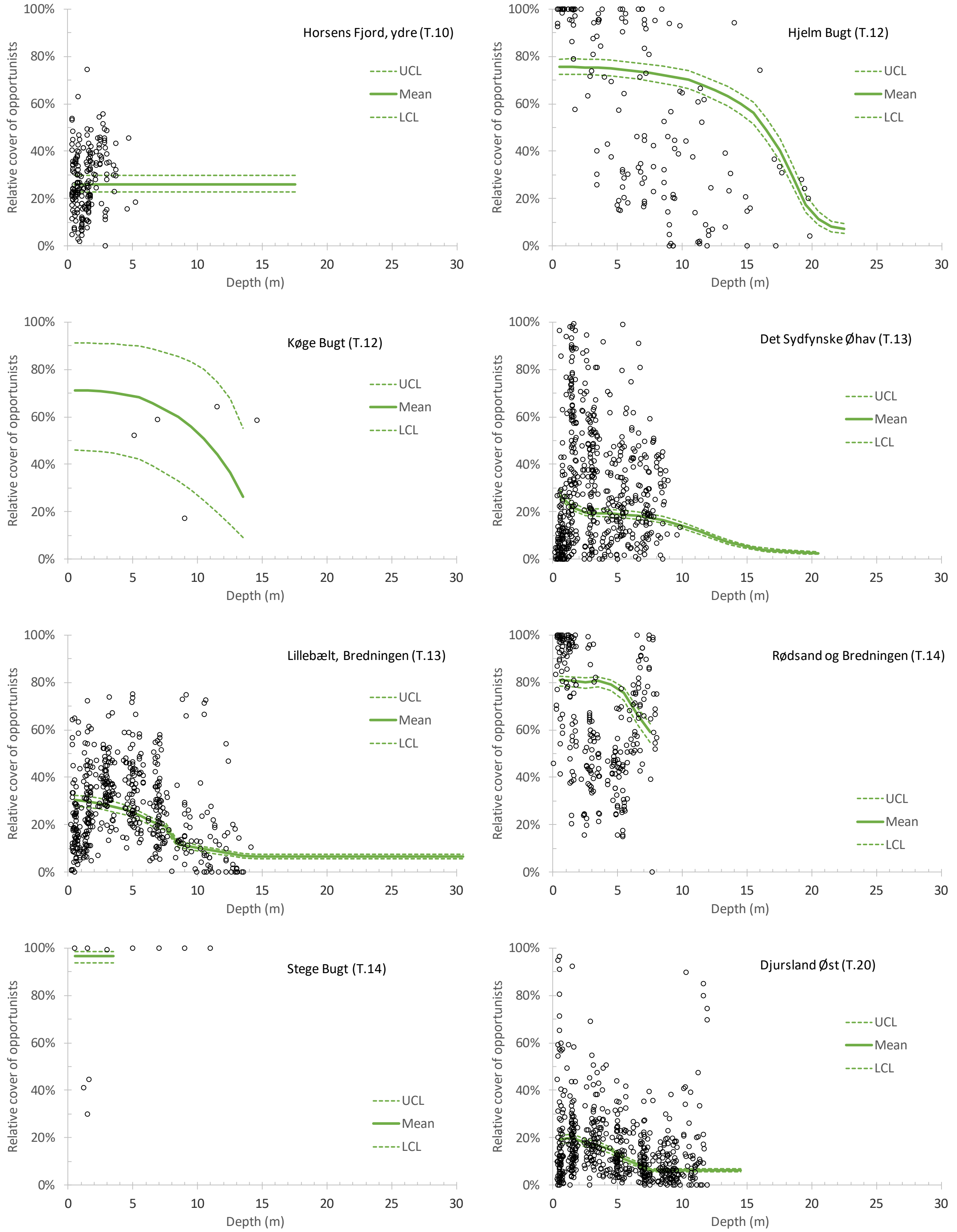


Figure 3.5 continues on next page.

Figure 3.5 continues on next page.

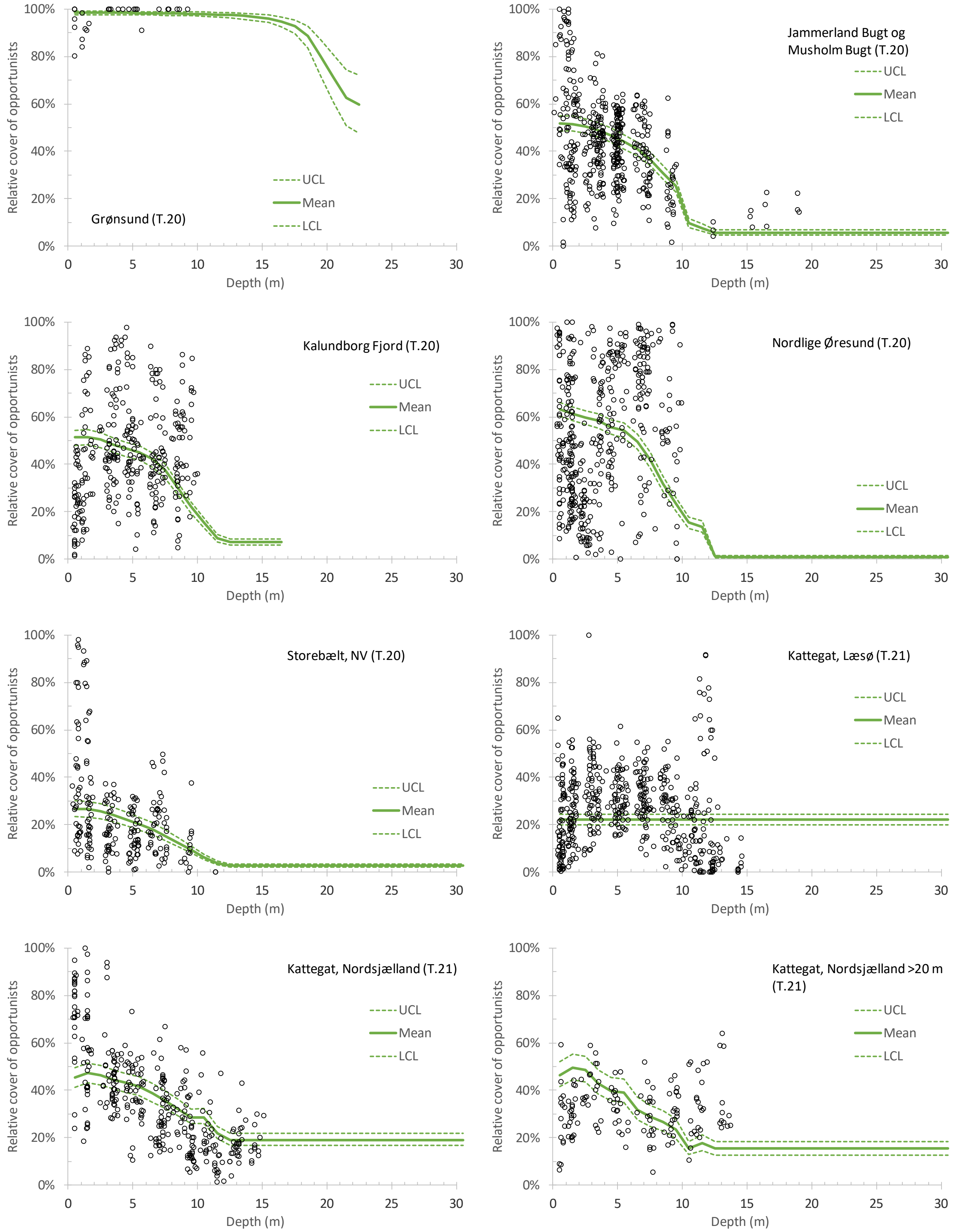


Figure 3.5 continues on next page.

Figure 3.5 continued.

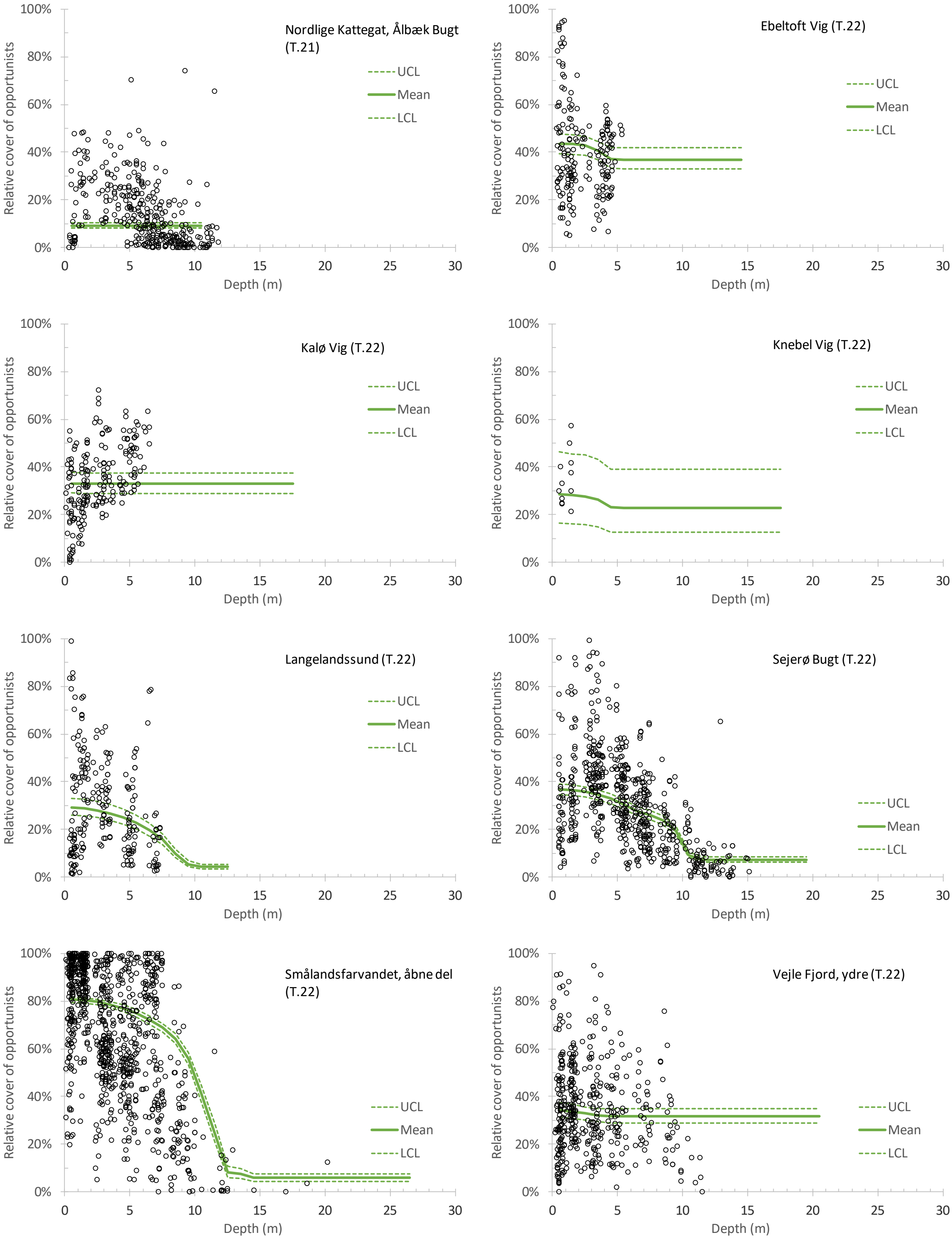


Figure 3.5 continues on next page.

Figure 3.5 continued.

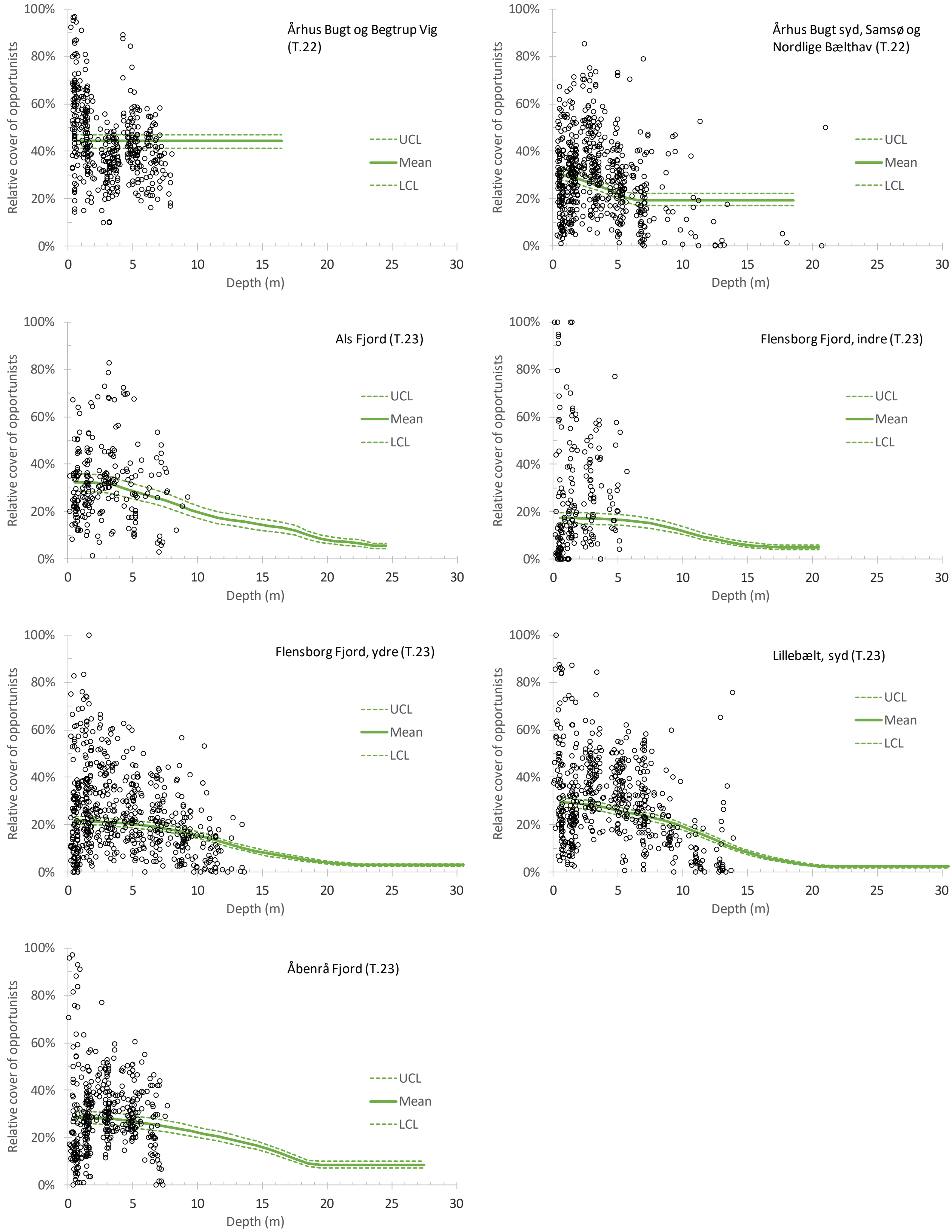


Figure 3.5. Observed relative cover of opportunists versus depth for the 37 investigated water bodies in coastal types (kystvandstype = Bælthav, Kattegat, Østersø). The water bodies are shown with ascending type number. For each water body the estimated depth (i.e. salinity) relationship from the non-linear model (*Eq. 2.7*) without sea urchins ($cover_{seaurchin} = 0$) is shown (green solid line) with the 95 % confidence interval of the model (dashed lines). The estimated model and confidence interval represent the geometric mean, corresponding to the median distribution. Note that the depth relationship is predicted for the depth of the water column at the corresponding hydrochemistry station, i.e. for a few water bodies the model predictions do not extend to the deepest depths with macroalgae data.

3.2.3 Additional open-water areas

The six additional areas represent a large salinity gradient, which is also partially reflected in the relative cover of opportunists (*Fig. 3.6*). Not surprisingly, Østersøen and Skagerrak had the largest and lowest relative cover of opportunists, respectively, but Kattegat also had a considerable proportion of opportunists, even at depths below 15 m, which was unexpected compared to the two areas in Storebælt, characterized by lower salinity but also lower relative cover of opportunists. These six additional areas, all having large depth ranges and displaying diverse gradients in relative cover of opportunists, confirm the overall finding that establishing generic models to describe variations in this macroalgae indicator, based on samplings once per year, is indeed difficult.

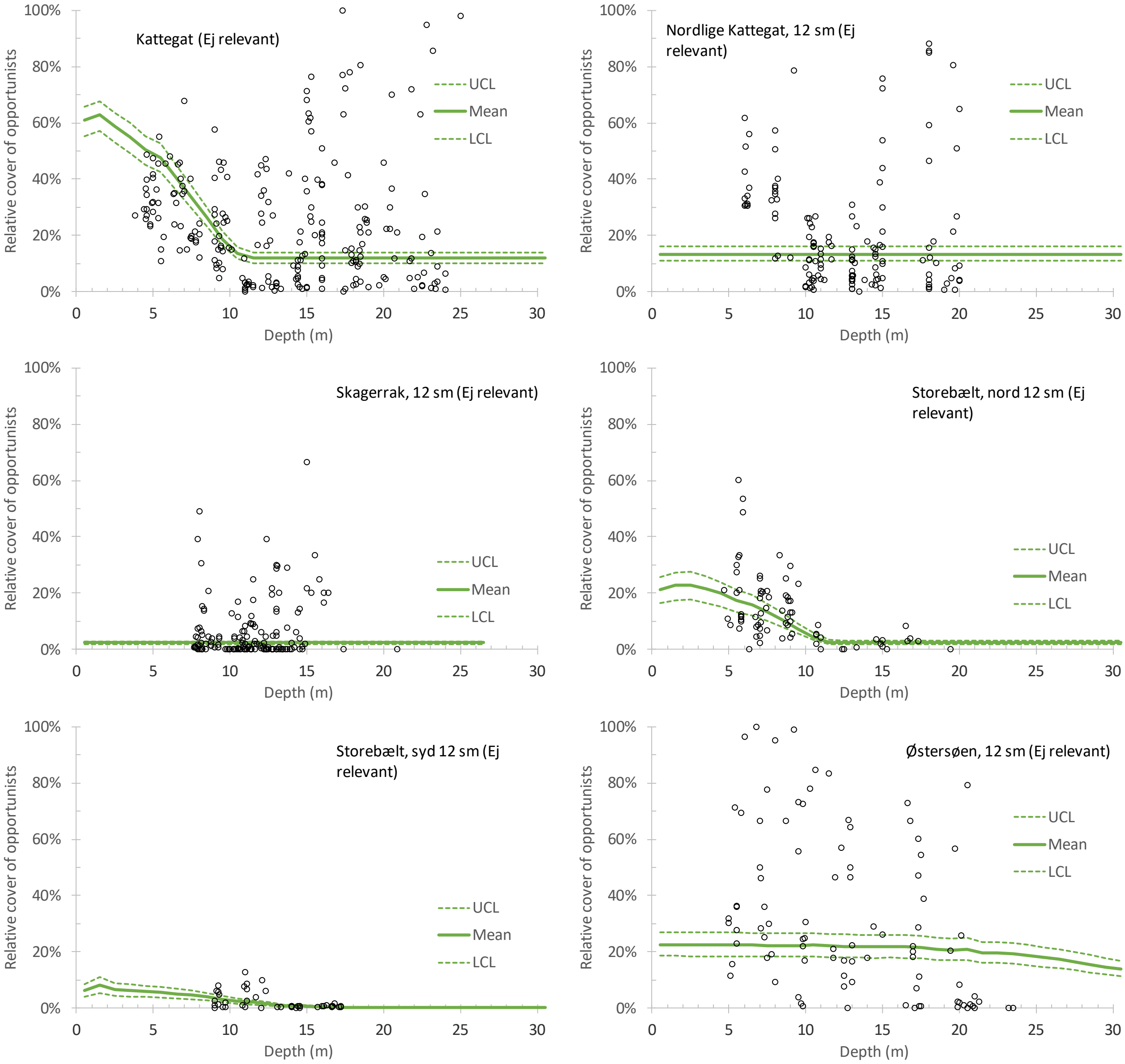


Figure 3.6. Observed cumulative cover (left panel) and perennial species richness (right panel) versus depth for the 6 additional areas outside the WFD baseline (these water bodies are denoted ‘Ej relevant’ in the Danish implementation of the WFD). For each water body the estimated depth (i.e. salinity) relationship from the non-linear model (*Eq. 2.7*) without sea urchins ($cover_{seaurchin} = 0$) is shown (green solid line) with the 95 % confidence interval of the model (dashed lines). The estimated model and confidence interval represent the geometric mean, corresponding to the median distribution. Note that the depth relationship is predicted for the depth of the water column at the corresponding hydrochemistry station, i.e. for a few water bodies the model predictions do not extend to the deepest depths with macroalgae data.

3.3 Accounting for other effects

In addition to water body-specific depth gradients shown above, the macroalgae indicators exhibited variations in response to physical exposure, cover of sea urchins and the specific year of monitoring (*Eqs. 2.5* and *2.7*).

The effect of sea urchins was not estimated here, but the parameter estimates from Carstensen &Dahl (2019) were used expressing a decline in cumulative cover and number of perennial species with increasing cover of sea urchins, whereas there was a slight increase in the relative cover of opportunists with increasing cover of sea urchins (*Fig. 3.7a*). Explanations for these relationships are found in Carstensen & Dahl (2019).

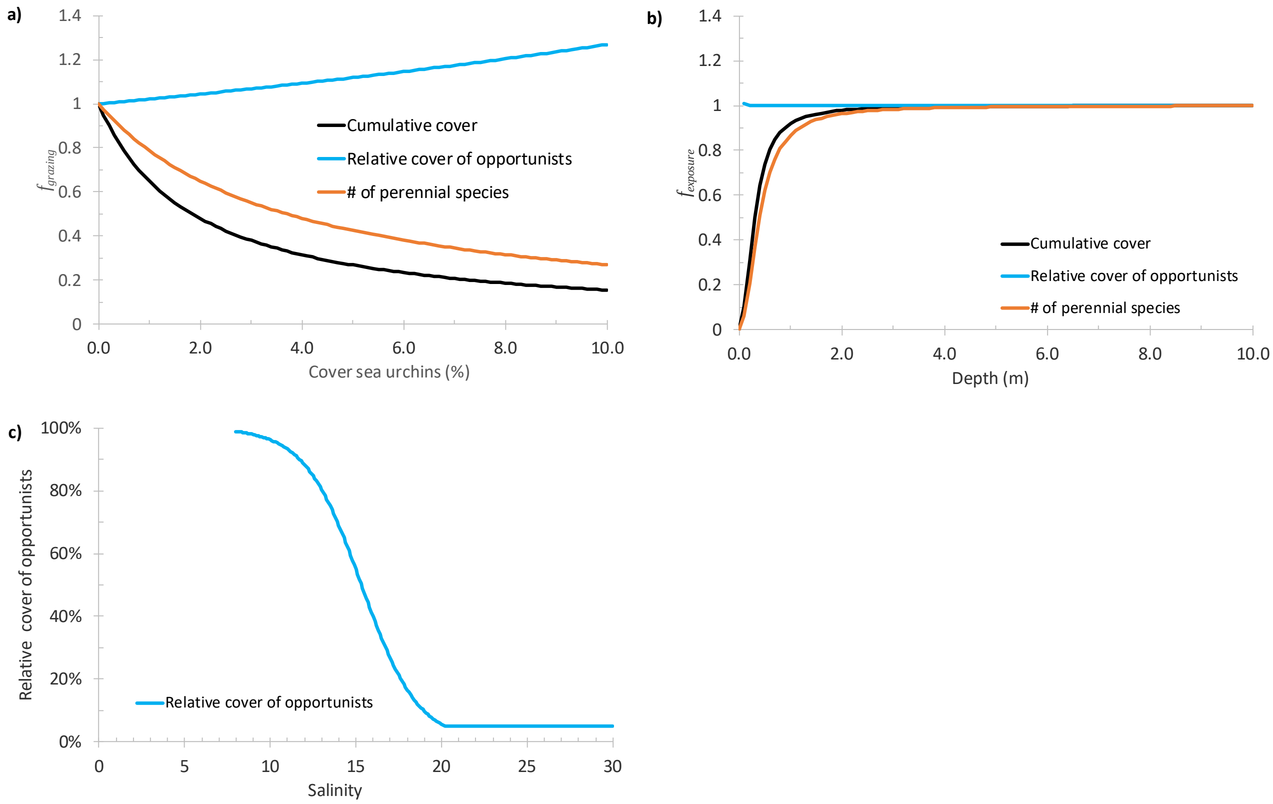


Figure 3.7. Modelled effects of sea urchins (a), physical exposure (b) and salinity (c) on cumulative cover, number of perennial species and relative cover of opportunists for all sites (*Table 2.1*). Relationships for sea urchins were taken from Carstensen & Dahl (2019), relationships for physical exposure were estimated from the models (*Eqs. 2.5* and *2.7*), and relationship for salinity was estimated from *Eq. 2.6* with a back-transformation of the logistic function (cf. *Eq. 2.7*).

The effect of physical exposure was most pronounced at depth shallower than 2 m for cumulative cover and number of perennial species, whereas there was no apparent effect on the proportion of opportunists (*Fig. 3.7b*). These two relationships are similar to those estimated in Carstensen & Dahl (2019), whereas the relationship is different for the relative cover of opportunists.

The effect of salinity for describing changes in the relative cover of opportunists with depth was a non-linear function (*Eq. 2.6*) with an estimated salinity threshold of $\hat{S}_T = 20.2$, i.e. the relative cover of opportunists gradually decreased with increasing salinity up to 20.2 and remained constant at higher salinity (*Fig. 3.7c*). The model suggests that salinity can induce a major change in the relative cover of opportunists in the salinity range from 10 to 20.

Interannual variations in the models for the three indicators, described by parameter k_{year}, were modest and did not show any particular pattern over time (*Fig. 3.8*). The attenuation of cumulative cover and number of perennial species varied approximately 5 % over the 12 years, whereas variability in the relatively cover of opportunists was slightly higher (14 %). All three indicators showed improving conditions (lower attenuation and lower relative cover of opportunists) over time, although none of the trends were significant.

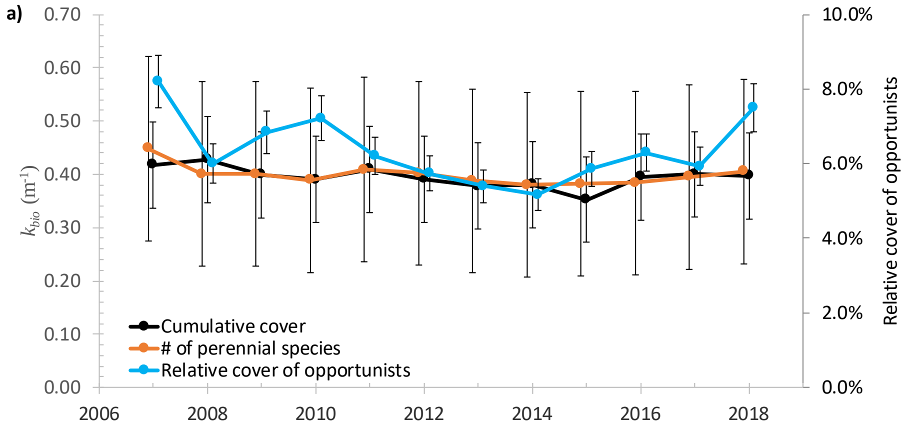


Figure 3.8. Estimated effect of the k_{year} factor in the models for cumulative cover, number of perennial species and relative cover of opportunists displayed for the average of all water bodies (cumulative cover and number of perennial species) and the average of all sites with a salinity above the estimated salinity threshold ($\hat{S}_T$ = 20.2). Error bars show the standard error of the estimates.

3.4 Spatial variation related to environmental conditions

Spatial variability among water bodies was expressed in the models for the three macroalgae indices by the parameters C_{max} and k_{bio} for cumulative cover and number of perennial species, and by P_{site} for the relative cover of opportunists. From the model formulations it is assumed that estimates of maximum indicator level (C_{max}) describe natural variations, whereas estimates of attenuation in macroalgae indicator level with depth (k_{bio}) and between sites (P_{site}) describe effect of human disturbance. These assumptions will be tested in the following by investigating the spatial variation in these parameters versus salinity, light and nutrient conditions.

3.4.1 Spatial variations in maximum indicator level (C_{max})

Macroalgae species richness increases with salinity, which also affects cumulative cover, because a more diverse macroalgae community can more easily build a multi-layered structure. Therefore, it is expected that C_{max}, describing the cumulative cover or number of perennial species when these are not limited by grazing, physical exposure or light, is related to salinity.

The maximum cumulative cover and maximum number of perennial species were both significantly related to salinity (*Fig. 3.9*). The five water bodies in Limfjorden and the open-water Skagerrak were not included, because sea urchins exert a strong, albeit not quantitatively monitored, grazing pressure on macroalgae in Limfjorden (Carstensen & Dahl 2019) and C_{max} estimates from Skagerrak were extrapolated from deeper observations. Overall, without grazing pressure, physical exposure and light limitation, cumulative cover is expected to increase from 100 % in the most brackish water bodies to 200 % in the more saline water bodies. Changes in the number of perennial species over the same salinity range are even more drastic, from 2 in brackish water to around 14 in waters with a salinity of 30. The present relationships are stronger than those reported in Carstensen & Dahl (2019).

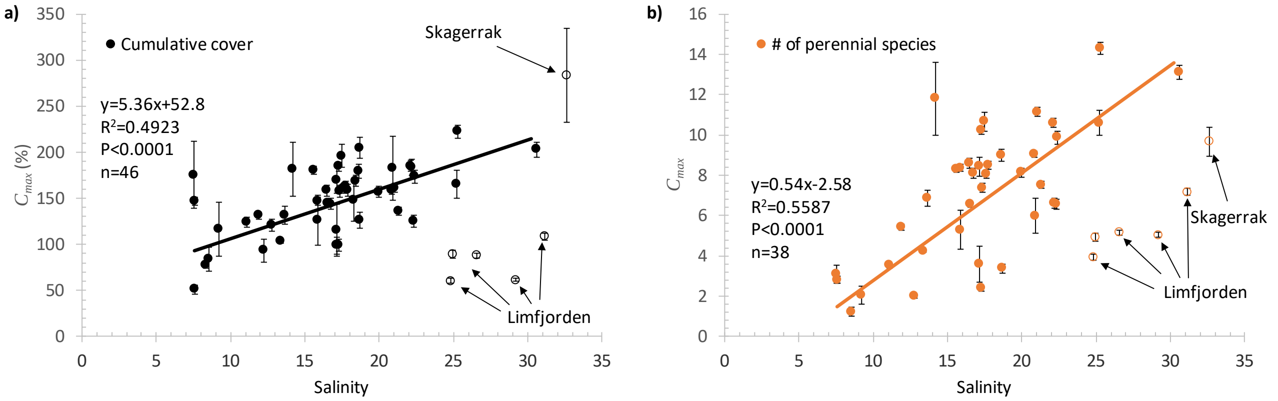


Figure 3.9. Estimates of C_{max} for cumulative cover (a) and number of perennial species (b) versus salinity for different water bodies. Error bars show the standard error of the estimates. Open symbols are not included in the weighted least squares regressions.

3.4.2 Spatial variations in attenuation of macroalgae with depth (k_{bio})

At deeper depths, cumulative cover and number of perennial species become limited by shading and therefore the attenuation parameter for these two macroalgae indicators (k_{bio}) is expected to be related with the light attenuation coefficient (k_d).

The attenuation of cumulative cover and number of perennial species was clearly related to light attenuation (*Fig. 3.10*). The attenuation of the macroalgae indicators with depth typically ranged from 0.1 to 1.2, a range similar to that exhibited by the light attenuation coefficient. Parameter estimates (k_{bio}) from the model (*Eq. 2.5*) that were not significant ($P > 0.05$) were excluded from the regression. For cumulative cover, the k_{bio} estimates for Faaborg Fjord, Haderslev Fjord and Hejlsminde Nor were not well determined (not significantly different from zero) and therefore not included, whereas for the number of perennial species, the k_{bio} estimates for Hejlsminde Nor, Smålandsfarvandet and Roskilde Fjord indre were also associated with large uncertainty and not included. The non-significant estimates are likely primarily due to low number of observations, particularly at deeper depths where light limitation becomes important.

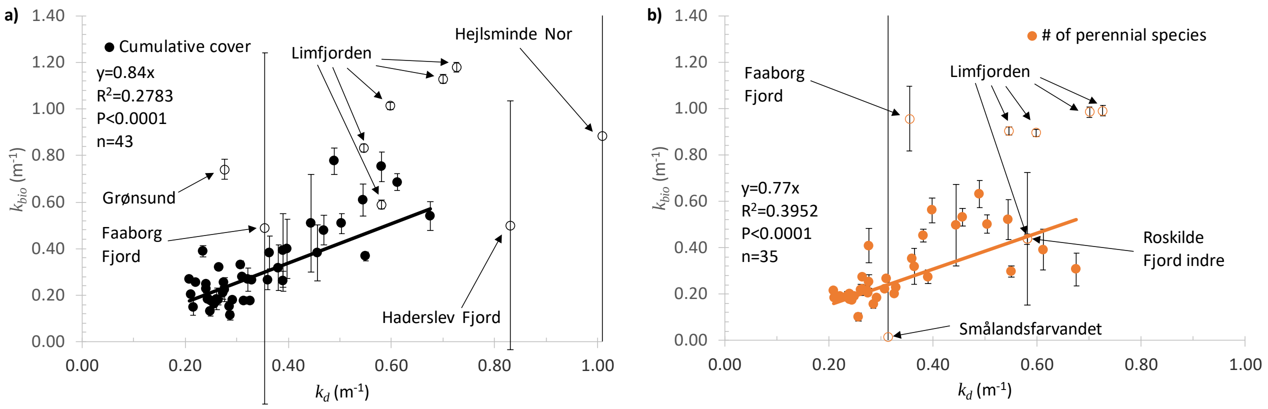


Figure 3.10. Estimates of k_{bio} for cumulative cover (a) and number of perennial species (b) versus light attenuation for different water bodies. Error bars show the standard error of the estimates. Open symbols are not included in the weighted least squares regressions.Hejlsminde Nor is not shown for the number of perennial species.

Furthermore, estimates from Limfjorden were excluded due to the potential bias introduced by sea urchin grazing (cf. *Fig. 3.9*). The relatively high k_{bio} estimates in Limfjorden (the only water body that follows the general pattern is Nissum Bredning) indicate that macroalgae is disappearing faster than light is attenuated, suggesting that sea urchins exert stronger grazing on deeper occurrences of macroalgae, where salinity is higher. The most dominant sea urchin in Limfjorden (*Psammechinus miliaris*) is a marine organism sensitive to oligo- and mesohaline conditions (Lawrence 2001). Finally, k_{bio} estimates from Grønsund (cumulative cover) and Faaborg Fjord (number of perennial species) were also excluded from the regression, as these estimates were based on few observations and deviated markedly from the overall pattern. However, the exclusion of these two outliers only had a marginal effect on the regressions.

For both regressions the estimated intercepts were not significant (P = 0.1913 and P = 0.3333, respectively) and therefore, a linear model with zero intercept was employed (*Fig. 3.10*). Intuitively, proportionality between k_{bio} and k_d seems logical as k_{bio} expresses changes with depth that are mainly governed by light. Indeed, if the response of macroalgae was identical to that of light, a slope parameter of 1 would be expected. However, the two slopes were significantly lower than 1 (P < 0.0001 for both), indicating that the attenuation of macroalgae variables was less steep than that of light, i.e. declines of cumulative cover and number of perennial species with depth were slower than the attenuation of light. The most likely explanation for this phenomenon is the ability of the macroalgae community to utilise different wavelengths. Whereas k_d is estimated based on a broad spectrum of wavelengths and thus represent an average attenuation of these, blue light penetrates deeper as opposed to red light that is attenuated more rapidly. The macroalgae community is adapted to these changes in the availability of different wavelengths, changing typically from green and brown algae at shallower depth to red algae that have pigments for utilising blue light penetrating deeper (Markager & Sand-Jensen 1992; Gattuso et al. 2006). Hence, this adaptation of the macroalgae community to different wavelengths is reflected in the lower slope estimate.

3.4.3 Spatial variations between sites (P_{site})

The relative cover of opportunists has been shown to correlate with salinity and nutrient status (Carstensen et al. 2008, 2014), although these relationships were based on a simpler and different model. In the present model (*Eq. 2.7*), the effect of salinity was used to describe individual point observations, since salinity often changes with depth at transects in the open waters. Therefore, we tested if the P_{site} parameter, describing the relative cover of opportunists when variations due to changes in salinity, physical exposure and grazing are accounted for, was related to different environmental variables representing pressures, suggested to regulate the composition of the macroalgae community. However, variations in the relative cover of opportunists did not correlate with light conditions or nutrient status, despite broad ranges in these suggested pressures (*Fig. 3.11*).

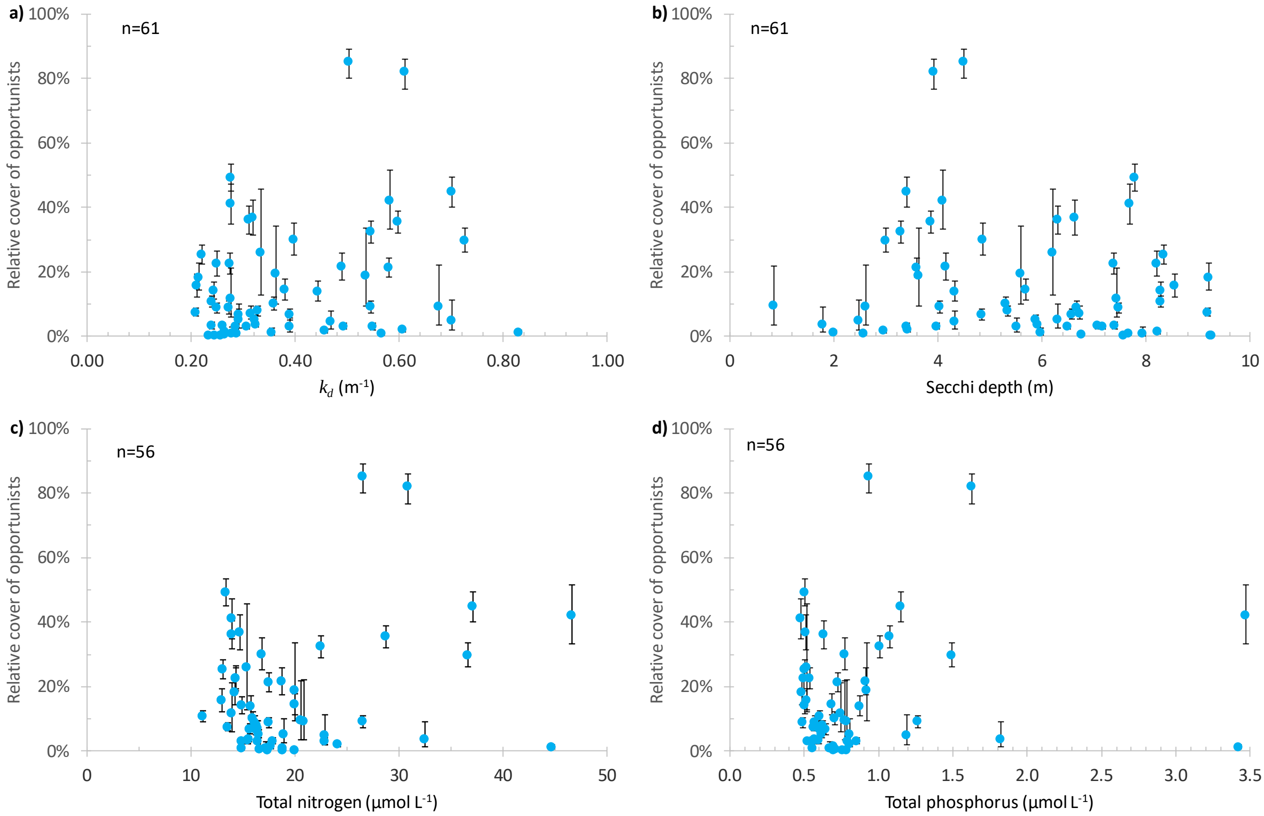


Figure 3.11. Estimates of P_{site} versus different environmental variables hypothesized to stimulate the dominance of opportunistic species: a) light attenuation, b) Secchi depth, c) total nitrogen, and d) total phosphorus. Error bars show the standard error of the estimates.

3.5 Estimating macroalgae indicators

For illustrating the estimation of the macroalgae indicators in practice, 10 water bodies were selected where k_{bio} parameters were estimable (*Figs. 3.1-3.2*), and where sufficient data were available in each of the two 6-year periods (2007-2012 and 2013-2018). For these water bodies, the parameters k_{bio} and C_{max} were estimated separately for each of the two 6-year periods for both macroalgae cumulative cover and number of perennial species.

For most of the water bodies, each of the two parameters (C_{max} and k_{bio}) showed similar estimates for the two 6-year periods (*Fig. 3.12*). Cumulative cover attenuation with depth improved (i.e. decreasing k_{bio}) in four of the 10 examples (Djursland Øst, Flensborg Fjord ydre, Nordlige Øresund, and Skive Fjord), whereas it worsened in Odense Fjord ydre. Similarly, the attenuation of the number of perennial species improved (i.e. decreasing k_{bio}) in six out of the 10 examples (Djursland Øst, Flensborg Fjord ydre, Kalundborg Fjord, Sejerø Bugt, Skive Fjord, and Storebælt NV), whereas the status of the number of perennial species worsened in Nordlige Øresund and Odense Fjord ydre.

It was not possible to estimate three out of 20 k_{bio} parameters for cumulative cover (Kalundborg Fjord, 2013-2018; Storebælt NV, 2007-2012; Århus Bugt and Begtrup Vig, 2013-2018) and one out of 20 k_{bio} parameters for number of perennial species (Århus Bugt and Begtrup Vig, 2013-2018). In these cases, k_{bio} could not be estimated due to the lack of deeper data representing the light-limited phase of the depth gradient. This highlights that the macroalgae indicators can only be estimated if monitoring data include observations at depths where light regulates cumulative cover and number of perennial species. It should also be noticed that for some water bodies and 6-year periods, the number of observations representing the light-regulated phase is limited and this will produce more uncertain k_{bio} estimates.

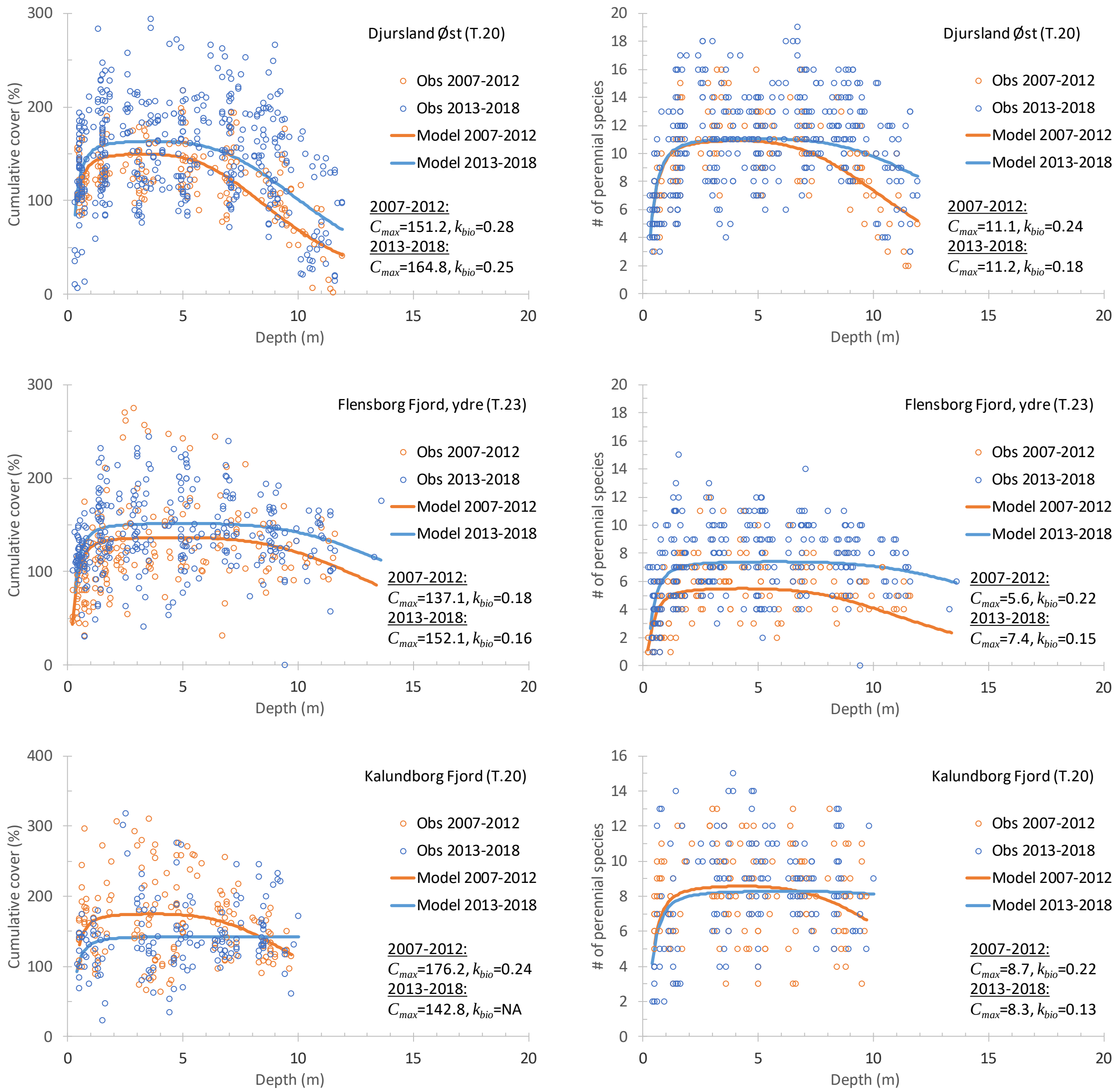


Figure 3.12 contiues on next page.

Figure 3.12 contiued.

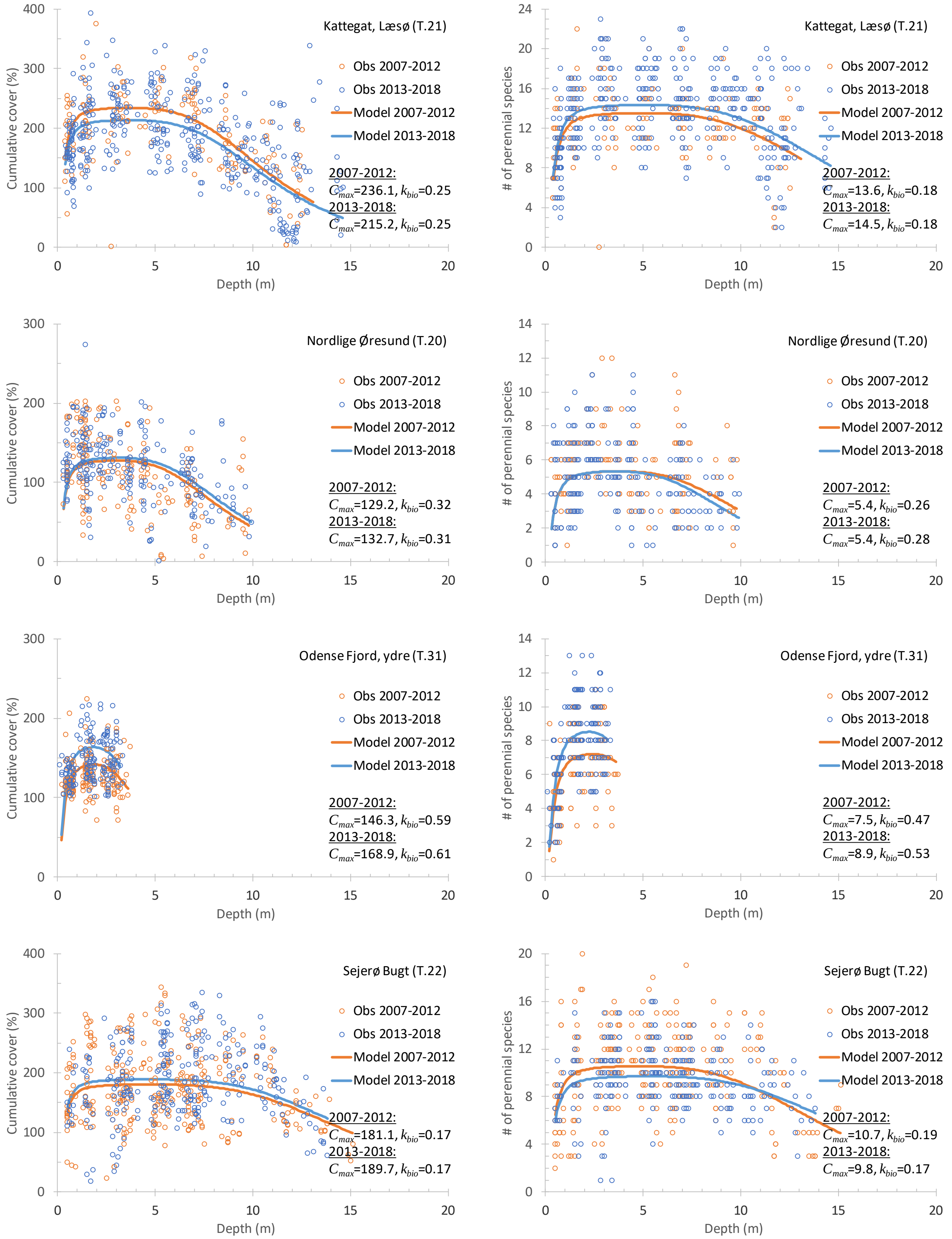


Figure 3.12 contiues on next page.

Figure 3.12 contiued.

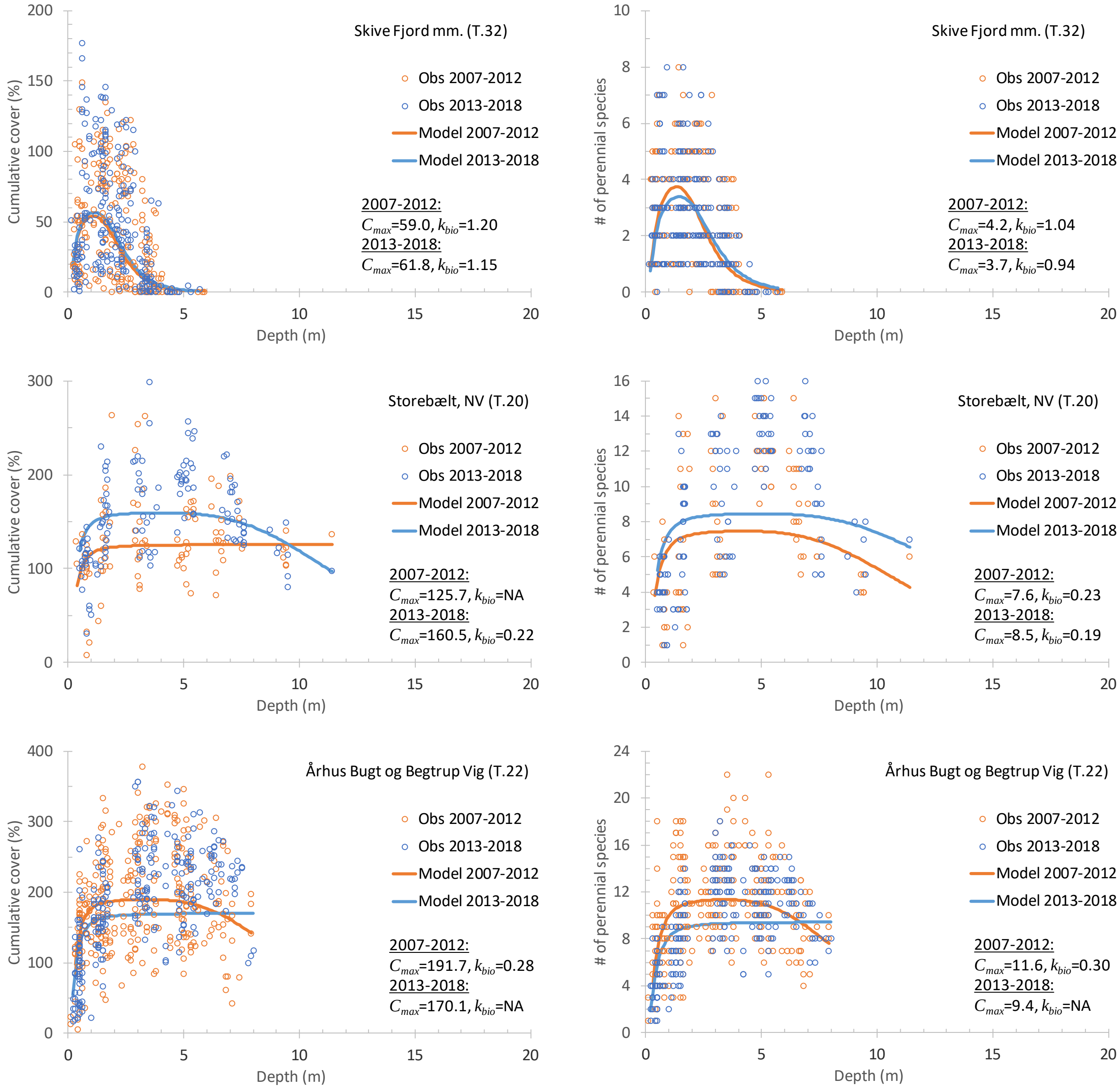


Figure 3.12. Observed cumulative cover (left panel) and perennial species richness (right panel) versus depth for 10 selected water bodies for illustrating the indicator estimation approach. The two parameters (C_{max} and k_{bio}) were estimated for two separate 6-year periods for each of the water bodies separately (given as inserts). Other parameters were fixed to the values estimated from the entire data set. Note that k_{bio} could not be estimated for some 6-year periods, listed as NA.

3.6 Macroalgae indicator class boundaries

The parameterization of the three macroalgae models separated variations into natural/non-human (physical exposure, grazing and salinity) and human perturbations. The attenuation of cumulative cover and number of perennial species (k_{bio}) was clearly linked to light conditions, showing that water bodies with poorer light conditions experienced steeper declines in cumulative cover and number of perennial species (*Fig. 3.10*). These two linear relationships between k_d and k_{bio} were used to translate reference conditions and class boundaries for k_d (*Table 3.1*) into values for k_{bio} (*Tables 3.2* and *3.3*). However, it was not possible to demonstrate a human-induced effect on the relative cover of opportunists, as this macroalgae indicator appeared to be controlled almost entirely by salinity. The translations of reference conditions and class boundaries through the regressions are not only statistically significant, but also rest on basic principles of light attenuation and the expected response of the macroalgae community to changing light conditions (see *Section 3.4*). Thus, it is plausible that the translation will apply in general, with the exception of Limfjorden, which deviated strongly from the overall patterns (*Fig. 3.10*). Although reference conditions and class boundaries for water bodies in Limfjorden in principle could be calculated using the established regression (*Fig. 3.10*), the scientific understanding of the large deviations is lacking. Hence, further analyses of macroalgae responses to light conditions, sea urchins and possibly also substrate conditions are required to develop targeted reference conditions and class boundaries for Limfjorden.

Table 3.1. Translated reference conditions and class boundaries (HG = High-Good, GM = Good-Moderate, MP = Moderate-Poor, PB = Poor-Bad) for the light attenuation coefficient (in m^{-1}) calculated from reference conditions (RC) for eelgrass main depth limits (in m). Eelgrass reference conditions are missing for 11 out the 109 Danish coastal water bodies.

WB id	Water body	Type	Eelgrass	Light attenuation coefficient (k_d)				
			RC	RC	HG	GM	MP	PB
1	Roskilde Fjord, ydre	T.17	7.5	0.24	0.27	0.33	0.49	0.98
2	Roskilde Fjord, indre	T.27	4.9	0.37	0.42	0.51	0.75	1.50
6	Nordlige Øresund	T.20	8.5	0.22	0.24	0.29	0.43	0.86
16	Korsør Nor	T.18	5.2	0.35	0.39	0.48	0.70	1.41
17	Basnæs Nor	T.16	5.6	0.33	0.36	0.44	0.65	1.31
18	Holsteinsborg Nor	T.16	5.6	0.33	0.36	0.44	0.65	1.31
24	Isefjord, ydre	T.17	7.5	0.24	0.27	0.33	0.49	0.98
25	Skælskør Fjord og Nor	T.18	5.3	0.35	0.38	0.47	0.69	1.38
28	Sejerøbugt	T.22	13.3	0.14	0.15	0.19	0.28	0.55
29	Kalundborg Fjord	T.20	11.0	0.17	0.19	0.23	0.33	0.67
34	Smålandsfarvandet, syd	T.9	5.8	0.31	0.35	0.43	0.63	1.26
35	Karrebæk Fjord	T.29	5.6	0.33	0.36	0.44	0.65	1.31
36	Dybsø Fjord	T.18	5.5	0.33	0.37	0.45	0.67	1.33
37	Avnø Fjord	T.9	6.3	0.29	0.32	0.39	0.58	1.16
38	Guldborgssund	T.9	5.3	0.35	0.38	0.47	0.69	1.38
44	Hjelm Bugt	T.12	9.8	0.19	0.21	0.25	0.37	0.75
45	Grønsund	T.20	11.2	0.16	0.18	0.22	0.33	0.65
46	Fakse Bugt	T.12	7.3	0.25	0.28	0.34	0.50	1.00
47	Præstø Fjord	T.17	5.4	0.34	0.38	0.46	0.68	1.36
48	Stege Bugt	T.14	4.1	0.44	0.49	0.60	0.89	1.77
49	Stege Nor	T.24	5.3	0.35	0.38	0.47	0.69	1.38
56	Østersøen, Bornholm	T.5	10.2	0.18	0.20	0.24	0.36	0.72
57	Østersøen, Christiansø	T.5	10.2	0.18	0.20	0.24	0.36	0.72

Table 3.1 continues on next page.

Table 3.1 continued.

WB id	Water body	Type	Eelgrass	Light attenuation coefficient (k_d)				
			RC	RC	HG	GM	MP	PB
59	Nærå Strand	T.25	5.2	0.35	0.39	0.48	0.70	1.41
62	Lillestrand	T.11	6.0	0.31	0.34	0.41	0.61	1.22
68	Lindelse Nor	T.16	6.4	0.29	0.32	0.39	0.57	1.15
72	Kløven	T.16	6.5	0.28	0.31	0.38	0.56	1.13
74	Bredningen	T.38	6.2	0.30	0.33	0.40	0.59	1.18
80	Gamborg Fjord	T.13	7.6	0.24	0.27	0.33	0.48	0.96
82	Aborg Minde Nor	T.38	6.2	0.30	0.33	0.40	0.59	1.18
83	Holckenhavn Fjord	T.33	5.8	0.32	0.35	0.43	0.63	1.26
84	Kerteminde Fjord	T.18	5.7	0.32	0.36	0.43	0.64	1.29
85	Kertinge Nor	T.18	5.2	0.35	0.39	0.48	0.70	1.41
86	Nyborg Fjord	T.6	8.4	0.22	0.24	0.29	0.44	0.87
87	Helnæs Bugt	T.13	7.7	0.24	0.26	0.32	0.48	0.95
89	Lunkebugten	T.7	7.9	0.23	0.26	0.31	0.46	0.93
90	Langelandssund	T.22	9.5	0.19	0.21	0.26	0.39	0.77
92	Odense Fjord, ydre	T.31	5.6	0.33	0.36	0.44	0.65	1.31
93	Odense Fjord, indre	T.35	5.4	0.34	0.38	0.46	0.68	1.36
95	Storebælt, SV	T.20	10.6	0.17	0.19	0.23	0.35	0.69
96	Storebælt, NV	T.20	10.5	0.17	0.19	0.24	0.35	0.70
101	Genner Bugt	T.20	10.9	0.17	0.19	0.23	0.34	0.67
102	Åbenrå Fjord	T.23	12.9	0.14	0.16	0.19	0.28	0.57
103	Als Fjord	T.23	10.5	0.17	0.19	0.24	0.35	0.70
104	Als Sund	T.17	7.7	0.24	0.26	0.32	0.48	0.95
105	Augustenborg Fjord	T.17	6.9	0.27	0.30	0.36	0.53	1.06
106	Haderslev Fjord	T.36	7.1	0.26	0.29	0.35	0.52	1.03
107	Juvre Dyb	T.1	IR					
108	Avnø Vig	T.8	6.3	0.29	0.32	0.39	0.58	1.16
109	Hejlsminde Nor	T.35	6.6	0.28	0.31	0.38	0.56	1.11
110	Nybøl Nor	T.17	7.5	0.24	0.27	0.33	0.49	0.98
111	Lister Dyb	T.1	IR					
113	Flensborg Fjord, indre	T.23	8.9	0.21	0.23	0.28	0.41	0.82
114	Flensborg Fjord, ydre	T.23	13.0	0.14	0.16	0.19	0.28	0.56
119	Vesterhavet, syd	T.1	IR					
120	Knudedyb	T.1	IR					
121	Grådyb	T.1	IR					
122	Vejle Fjord, ydre	T.22	9.9	0.19	0.21	0.25	0.37	0.74
123	Vejle Fjord, indre	T.28	7.6	0.24	0.27	0.33	0.48	0.96
124	Kolding Fjord, indre	T.28	7.4	0.25	0.28	0.33	0.50	0.99
125	Kolding Fjord, ydre	T.17	7.4	0.25	0.28	0.33	0.50	0.99
127	Horsens Fjord, ydre	T.10	11.5	0.16	0.18	0.22	0.32	0.64
128	Horsens Fjord, indre	T.10	8.0	0.23	0.25	0.31	0.46	0.92
129	Nissum Fjord, ydre	T.15	5.4	0.34	0.38	0.46	0.68	1.36
130	Nissum Fjord, mellem	T.15	IR					
131	Nissum Fjord, Felsted Kog	T.26	IR					
132	Ringkøbing Fjord	T.37	4.2	0.44	0.48	0.59	0.87	1.75
133	Vesterhavet, nord	T.2	IR					
136	Randers Fjord, indre	T.4						
137	Randers Fjord, ydre	T.3						
138	Hevring Bugt	T.22	9.2	0.20	0.22	0.27	0.40	0.80
139	Anholt	T.21	11.6	0.16	0.18	0.21	0.32	0.63
140	Djursland Øst	T.20	10.4	0.18	0.20	0.24	0.35	0.70

Table 3.1 continues on next page.

Table 3.1 continued.

WB id	Water body	Type	Eelgrass	Light attenuation coefficient (k_d)				
			RC	RC	HG	GM	MP	PB
141	Ebeltoft Vig	T.22	9.9	0.19	0.21	0.25	0.37	0.74
142	Stavns Fjord	T.22	10.0	0.18	0.20	0.25	0.37	0.73
144	Knebel Vig	T.22	9.0	0.20	0.23	0.28	0.41	0.81
145	Kalø Vig, indre	T.22	9.5	0.19	0.21	0.26	0.39	0.77
146	Norsminde Fjord	T.33	5.6	0.33	0.36	0.44	0.65	1.31
147	Århus Bugt og Begtrup Vig	T.22	9.4	0.19	0.22	0.26	0.39	0.78
154	Kattegat, Læsø	T.21	10.0	0.18	0.20	0.25	0.37	0.73
157	Skive Fjord mm.	T.32	5.5	0.33	0.37	0.45	0.67	1.33
158	Hjarbæk Fjord	T.36	5.6	0.33	0.37	0.45	0.66	1.32
159	Mariager Fjord, indre	T.30	7.7	0.24	0.27	0.32	0.48	0.96
160	Mariager Fjord, ydre	T.25	5.2	0.36	0.39	0.48	0.71	1.42
165	Isefjord, indre	T.17	5.2	0.35	0.39	0.48	0.70	1.41
200	Kattegat, Nordsjælland	T.21	12.4	0.15	0.16	0.20	0.30	0.59
201	Køge Bugt	T.12	9.5	0.19	0.21	0.26	0.39	0.77
204	Jammerland og Musholm Bugt	T.20	13.0	0.14	0.16	0.19	0.28	0.56
205	Kattegat, Nordsjælland > 20 m	T.21	11.6	0.16	0.18	0.21	0.32	0.63
206	Smålandsfarvandet, åbne del	T.22	9.5	0.19	0.21	0.26	0.39	0.77
207	Nakskov Fjord	T.14	6.1	0.30	0.33	0.41	0.60	1.20
208	Femerbælt	T.6	7.5	0.24	0.27	0.33	0.49	0.98
209	Rødsand	T.14	5.5	0.33	0.37	0.45	0.67	1.33
212	Faaborg Fjord	T.7	8.7	0.21	0.23	0.28	0.42	0.84
214	Det Sydfynske Øhav	T.13	11.2	0.16	0.18	0.22	0.33	0.65
216	Lillebælt, syd	T.23	9.6	0.19	0.21	0.26	0.38	0.76
217	Lillebælt, Bredningen	T.13	11.5	0.16	0.18	0.22	0.32	0.64
219	Århus Bugt syd, Samsø og Nordlige Bælthav	T.22	11.5	0.16	0.18	0.22	0.32	0.64
221	Skagerrak	T.2						
222	Kattegat, Aalborg Bugt	T.21	10.4	0.18	0.20	0.24	0.35	0.70
224	Nordlige Lillebælt	T.22	9.3	0.20	0.22	0.27	0.39	0.79
225	Nordlige Kattegat, Ålbæk Bugt	T.21	12.8	0.14	0.16	0.19	0.29	0.57
231	Snævringen	T.6	12.8	0.14	0.16	0.19	0.29	0.57
232	Nissum Bredning	T.19	6.2	0.30	0.33	0.40	0.59	1.18
233	Kås bredning	T.19	7.8	0.23	0.26	0.32	0.47	0.94
234	Løgstør Bredning	T.17	6.5	0.28	0.31	0.38	0.56	1.13
235	Nibe Bredning og Langerak	T.31	4.7	0.39	0.43	0.53	0.78	1.56
236	Thisted Bredning	T.34	6.5	0.28	0.31	0.38	0.56	1.13
238	Halkær Bredning	T.36	5.3	0.35	0.38	0.47	0.69	1.38

Table 3.2. Proposed reference conditions (RC) and class boundaries (HG = High-Good, GM = Good-Moderate, MP = Moderate-Poor, PB = Poor-Bad) for the attenuation of cumulative cover with depth (in m^{-1}) calculated from reference conditions for eelgrass main depth limits (in m) through k_d. Reference conditions and class boundaries have not been calculated for 11 out the 109 Danish coastal water bodies due to missing reference conditions for eelgrass. Values for water bodies without reference condition for eelgrass could not be calculated and values for water bodies in Limfjorden are not shown.

WB id	Water body	Type	Eelgrass	Cumulative cover attenuation coefficient (k_{bio})				
			RC	RC	HG	GM	MP	PB
1	Roskilde Fjord, ydre	T.17	7.5	0.21	0.23	0.28	0.41	0.83
2	Roskilde Fjord, indre	T.27	4.9	0.32	0.35	0.43	0.63	1.27
6	Nordlige Øresund	T.20	8.5	0.18	0.20	0.25	0.36	0.73
16	Korsør Nor	T.18	5.2	0.30	0.33	0.40	0.60	1.19
17	Basnæs Nor	T.16	5.6	0.28	0.31	0.37	0.55	1.11
18	Holsteinsborg Nor	T.16	5.6	0.28	0.31	0.37	0.55	1.11
24	Isefjord, ydre	T.17	7.5	0.21	0.23	0.28	0.41	0.83
25	Skælskør Fjord og Nor	T.18	5.3	0.29	0.33	0.40	0.59	1.17
28	Sejerøbugt	T.22	13.3	0.12	0.13	0.16	0.23	0.47
29	Kalundborg Fjord	T.20	11.0	0.14	0.16	0.19	0.28	0.56
34	Smålandsfarvandet, syd	T.9	5.8	0.27	0.30	0.36	0.53	1.07
35	Karrebæk Fjord	T.29	5.6	0.28	0.31	0.37	0.55	1.11
36	Dybsø Fjord	T.18	5.5	0.28	0.31	0.38	0.56	1.13
37	Avnø Fjord	T.9	6.3	0.25	0.27	0.33	0.49	0.98
38	Guldborgssund	T.9	5.3	0.29	0.33	0.40	0.59	1.17
44	Hjelm Bugt	T.12	9.8	0.16	0.18	0.21	0.32	0.63
45	Grønsund	T.20	11.2	0.14	0.15	0.19	0.28	0.55
46	Fakse Bugt	T.12	7.3	0.21	0.24	0.29	0.42	0.85
47	Præstø Fjord	T.17	5.4	0.29	0.32	0.39	0.57	1.15
48	Stege Bugt	T.14	4.1	0.37	0.42	0.51	0.75	1.50
49	Stege Nor	T.24	5.3	0.29	0.33	0.40	0.59	1.17
56	Østersøen, Bornholm	T.5	10.2	0.15	0.17	0.21	0.30	0.61
57	Østersøen, Christiansø	T.5	10.2	0.15	0.17	0.21	0.30	0.61
59	Nærå Strand	T.25	5.2	0.30	0.33	0.40	0.60	1.19
62	Lillestrand	T.11	6.0	0.26	0.29	0.35	0.52	1.03
68	Lindelse Nor	T.16	6.4	0.24	0.27	0.33	0.48	0.97
72	Kløven	T.16	6.5	0.24	0.27	0.32	0.48	0.95
74	Bredningen	T.38	6.2	0.25	0.28	0.34	0.50	1.00
80	Gamborg Fjord	T.13	7.6	0.20	0.23	0.28	0.41	0.82
82	Aborg Minde Nor	T.38	6.2	0.25	0.28	0.34	0.50	1.00
83	Holckenhavn Fjord	T.33	5.8	0.27	0.30	0.36	0.53	1.07
84	Kerteminde Fjord	T.18	5.7	0.27	0.30	0.37	0.54	1.09
85	Kertinge Nor	T.18	5.2	0.30	0.33	0.40	0.60	1.19
86	Nyborg Fjord	T.6	8.4	0.18	0.21	0.25	0.37	0.74
87	Helnæs Bugt	T.13	7.7	0.20	0.22	0.27	0.40	0.81
89	Lunkebugten	T.7	7.9	0.20	0.22	0.27	0.39	0.79
90	Langelandssund	T.22	9.5	0.16	0.18	0.22	0.33	0.65
92	Odense Fjord, ydre	T.31	5.6	0.28	0.31	0.37	0.55	1.11
93	Odense Fjord, indre	T.35	5.4	0.29	0.32	0.39	0.57	1.15
95	Storebælt, SV	T.20	10.6	0.15	0.16	0.20	0.29	0.59
96	Storebælt, NV	T.20	10.5	0.15	0.16	0.20	0.30	0.59
101	Genner Bugt	T.20	10.9	0.14	0.16	0.19	0.28	0.57
102	Åbenrå Fjord	T.23	12.9	0.12	0.13	0.16	0.24	0.48
103	Als Fjord	T.23	10.5	0.15	0.16	0.20	0.30	0.59
104	Als Sund	T.17	7.7	0.20	0.22	0.27	0.40	0.81
105	Augustenborg Fjord	T.17	6.9	0.22	0.25	0.30	0.45	0.90

Table 3.2 continues on next page.

Table 3.2 continued.

WB id	Water body	Type	Eelgrass	Cumulative cover attenuation coefficient (k_{bio})				
			RC	RC	HG	GM	MP	PB
106	Haderslev Fjord	T.36	7.1	0.22	0.24	0.30	0.44	0.87
108	Avnø Vig	T.8	6.3	0.25	0.27	0.33	0.49	0.98
109	Hejlsminde Nor	T.35	6.6	0.24	0.26	0.32	0.47	0.94
110	Nybøl Nor	T.17	7.5	0.21	0.23	0.28	0.41	0.83
113	Flensborg Fjord, indre	T.23	8.9	0.17	0.19	0.24	0.35	0.70
114	Flensborg Fjord, ydre	T.23	13.0	0.12	0.13	0.16	0.24	0.48
122	Vejle Fjord, ydre	T.22	9.9	0.16	0.17	0.21	0.31	0.63
123	Vejle Fjord, indre	T.28	7.6	0.20	0.23	0.28	0.41	0.82
124	Kolding Fjord, indre	T.28	7.4	0.21	0.23	0.28	0.42	0.84
125	Kolding Fjord, ydre	T.17	7.4	0.21	0.23	0.28	0.42	0.84
127	Horsens Fjord, ydre	T.10	11.5	0.13	0.15	0.18	0.27	0.54
128	Horsens Fjord, indre	T.10	8.0	0.19	0.22	0.26	0.39	0.78
129	Nissum Fjord, ydre	T.15	5.4	0.29	0.32	0.39	0.57	1.15
132	Ringkøbing Fjord	T.37	4.2	0.37	0.41	0.50	0.74	1.48
138	Hevring Bugt	T.22	9.2	0.17	0.19	0.23	0.34	0.67
139	Anholt	T.21	11.6	0.13	0.15	0.18	0.27	0.53
140	Djursland Øst	T.20	10.4	0.15	0.17	0.20	0.30	0.60
141	Ebeltoft Vig	T.22	9.9	0.16	0.17	0.21	0.31	0.63
142	Stavns Fjord	T.22	10.0	0.16	0.17	0.21	0.31	0.62
144	Knebel Vig	T.22	9.0	0.17	0.19	0.23	0.34	0.69
145	Kalø Vig, indre	T.22	9.5	0.16	0.18	0.22	0.33	0.65
146	Norsminde Fjord	T.33	5.6	0.28	0.31	0.37	0.55	1.11
147	Århus Bugt og Begtrup Vig	T.22	9.4	0.17	0.18	0.22	0.33	0.66
154	Kattegat, Læsø	T.21	10.0	0.16	0.17	0.21	0.31	0.62
159	Mariager Fjord, indre	T.30	7.7	0.20	0.23	0.27	0.41	0.81
160	Mariager Fjord, ydre	T.25	5.2	0.30	0.33	0.41	0.60	1.20
165	Isefjord, indre	T.17	5.2	0.30	0.33	0.40	0.60	1.19
200	Kattegat, Nordsjælland	T.21	12.4	0.13	0.14	0.17	0.25	0.50
201	Køge Bugt	T.12	9.5	0.16	0.18	0.22	0.33	0.65
204	Jammerland og Musholm Bugt	T.20	13.0	0.12	0.13	0.16	0.24	0.48
205	Kattegat, Nordsjælland > 20 m	T.21	11.6	0.13	0.15	0.18	0.27	0.53
206	Smålandsfarvandet, åbne del	T.22	9.5	0.16	0.18	0.22	0.33	0.65
207	Nakskov Fjord	T.14	6.1	0.25	0.28	0.34	0.51	1.02
208	Femerbælt	T.6	7.5	0.21	0.23	0.28	0.41	0.83
209	Rødsand	T.14	5.5	0.28	0.31	0.38	0.56	1.13
212	Faaborg Fjord	T.7	8.7	0.18	0.20	0.24	0.36	0.71
214	Det Sydfynske Øhav	T.13	11.2	0.14	0.15	0.19	0.28	0.55
216	Lillebælt, syd	T.23	9.6	0.16	0.18	0.22	0.32	0.65
217	Lillebælt, Bredningen	T.13	11.5	0.13	0.15	0.18	0.27	0.54
219	Århus Bugt syd, Samsø og Nordlige Bælthav	T.22	11.5	0.13	0.15	0.18	0.27	0.54
222	Kattegat, Aalborg Bugt	T.21	10.4	0.15	0.17	0.20	0.30	0.60
224	Nordlige Lillebælt	T.22	9.3	0.17	0.19	0.23	0.33	0.67
225	Nordlige Kattegat, Ålbæk Bugt	T.21	12.8	0.12	0.13	0.16	0.24	0.48
231	Snævringen	T.6	12.8	0.12	0.13	0.16	0.24	0.48

Table 3.3. Proposed reference conditions (RC) and class boundaries ((HG = High-Good, GM = Good-Moderate, MP = Moderate-Poor, PB = Poor-Bad) for the attenuation of number of perennial species with depth (in m^{-1}) calculated from reference conditions for eelgrass main depth limits (in m) through k_d. Reference conditions and class boundaries have not been calculated for 11 out the 109 Danish coastal water bodies due to missing reference conditions for eelgrass. Values for water bodies without reference condition for eelgrass could not be calculated and values for water bodies in Limfjorden are not shown.

WB id	Water body	Type	Eelgrass	Number of perennial species attenuation coefficient (k_{bio})				
			RC	RC	HG	GM	MP	PB
1	Roskilde Fjord, ydre	T.17	7.5	0.19	0.21	0.25	0.38	0.75
2	Roskilde Fjord, indre	T.27	4.9	0.29	0.32	0.39	0.58	1.15
6	Nordlige Øresund	T.20	8.5	0.17	0.18	0.22	0.33	0.66
16	Korsør Nor	T.18	5.2	0.27	0.30	0.37	0.54	1.09
17	Basnæs Nor	T.16	5.6	0.25	0.28	0.34	0.50	1.01
18	Holsteinsborg Nor	T.16	5.6	0.25	0.28	0.34	0.50	1.01
24	Isefjord, ydre	T.17	7.5	0.19	0.21	0.25	0.38	0.75
25	Skælskør Fjord og Nor	T.18	5.3	0.27	0.30	0.36	0.53	1.07
28	Sejerøbugt	T.22	13.3	0.11	0.12	0.14	0.21	0.42
29	Kalundborg Fjord	T.20	11.0	0.13	0.14	0.17	0.26	0.51
34	Smålandsfarvandet, syd	T.9	5.8	0.24	0.27	0.33	0.49	0.97
35	Karrebæk Fjord	T.29	5.6	0.25	0.28	0.34	0.50	1.01
36	Dybsø Fjord	T.18	5.5	0.26	0.29	0.35	0.51	1.03
37	Avnø Fjord	T.9	6.3	0.22	0.25	0.30	0.45	0.90
38	Guldborgssund	T.9	5.3	0.27	0.30	0.36	0.53	1.07
44	Hjelm Bugt	T.12	9.8	0.14	0.16	0.19	0.29	0.58
45	Grønsund	T.20	11.2	0.13	0.14	0.17	0.25	0.50
46	Fakse Bugt	T.12	7.3	0.19	0.21	0.26	0.39	0.77
47	Præstø Fjord	T.17	5.4	0.26	0.29	0.35	0.52	1.05
48	Stege Bugt	T.14	4.1	0.34	0.38	0.46	0.68	1.36
49	Stege Nor	T.24	5.3	0.27	0.30	0.36	0.53	1.07
56	Østersøen, Bornholm	T.5	10.2	0.14	0.15	0.19	0.28	0.55
57	Østersøen, Christiansø	T.5	10.2	0.14	0.15	0.19	0.28	0.55
59	Nærå Strand	T.25	5.2	0.27	0.30	0.37	0.54	1.09
62	Lillestrand	T.11	6.0	0.24	0.26	0.32	0.47	0.94
68	Lindelse Nor	T.16	6.4	0.22	0.25	0.30	0.44	0.88
72	Kløven	T.16	6.5	0.22	0.24	0.29	0.43	0.87
74	Bredningen	T.38	6.2	0.23	0.25	0.31	0.46	0.91
80	Gamborg Fjord	T.13	7.6	0.19	0.21	0.25	0.37	0.74
82	Aborg Minde Nor	T.38	6.2	0.23	0.25	0.31	0.46	0.91
83	Holckenhavn Fjord	T.33	5.8	0.24	0.27	0.33	0.49	0.97
84	Kerteminde Fjord	T.18	5.7	0.25	0.28	0.33	0.50	0.99
85	Kertinge Nor	T.18	5.2	0.27	0.30	0.37	0.54	1.09
86	Nyborg Fjord	T.6	8.4	0.17	0.19	0.23	0.34	0.67
87	Helnæs Bugt	T.13	7.7	0.18	0.20	0.25	0.37	0.73
89	Lunkebugten	T.7	7.9	0.18	0.20	0.24	0.36	0.71
90	Langelandssund	T.22	9.5	0.15	0.17	0.20	0.30	0.59
92	Odense Fjord, ydre	T.31	5.6	0.25	0.28	0.34	0.50	1.01
93	Odense Fjord, indre	T.35	5.4	0.26	0.29	0.35	0.52	1.05
95	Storebælt, SV	T.20	10.6	0.13	0.15	0.18	0.27	0.53
96	Storebælt, NV	T.20	10.5	0.13	0.15	0.18	0.27	0.54
101	Genner Bugt	T.20	10.9	0.13	0.14	0.18	0.26	0.52
102	Åbenrå Fjord	T.23	12.9	0.11	0.12	0.15	0.22	0.44
103	Als Fjord	T.23	10.5	0.13	0.15	0.18	0.27	0.54
104	Als Sund	T.17	7.7	0.18	0.20	0.25	0.37	0.73

Table 3.3 continues on next page.

Table 3.3 continued.

WB id	Water body	Type	Eelgrass	Number of perennial species attenuation coefficient (k_{bio})				
			RC	RC	HG	GM	MP	PB
105	Augustenborg Fjord	T.17	6.9	0.20	0.23	0.28	0.41	0.82
106	Haderslev Fjord	T.36	7.1	0.20	0.22	0.27	0.40	0.80
108	Avnø Vig	T.8	6.3	0.22	0.25	0.30	0.45	0.90
109	Hejlsminde Nor	T.35	6.6	0.21	0.24	0.29	0.43	0.86
110	Nybøl Nor	T.17	7.5	0.19	0.21	0.25	0.38	0.75
113	Flensborg Fjord, indre	T.23	8.9	0.16	0.18	0.21	0.32	0.63
114	Flensborg Fjord, ydre	T.23	13.0	0.11	0.12	0.15	0.22	0.43
122	Vejle Fjord, ydre	T.22	9.9	0.14	0.16	0.19	0.29	0.57
123	Vejle Fjord, indre	T.28	7.6	0.19	0.21	0.25	0.37	0.74
124	Kolding Fjord, indre	T.28	7.4	0.19	0.21	0.26	0.38	0.76
125	Kolding Fjord, ydre	T.17	7.4	0.19	0.21	0.26	0.38	0.76
127	Horsens Fjord, ydre	T.10	11.5	0.12	0.14	0.17	0.25	0.49
128	Horsens Fjord, indre	T.10	8.0	0.18	0.20	0.24	0.35	0.71
129	Nissum Fjord, ydre	T.15	5.4	0.26	0.29	0.35	0.52	1.05
132	Ringkøbing Fjord	T.37	4.2	0.34	0.37	0.45	0.67	1.34
138	Hevring Bugt	T.22	9.2	0.15	0.17	0.21	0.31	0.61
139	Anholt	T.21	11.6	0.12	0.14	0.16	0.24	0.49
140	Djursland Øst	T.20	10.4	0.14	0.15	0.18	0.27	0.54
141	Ebeltoft Vig	T.22	9.9	0.14	0.16	0.19	0.29	0.57
142	Stavns Fjord	T.22	10.0	0.14	0.16	0.19	0.28	0.56
144	Knebel Vig	T.22	9.0	0.16	0.17	0.21	0.31	0.63
145	Kalø Vig, indre	T.22	9.5	0.15	0.17	0.20	0.30	0.59
146	Norsminde Fjord	T.33	5.6	0.25	0.28	0.34	0.50	1.01
147	Århus Bugt og Begtrup Vig	T.22	9.4	0.15	0.17	0.20	0.30	0.60
154	Kattegat, Læsø	T.21	10.0	0.14	0.16	0.19	0.28	0.56
159	Mariager Fjord, indre	T.30	7.7	0.18	0.20	0.25	0.37	0.74
160	Mariager Fjord, ydre	T.25	5.2	0.27	0.30	0.37	0.55	1.09
165	Isefjord, indre	T.17	5.2	0.27	0.30	0.37	0.54	1.09
200	Kattegat, Nordsjælland	T.21	12.4	0.11	0.13	0.15	0.23	0.46
201	Køge Bugt	T.12	9.5	0.15	0.17	0.20	0.30	0.59
204	Jammerland og Musholm Bugt	T.20	13.0	0.11	0.12	0.15	0.22	0.43
205	Kattegat, Nordsjælland > 20 m	T.21	11.6	0.12	0.14	0.16	0.24	0.49
206	Smålandsfarvandet, åbne del	T.22	9.5	0.15	0.17	0.20	0.30	0.59
207	Nakskov Fjord	T.14	6.1	0.23	0.26	0.31	0.46	0.93
208	Femerbælt	T.6	7.5	0.19	0.21	0.25	0.38	0.75
209	Rødsand	T.14	5.5	0.26	0.29	0.35	0.51	1.03
212	Faaborg Fjord	T.7	8.7	0.16	0.18	0.22	0.32	0.65
214	Det Sydfynske Øhav	T.13	11.2	0.13	0.14	0.17	0.25	0.50
216	Lillebælt, syd	T.23	9.6	0.15	0.16	0.20	0.29	0.59
217	Lillebælt, Bredningen	T.13	11.5	0.12	0.14	0.17	0.25	0.49
219	Århus Bugt syd, Samsø og Nordlige Bælthav	T.22	11.5	0.12	0.14	0.17	0.25	0.49
222	Kattegat, Aalborg Bugt	T.21	10.4	0.14	0.15	0.18	0.27	0.54
224	Nordlige Lillebælt	T.22	9.3	0.15	0.17	0.21	0.30	0.61
225	Nordlige Kattegat, Ålbæk Bugt	T.21	12.8	0.11	0.12	0.15	0.22	0.44
231	Snævringen	T.6	12.8	0.11	0.12	0.15	0.22	0.44

3.7 Classification of macroalgae indicators in practice

Given the class boundaries (*Tables 3.2* and *3.3*) and the estimated macroalgae indicators, the status of macroalgae can be assessed based on the indicators' cumulative cover and number of perennial species (*Fig. 3.13*). The classification principles are illustrated with two examples, Odense Fjord ydre and Kattegat Læsø, representing two different types of water bodies.

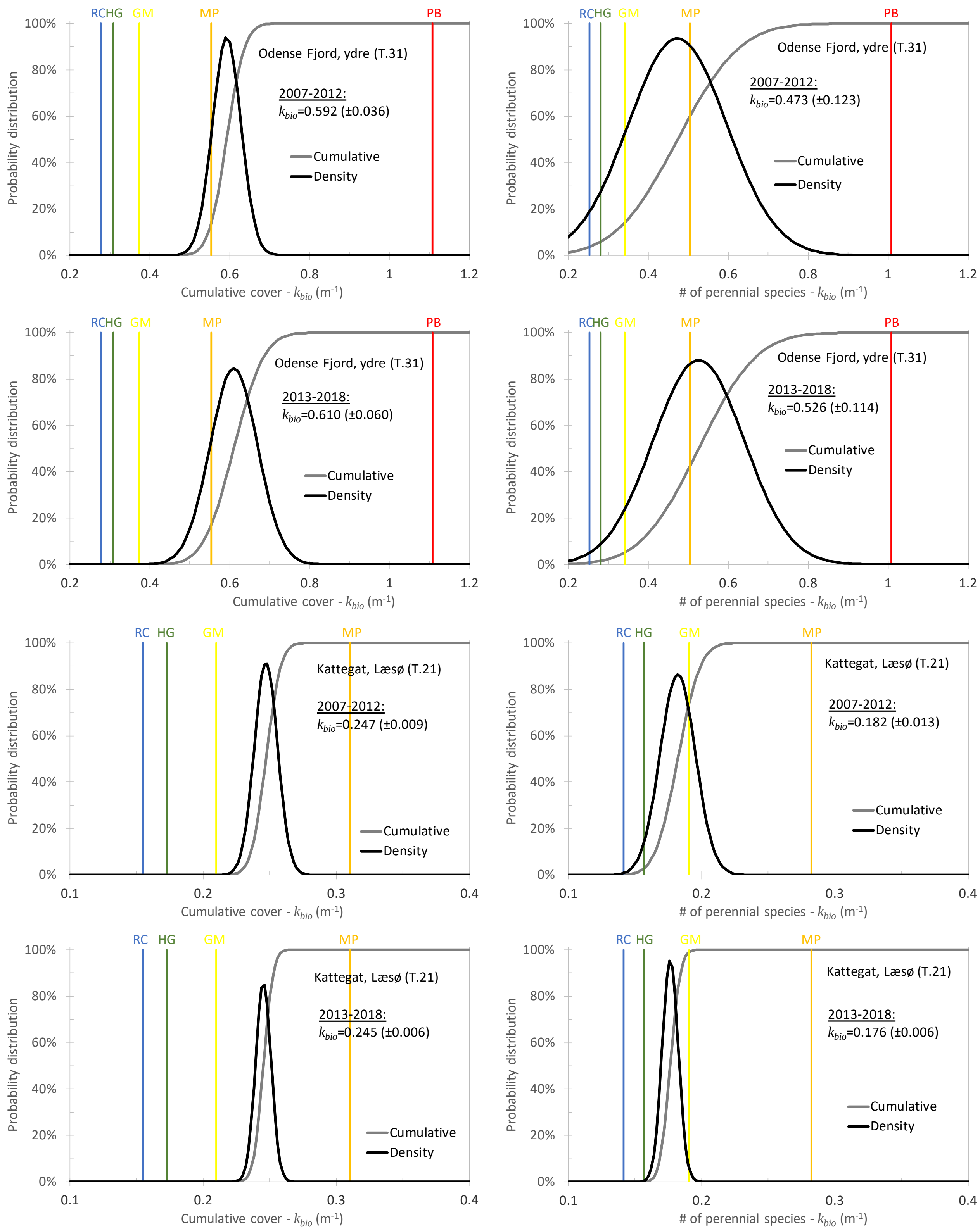


Figure 3.13. Estimated macroalgae indicators for cumulative cover (left) and number of perennial species (right) compared to suggested class boundaries (*Tables 3.2* and *3.3*). The indicator distributions are shown with both the probability density function and the cumulative density function (calculated from the normal distribution with mean and standard error of k_{bio} shown as inserts), and the indicator mean and standard error of the mean are listed as inserts. Class boundaries: RC = Reference Condition, HG = High-Good, GM = Good-Moderate, MP = Moderate-Poor, PB = Poor-Bad. The PB boundary for Kattegat Læsø is not shown.

In Odense Fjord ydre, k_{bio} for cumulative cover was estimated to 0.592 m^{-1} and 0.610 m^{-1} for the first and second 6-year period, respectively. The standard errors of these estimates were ± 0.036 m^{-1} and ± 0.060 m^{-1}, respectively, showing that the k_{bio} indicator for cumulative cover was well determined for both periods. For both periods, the indicator distribution was located mainly in the poor status class, although there was still 14 % and 17 % probability of achieving moderate status class, respectively. Thus, the status is that macroalgae cumulative cover in Odense Fjord is poor with 86 % and 83 % confidence.

Similar k_{bio} estimates for the number of perennial species were obtained for Odense Fjord ydre (0.473 m^{-1} and 0.526 m^{-1} for the two periods with standard errors of ± 0.123 m^{-1} and ± 0.114 m^{-1}). These two parameters were estimated with relatively higher uncertainty, reflected in the broad distributions spanning even across the reference condition. These indicator estimates suggest that the status is moderate (evaluated by the median) for the first period and poor for the second, although the probabilities of poor and moderate status, respectively, were also high. There was a minor probability of achieving both high (6 % and 2 %) and good (8 % and 4 %) status.

So, for Odense Fjord ydre, the cumulative cover of macroalgae indicated poor status in both periods, while the number of perennial macroalgal species indicated moderate and poor status.

For Kattegat Læsø, k_{bio} for cumulative cover was estimated to 0.247 m^{-1} and 0.245 m^{-1} with standard errors of 0.009 m^{-1} and 0.006 m^{-1} for the first and second 6-year period, respectively. The distributions of these two indicator estimates were located in the moderate status class. For both periods the probability of moderate status was 100 %.

The k_{bio} estimates for the number of perennial species were 0.182 m^{-1} and 0.176 m^{-1} for Kattegat Læsø in the two periods with standard errors of 0.013 m^{-1} and 0.006 m^{-1}, respectively. These indicator distributions were mostly within the good status class (71 % and 99 %, respectively). Correspondingly, the probability of moderate status class was 26 % and 1 % for the two periods, respectively, whereas there was 3 % probability of high status in the first period. Hence, the number of perennial species was most likely in good status for the two periods.

So, for Kattegat, the cumulative cover of macroalgae indicated moderate status while the number of perennial macroalgal species indicated good status.

3.8 Combining indicators for macroalgae and eelgrass

Three indicators can be used for assessing the status of the BQE 'macroalgae and angiosperms': 1) macroalgae cumulative cover, 2) number of perennial macroalgae species, and 3) eelgrass main depth limit. The aggregation principles outlined in Section 2.4 are exemplified with macroalgae indicators estimated for Odense Fjord ydre and Kattegat Læsø (*Fig. 3.13*) supplemented with the means and standard errors of the mean for eelgrass main depth limits for the same water bodies and periods.

The first step in the BQE assessment was to translate k_{bio} estimates for cumulative cover and number of perennial species into standardised EQR values (*Fig. 3.14*) using the piecewise linear transformation (*Fig. 2.4*) based on suggested boundary values (*Tables 3.2* and *3.3*). This transformation resulted in a reversal of the scale order, as EQR was negatively related to k_{bio}, i.e. increasing k_{bio} indicates a worsening ecological status. Importantly, however, the probability distribution among classes was maintained with the transformation.

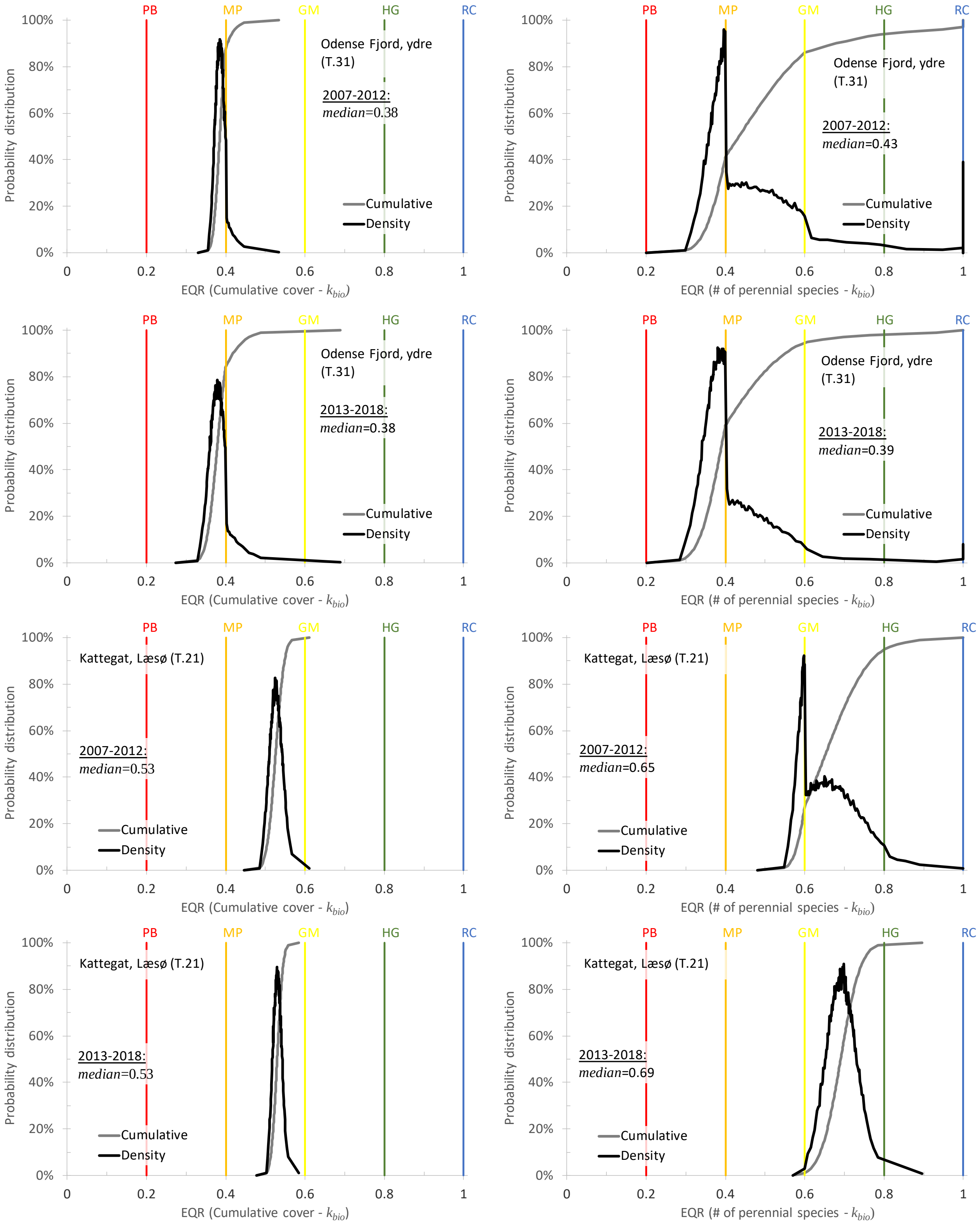


Figure 3.14. Distributions of estimated macroalgae indicators for cumulative cover (left) and number of perennial species (right) translated into the standardised EQR scale using suggested class boundaries (*Tables 3.2* and *3.3*). The EQR distributions are shown with both the probability density function and the cumulative density function, and the indicator median (face value approach) is shown as insert. Class boundaries: RC = Reference Condition, HG = High-Good, GM = Good-Moderate, MP = Moderate-Poor, PB = Poor-Bad.

For Odense Fjord ydre, the standardized EQR distributions for the two periods had median values of 0.38 (for both periods) for cumulative cover and 0.43 and 0.39 for number of perennial species. The distributions for cumulative cover were almost entirely within the poor status class, whereas the distributions for number of perennial species overlapped the poor-moderate boundary. It should be noted that the distributions for number of perennial species displayed a peak at EQR = 1, since the EQR transformation truncates values lower than the reference condition. For Kattegat Læsø, the standardized EQR distributions for the two periods had median values of 0.53 (both periods) for cumulative cover and 0.65 and 0.69 for number of perennial species. Cumulative cover had distributions almost entirely within the moderate status, whereas the distributions for number of perennial species were mainly in the good status class.

Eelgrass main depth limit had estimated means of 2.37 m and 2.49 m for the two periods in Odense Fjord ydre, and means of 4.92 m and 5.77 m for the two periods in Kattegat Læsø. The standard errors of these means were ±0.14, ±0.16, ±0.11 and ±0.28 m, respectively. After transformation into the standardized EQR scale, the means of the eelgrass main depth limit indicator were 0.34 and 0.36 for the two periods in Odense Fjord ydre and 0.39 and 0.46 for the two periods in Kattegat Læsø (*Fig. 3.15*). These results suggest that ecological status for eelgrass was poor in Odense Fjord ydre for both periods, whereas status changed from poor to moderate in Kattegat Læsø.

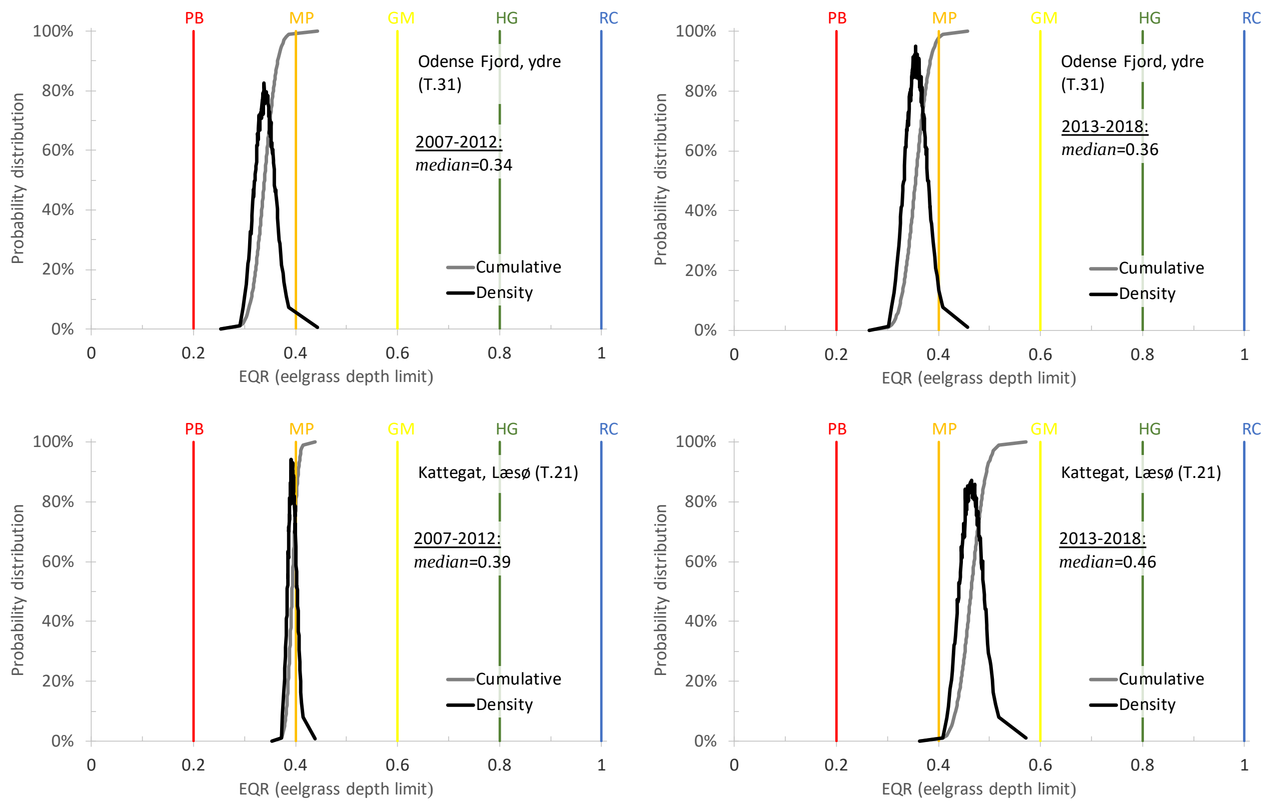


Figure 3.15. Distributions of estimated eelgrass depth limit translated into the standardised EQR scale using established boundaries (Bekendtgørelse nr. 1001, 2016). The EQR distributions are shown with both the probability density function and the cumulative density function, and the indicator median (face value approach) is shown as insert. Class boundaries: RC = Reference Condition, HG = High-Good, GM = Good-Moderate, MP = Moderate-Poor, PB = Poor-Bad.

Second step in the BQE assessment was to aggregate cumulative cover and number of perennial species into a combined macroalgae indicator. This aggregation was done by averaging the standardised EQR distributions (*Fig. 3.16*).

For Odense Fjord ydre, the two indicator distributions were different with a narrow distribution for cumulative cover and a broader distribution for number of perennial species (*Fig. 3.14*). For the first period (2007-2012) the resulting distribution, the average of the two indicators, had a median of 0.41 (moderate status) and a marked second peak around 0.7, which resulted from the spike in the distribution at EQR = 1 for number of perennial species (*Fig. 3.16*). This latter feature was not pronounced for the second period, having a median of 0.39 (poor status), since only a minor part of the indicator distribution was truncated at EQR = 1 (*Fig. 3.14*).

For Kattegat Læsø, the two input distributions for the aggregation were slightly different and the resulting distribution represents a compromise between the two. Whereas cumulative cover was distributed in the middle of the moderate status class, the number of perennial species was distributed in the good status class (*Fig. 3.14*). Consequently, the combined distribution for macroalgae was distributed around the good-moderate boundary with a median suggesting moderate status in the first period (with 60 % confidence) and good status in the second period (with 68 % confidence) (*Fig. 3.16*).

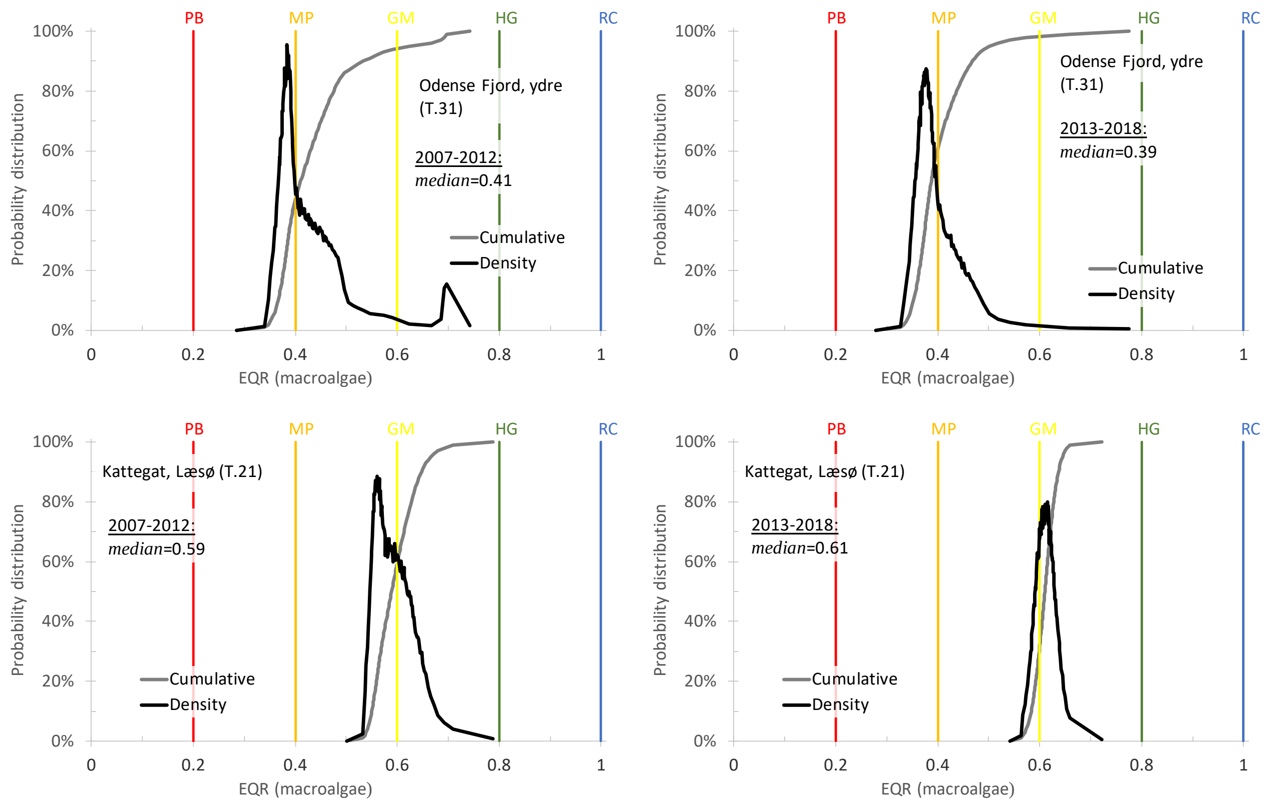


Figure 3.16. Distributions of the combined macroalgae indicator on the standardised EQR scale. The EQR distributions are shown with both the probability density function and the cumulative density function, and the indicator mean and standard error of the mean are listed as inserts. Class boundaries: RC = Reference Condition, HG = High-Good, GM = Good-Moderate, MP = Moderate-Poor, PB = Poor-Bad.

The final step in the BQE assessment was to aggregate the combined macroalgae indicator (*Fig. 3.16*) and eelgrass main depth limit (*Fig. 3.15*) into an overall benthic vegetation indicator (*Fig. 3.17*) by averaging the standardised distributions.

For Odense Fjord ydre, the macroalgae distribution was located around the moderate-poor boundary and the eelgrass distribution was located in the poor status class and therefore, the combined distribution was located primarily in the poor status class (with 74 % and 82 % confidence for the two periods, respectively). Overall, the status of the BQE 'Macroalgae and angiosperms' remained unaltered between the two periods, because the improvements in eelgrass depth limits were outbalanced by decreases in number of perennial species.

For Kattegat Læsø, the combined indicator for macroalgae and angiosperms was moderate with high confidence (100 % and 99 % for the two periods, respectively), which was a compromise between eelgrass distributions around the moderate-poor boundary (*Fig. 3.15*) and macroalgae distributions around the good-moderate boundary (*Fig. 3.16*). Kattegat Læsø showed increasing status (assessed by the median) from 2007-2012 to 2013-2018, which was driven by improved status of number of perennial species and eelgrass main depth limit.

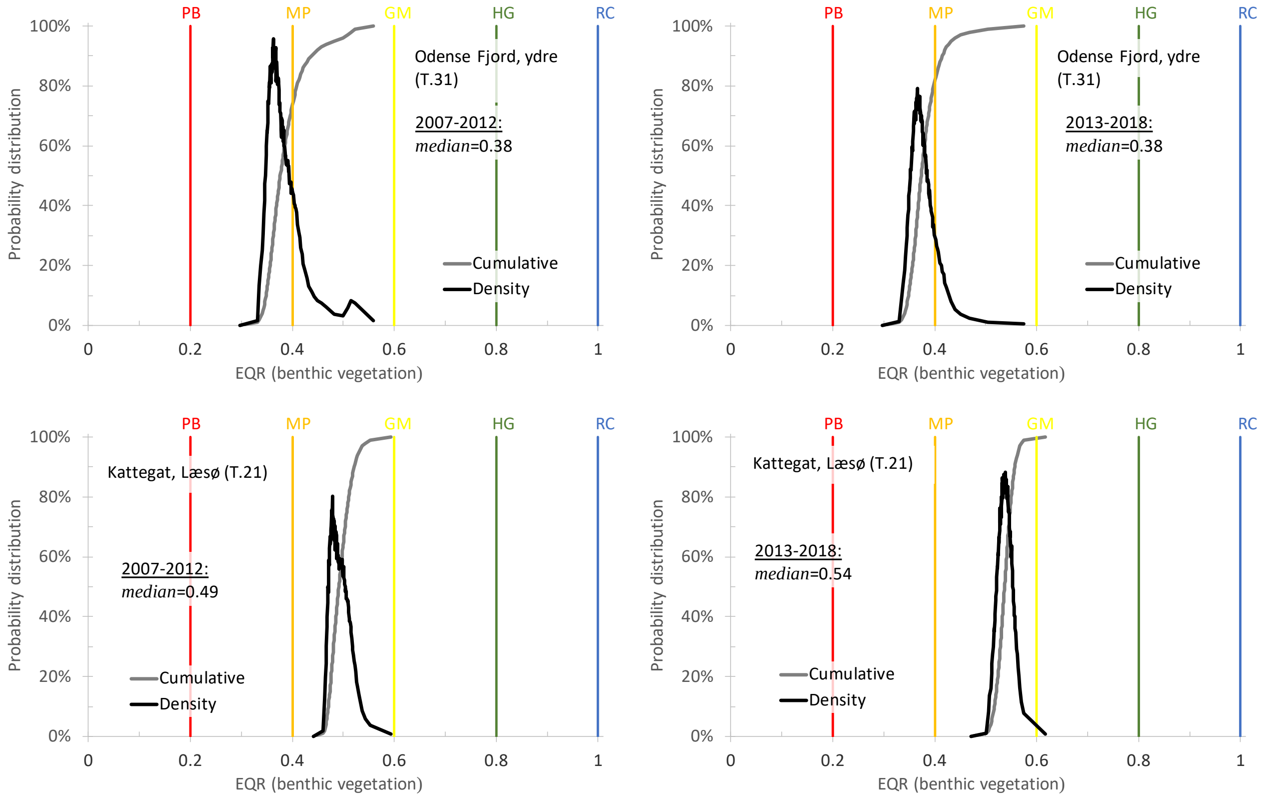


Figure 3.17. Distributions of the combined benthic vegetation indicator on the standardised EQR scale. The EQR distributions are shown with both the probability density function and the cumulative density function, and the indicator mean and standard error of the mean are listed as inserts. Class boundaries: RC = Reference Condition, HG = High-Good, GM = Good-Moderate, MP = Moderate-Poor, PB = Poor-Bad.

4 Conclusions

In this report, three macroalgae indices (cumulative cover, number of perennial species and relative cover of opportunists) have been compiled from Danish monitoring data covering 58 water bodies distributed over 23 types. These indices have been analysed using a non-linear statistical model, disentangling effects of physical exposure, light limitation, sea urchin grazing and salinity-dependent responses. The cover of sea urchins is not consistently monitored in the Danish coastal monitoring programme, but the grazing effect has been estimated from stone reefs in the open waters (Carstensen & Dahl 2019) and was included in the present analysis. It has been demonstrated how macroalgae indicators that respond to eutrophication can be derived from the models and made operational. Finally, it has also been shown how macroalgae indicators can be combined with indicators for angiosperms, providing a complete assessment of the WFD biological quality element 'macroalgae and angiosperms'.

Based on the results from the analyses in the present report, it is concluded:

- Cumulative cover and the number of perennial species exhibit marked depth gradients with three distinct phases: 1) reduced cover/species due to physical exposure near the surface, 2) maximum cover/species at intermediate depth with minimal physical exposure and light limitation, and 3) reduced cover/species at deeper depths due to light limitation.
- Physical exposure has a significant reducing effect on the cumulative cover and the number of perennial species in shallow waters, most pronounced for depths shallower than 2 m.
- Light conditions regulate the cumulative macroalgae cover and the number of perennial species at deeper depths, reducing the number of species and the cumulative cover exponentially with depth (k_{bio}) in a manner similar to the attenuation of light (k_d). The steepest declines in the number of perennial species and the cumulative cover (large k_{bio}) were observed at locations with poor light conditions (large k_d), indicating eutrophication.
- Salinity is an important factor influencing variations in all three macroalgae indices. The maximum cumulative cover and the maximum number of perennial species increase with salinity, exhibiting more than a doubling over the studied salinity range. This implies that macroalgae communities are richer in saline waters, allowing multilayered species structures. The relative cover of opportunists also changed with salinity, with brackish water hosting a larger proportion of opportunists than saline waters. The strong relationships with salinity for all three indices highlight the importance of considering salinity-induced variations when comparing macroalgae data across WFD water bodies.
- Significant relationships between the attenuation of cumulative cover and number of perennial species with depth (k_{bio}) versus the light attenuation coefficient (k_d) document that k_{bio} constitutes a good indicator of eutrophication on the macroalgae community. Moreover, these relationships can be used to establish WFD class boundaries from boundaries for k_d that were derived from eelgrass reference conditions and class boundaries.
- The model parameters for relative cover of opportunists on suitable hard substrate did not show any significant relationship to light and nutrient conditions. This macroalgae index was primarily controlled by salinity conditions showing a marked change in the macroalgae community from

high proportion of opportunistic species at salinity < 10 to low proportion at salinity > 20. Hence, the relative cover of opportunists is not considered suitable as eutrophication operational indicator for assessing ecological status *sensu* the WFD.

- The ecological status of macroalgae can be assessed by means of aggregating k_{bio} estimates from cumulative cover and number of perennial species into a combined macroalgae indicator. Aggregation can be done by transforming the two k_{bio} estimates into a standardised EQR scale before averaging.
- The ecological status of the WFD's biological quality element 'macroalgae and angiosperms' can be assessed by aggregating the combined macroalgae indicator with the existing indicator for angiosperms (eelgrass main depth limit). Following a transformation of eelgrass main depth limit to a standardised EQR scale, aggregation can be done by averaging. This approach of aggregating indicators is generic and can be used in an extended version, in case additional indicators are developed and associated with reference conditions and class boundaries.
- Indicators for macroalgae and angiosperms are associated with uncertainty and should be described by their statistical distributions. Aggregation of indicator distributions can be done by Monte Carlo simulation.

5 Recommendations

For assessing the ecological status of the WFD's biological quality element 'Macroalgae and angiosperms', it is recommended that:

- The status of macroalgae is assessed by parameter estimates of the attenuation with depth (k_{bio}) for cumulative cover and number of perennial species. These two parameter estimates are termed macroalgae indicators.
- Class boundaries for these two macroalgae indicators are determined from boundaries for the attenuation of light (k_d), obtained from eelgrass main depth limit boundaries, through established linear relationships.
- A combined macroalgae indicator is determined by averaging the two macroalgae indicators after transformation to a standardised EQR scale.
- A combined indicator for the WFD's biological quality element 'Macroalgae and angiosperms' is determined by averaging the combined macroalgae indicator with the established eelgrass indicator main depth limit (or angiosperm main depth limit), after transformation to a standardised EQR scale.
- All indicators and their aggregates should be described through their statistical distribution.
- The status of the biological quality element 'Macroalgae and angiosperms' should be assessed as the median of the combined indicator for macroalgae and angiosperms.
- The probabilities of the different status classes for the combined indicator should be quantified and used in the overall assessment of ecological status.
- The macroalgae community in Limfjorden should be studied more closely in relation to other possible stressors than light conditions (e.g. sea urchin grazing, substrate, trawling), and this knowledge should be used as a basis for developing specific assessment methods for macroalgae in Limfjorden.

For improving the monitoring programme and data used for assessing the ecological status of macroalgae, it is recommended that:

- The cover of sea urchins is monitored consistently in order to reduce grazing effects on macroalgae in the status assessment. Including such information is likely to improve the estimation of k_{bio}.
- The depth ranges of macroalgae transects with suitable hard substrate are assessed in relation to expected attenuation in cumulative cover and number of perennial species. Transects that do not cover depths with light limitation provide little information for estimating the macroalgae indicators. Each water body should include at least one transect exhibiting light regulation. The results of the present report should be used for optimising macroalgae monitoring efforts.
- The potential of using information on 'non-firmly' attached macroalgae from the monitoring data for indicator development should be investigated.

6 References

Bekendtgørelse nr. 1001 (2016) Bekendtgørelse om overvågning af overfladevandets, grundvandets og beskyttede områders tilstand og om naturovervågning af internationale naturbeskyttelsesområder. https://www.retsinformation.dk/eli/lta/2016/1001

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. https://dce2.au.dk/pub/SR93.pdf

Carstensen J, Krause-Jensen D (2018) Indicators for ecological status of angiosperms and charophytes in Danish coastal waters. Research note from DCE – Danish Centre for Environment and Energy. https://pure.au.dk/portal/files/123732609/Blomsterplanter_indikatorer.pdf

Carstensen J, Dahl K (2019) Macroalgal indicators for Danish Natura 2000 habitats. Aarhus University, DCE – Danish Centre for Environment and Energy, 45 pp. Technical Report No. 142. http://dce2.au.dk/pub/TR142.pdf

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

Erichsen A, Møhlenberg F, Timmermann K, Christensen JPA, Göke C (2019) Gennemgang af grundlaget for afgrænsning, karakterisering og typeinddeling af kystvande i vandområdeplanerne. Report from DHI and Aarhus University. https://dce.au.dk/fileadmin/dce.au.dk/Udgivelser/Eksterne_udgivelser/Afgraensning-karakterisering-typologi.pdf

Gattuso J P, Gentili B, Duarte CM, Kleypas JA, Middelburg JJ, Antoine D (2006). Light availability in the coastal ocean: impact on the distribution of benthic photosynthetic organisms and their contribution to primary production. Biogeosciences 3:489–513.

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. https://dce2.au.dk/pub/SR253.pdf

Høgslund S, Carstensen J, Krause-Jensen D (2018) Historiske observationer af makroalger og blomsterplanter andre end ålegræs i danske kystområder. Notat fra DCE . https://dce.au.dk/fileadmin/dce.au.dk/Udgivelser/Notater_2018/Historiske_observationer_af_makroalger.pdf

Krause-Jensen D, Carstensen J (2018) Light requirements of marine rooted macrophytes. Research note from DCE – Danish Centre for Environment and Energy.
https://dce.au.dk/fileadmin/dce.au.dk/Udgivelser/Notater_2018/Light_requirements_of_marine_rooted_macrophytes.pdf

Lawrence JM (2001) Edible sea urchins: Biology and ecology. Development in Aquaculture and Fisheries 32. Elsevier, Amsterdam. 432 pp.

Markager S, Sand-Jensen K (1992) Light requirements and depth zonation of marine macroalgae. Marine Ecology Progress Series 88:83–92.

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

Timmermann K, Christensen JPA, Erichsen A (2020) Referenceværdier og grænseværdier for ålegræsdybdegrænser til brug for vandområdeplanerne. Aarhus Universitet, DCE – Nationalt Center for Miljø og Energi, 27 s.

WFD CIS Guidance Document No. 5 (2003). Transitional and Coastal Waters – Typology, Reference Conditions and Classification Systems. Published by the Directorate General Environment of the European Commission, Brussels, ISBN No. 92-894-5125-4, ISSN No. 1725–1087.

WFD CIS Guidance Document No. 7 (2003). Monitoring under the Water Framework Directive. Published by the Directorate General Environment of the European Commission, Brussels, ISBN No. 92-894-5127-0, ISSN No. 1725–1087.

WFD CIS Guidance Document No. 13 (2005). Overall Approach to the Classification of Ecological Status and Ecological Potential. Published by the Directorate General Environment of the European Commission, Brussels, ISBN No. 92-894-6968-4, ISSN No. 1725–1087.

MACROALGAE INDICATORS FOR ASSESSING ECOLOGICAL STATUS IN DANISH WFD WATER BODIES

This report investigates three macroalgae indices for assessing ecological status according to the Water Framework Directive using monitoring data from 58 water bodies in Denmark. The three indices are cumulative cover, perennial species richness and relative cover of opportunists. The different macroalgae indices were analysed with non-linear statistical models, describing regulation by light availability, salinity, grazing and physical exposure. These models separate anthropogenic disturbance from natural variations. The attenuation of cumulative cover and number of perennial species with depth constitute operational macroalgae indicators, which respond clearly to eutrophication status. Reference conditions and class boundaries, calculated from established values for light attenuation, as well as an aggregation scheme for combining macroalgae indicators with the indicator for eelgrass main depth limit are proposed. The macroalgae indicator based on the relative cover of opportunists was not operational, as it did not respond to any primary eutrophication variable.

ISBN: 978-87-7156-490-7
ISSN: 2245-019X