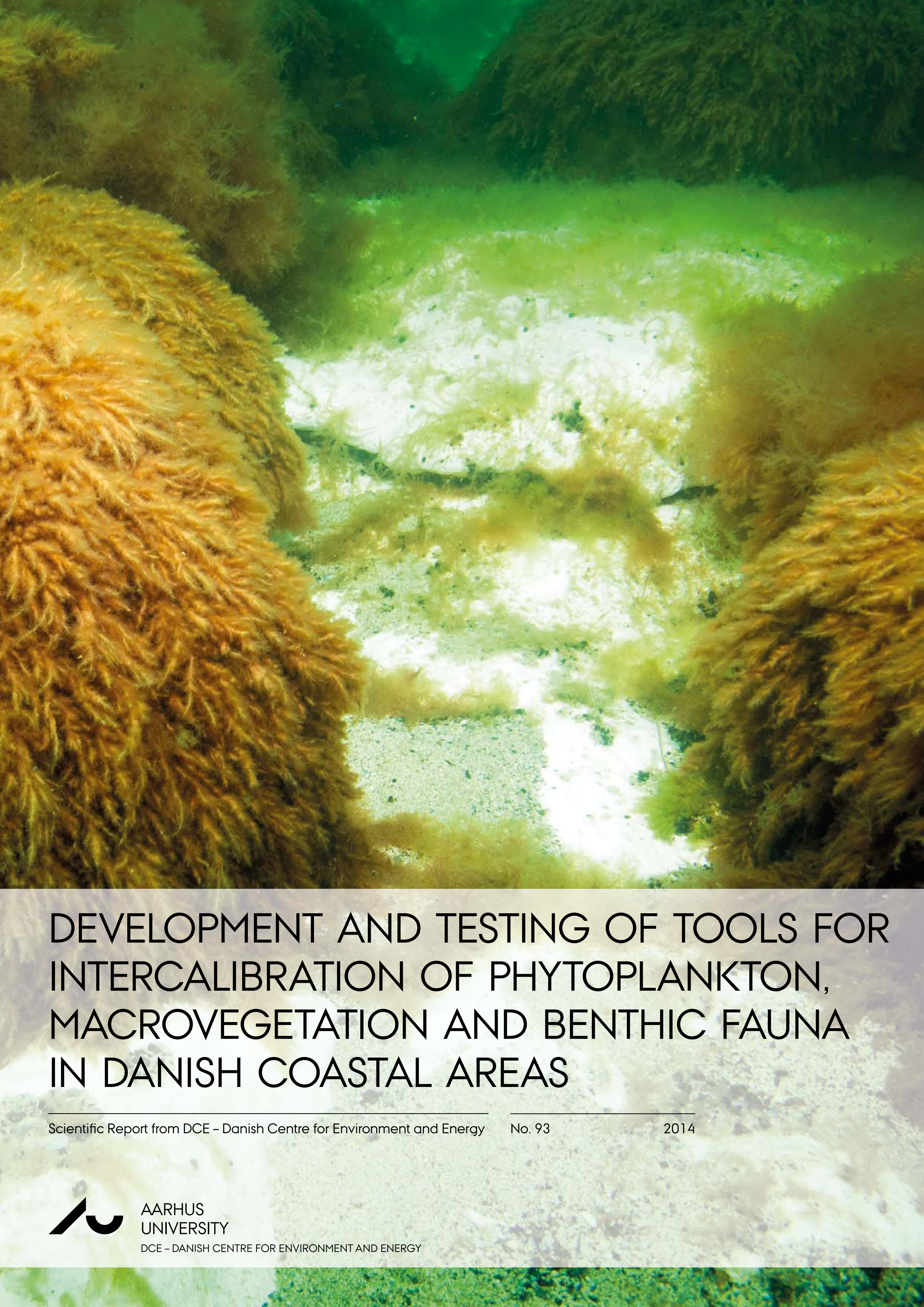

DEVELOPMENT AND TESTING OF TOOLS FOR INTERCALIBRATION OF PHYTOPLANKTON, MACROVEGETATION AND BENTHIC FAUNA IN DANISH COASTAL AREAS

Scientific Report from DCE – Danish Centre for Environment and Energy No. 93 2014

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Data sheet

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Abstract: This report contributes to the development of indicators and assessment tools for ecological status classification according to the European Water Framework Directive as well as the intercalibration of the phytoplankton biomass indicator with Sweden and Germany. For the open coastal waters in the Kattegat and the Sound Denmark and Sweden have similar reference conditions for summer chlorophyll, whereas class boundaries are more strict in Denmark. Different indicators for phytoplankton composition, based on the distribution of functional groups, have been tested and none of these indicators was found operational for assessing ecological status. Six indicators for macroalgae cover and composition have also been tested and three of these are suggested as the basis for an overall status assessment tool for macroalgae. The proposed assessment tool is modular and sufficiently flexible such that it can be readily adapted to other indicators and biological quality elements, for the WFD and other directives as well. The DKI indicator for benthic macrofauna integrates species sensitivity and diversity. Salinity is an important governing factor for diversity and therefore a salinity normalisation of the DKI is proposed.

Keywords: Water Framework Directive, phytoplankton, macroalgae, angiosperms, indicator development, statistical models, reference condition, status classification.

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Contents

Summary

This report describes the development of indicators and assessment tools for the Water Framework Directive's (WFD) marine biological quality elements: phytoplankton, macroalgae and benthic macrofauna. It also addresses intercalibration of these with Sweden and Germany.

Phytoplankton biomass, which is quantified as the summer chlorophyll *a* concentration, increases with nutrient levels, most significantly with the total nitrogen concentration. The suggested reference conditions in Danish and Swedish coastal waters, sharing the same typology, are similar, but Denmark has suggested a stricter boundary setting than Sweden, and these differences must be clarified.

Phytoplankton composition across coastal water bodies is mainly governed by differences in salinity, but increasing levels of total nitrogen also suggested a shift from dinoflagellates and other species to increasing dominance of diatoms. This shift is not perceived as a degradation of ecological status and the analyses of phytoplankton communities did not result in meaningful indicators sensitive to changes in nutrient levels. The scientific understanding of phytoplankton community responses to nutrient enrichment is not yet ripe for operational implementation in the WFD, and the implementation must await scientific advances in this field.

Macroalgae cover and composition have been described by six different indicators, which are not biased by differences in depth and substrate of the monitoring observations. Three of these indicators, selected according to their sensitivity to total nitrogen and the uncertainty involved in their estimation, are proposed as the basis for assessing the ecological status of this biological quality element.

An assessment tool is proposed for macroalgae, but the approach can be applied more generally to other biological quality elements. The idea is to transform all indicators to a common EQR-scale, where status classes are equidistantly distributed. Indicators for the same biological quality element can be combined by weighted average, once transformed to the common EQR-scale. A quality of the proposed tool is its transparency from the indicator level to the overall assessment.

The quality of soft sediment macrobenthic fauna may be measured by an index integrating diversity and sensitivity components of species, the Danish quality index DKI, albeit with restricted applicability.

An investigation was undertaken to identify important natural factors influencing local richness diversity (alpha) and consequently the Shannon diversity (H), one major component in the DKI index, to make the DKI operational in a wider context including Danish shallow coastal areas and estuaries. For this purpose, fauna data were used from an open sea area fringed by 17 estuaries. Both dispersal and environmental filtering were important in regulating alpha diversity in the estuaries, where salinity filtered primarily dispersive species. So although dispersal limitation may determine the species

composition of alpha, the level of alpha is determined by salinity. Thus, for the purpose of normalizing, DKI correction for salinity makes sense.

It is concluded that the original relationships between salinity and DKI components could be kept in the 'new' general version of DKIver2, re-described in this report with two minor amendments and more detailed suggestions of status determination procedure. It is recommended to use the salinity normalized DKIver2 index in general, and to use the 20th percentile of index data when evaluating status against a common set of boundaries for all types.

Sammenfatning

Denne rapport beskriver udviklingen af indikatorer og tilstandsvurderingsværktøjer til brug for vandrammedirektivets (VRD) marine biologiske kvalitetselementer: fytoplankton, makroalger og bentisk makrofauna. Rapporten behandler også interkalibreringen af disse kvalitetselementer med Sverige og Tyskland.

Biomassen af fytoplankton, kvantificeret som koncentrationen af klorofyl *a*, vokser med mængden af næringssalte, mest signifikant for mængden af total kvælstof. De foreslåede referenceværdier for kystnære områder i Danmark og Sverige med samme typologi er sammenlignelige, hvorimod grænseværdierne mellem kvalitetsklasser er mere restriktive i Danmark end i Sverige. Det er vigtigt for interkalibreringen, at denne forskel mellem grænseværdier afklares mellem landene.

Sammensætningen af fytoplankton i kystområder kan hovedsageligt forklares ved hjælp af salinitet. Derudover sker der et skift fra dinoflagellater til kiselalger ved stigende niveauer af total kvælstof. Da kiselalger generelt opfattes som gode alger med en effektiv overførsel af energi til højere trofiske niveauer, udtrykker en indikator baseret på forholdet mellem de to grupper ikke en forringelse af miljøkvaliteten ved stigende kvælstofniveauer. Vidensniveauet omkring styrende faktorer for fytoplanktonets sammensætning er endnu ikke tilstrækkeligt udviklet til, at der kan udvikles operationelle indikatorer til brug i VRD.

Dækningsgrader og sammensætning af makroalger er beskrevet ved hjælp af seks indikatorer, som korrigerer for forskelle i dybder og substrat i overvågningsdata. Tre af disse indikatorer, udvalgt som de mest følsomme over for ændringer i kvælstofniveauet og med den mindste usikkerhed, er foreslået som grundlag for at vurdere den økologiske tilstand af makroalgesamfundet.

Et tilstandsvurderingsværktøj er foreslået for makroalger, men værktøjet finder generel anvendelse for andre typer af biologiske kvalitetselementer og deres indikatorer. Grundtanken er at transformere alle indikatorværdier til en fælles EQR-skala, hvor tilstandsklasserne har samme inddeling. Indikatorerne kan derefter vægtes sammen til en samlet tilstandsvurdering for det biologiske kvalitetselement. En fordel ved dette værktøj er, at det er muligt at følge beregningerne trin for trin, fra indikator til samlet tilstandsvurdering.

Miljøstatus af bundfauna kan måles ved DKI-indekset, der integrerer diversitet og følsomhed over for forstyrrelser af arter, dog kun i områder med høj saltholdighed.

Med henblik på at gøre DKI mere generelt operationelt, også i lavvandede danske kystområder og fjorde, blev en analyse foretaget med det formål at identificere vigtige naturlige faktorer for diversiteten. Både spredningsrelaterede og miljørelaterede faktorer indvirker på alpha diversiteten i fjorde, og dermed også Shannons H, hvor arter med planktoniske larver spredes fra

åbne områder ind i fjordene, og hvor etableringens succes bestemmes af saltholdigheden. Eftersom flere arter kan etableres ved høj saltholdighed end ved lav saltholdighed, bliver diversiteten bestemt af saltholdigheden. Derfor er normalisering af DKI med hensyn til saltholdighed fornuftig.

Det anbefales, at man bruger de originale relationer mellem DKI-komponenter og saltholdighed fra konstruktionen af DKIver2. Indekset bliver beskrevet på ny i denne rapport med to tilføjelser til beregningen.

Det anbefales at indekset bliver benyttet generelt i danske farvande og at 20 % fraktilen af indeksdata bliver brugt med et fælles set af grænser med GM-grænsen 0,68 i stort set alle typer (undtaget type BC8, hvor grænsen er 0,72) i forbindelse med vurdering af status.

1 Introduction

This report documents the outcome of the Water Framework Directive (WFD) intercalibration project ‘Development and testing of tools for intercalibration of phytoplankton, benthic fauna and macrovegetation in Danish coastal areas’. The project is financed by the Danish Nature Agency.

The WFD aims to achieve at least a good ecological status in all European rivers, lakes and coastal waters and demands that the ecological status is quantified based primarily on biological indicators, i.e. phytoplankton and benthic flora and fauna. The WFD demands an evaluation of which water bodies are being at risk of failing to meet the good ecological status in 2015.

In order to assess the ecological status, it is necessary to identify biological indicators, which respond to environmental impact/anthropogenic pressures. Moreover, it is necessary to relate the levels of these indicators to biological status classes.

According to the WFD, the assessment systems for ecological status should be intercalibrated among member states belonging to the same geographical intercalibration group (GIG) and sharing the same typology. For the intercalibration of coastal waters, Denmark belongs to the intercalibration group for the Baltic Sea (Baltic GIG) and to the intercalibration group for the North-east Atlantic (NEA GIG). The first intercalibration phase took place from 2004-2007 and the second intercalibration phase took place from 2008-2012. The third intercalibration phase started in 2013 and will continue until 2016. The aim of the third intercalibration phase is to intercalibrate biological quality elements that were not covered in phase 1 and 2.

The overall objective of this project is to develop tools for assessing ecological status of biological quality elements, to intercalibrate class boundaries high/good and good/moderate for typologies shared with Sweden and Germany, and to contribute to the Danish reporting for the EU intercalibration.

The report is structured around three main chapters: one for each of the biological quality elements used to assess ecological status of coastal water bodies (phytoplankton, macroalgae and angiosperms, benthic fauna).

2 Phytoplankton

Phytoplankton is one of the three biological quality elements of the Water Framework Directive constituting the basis for the overall ecological status assessment. According to Annex V of the Directive, the ecological status of phytoplankton should be based on assessing 1) composition and abundance of phytoplankton taxa, 2) the average phytoplankton biomass, and 3) frequency and intensity of planktonic blooms. In this section, we will consider 1) indicators of phytoplankton biomass and its relationships to potential regulating physical-chemical factors and 2) indicators of the phytoplankton community composition and its relationships to potential physical-chemical factors. Indicators of phytoplankton biomass will be compared to Swedish values for the intercalibration areas NEA 8b.

In Danish coastal waters, phytoplankton production during the summer period is considered nitrogen-limited. Since production and biomass are strongly coupled, it is similarly expected that phytoplankton biomass will be limited by the amount of bioavailable nitrogen. The amount of bioavailable nitrogen cannot be readily measured, since dissolved inorganic nitrogen (DIN) is immediately bioavailable, dissolved organic nitrogen (DON) is partly bioavailable and some of the remaining particulate organic nitrogen (PON) can be made bioavailable through the microbial loop. Therefore, total nitrogen (TN) is considered the best proxy for the bioavailable nitrogen, although the fraction of non-labile nitrogen varies between coastal areas with a larger fraction in coastal waters influenced by outflowing water from the Baltic Sea. For comparison, relationships with total phosphorus are also shown.

In Danish coastal waters, the amount of available nutrients sets the limit for the phytoplankton biomass, whereas the composition of the phytoplankton community is based on the competitiveness of different species and the evolutionary strategies they have developed. Diatoms have fast growth rates when pulses of nutrients become available and they prefer a turbulent environment. Dinoflagellates are motile and particularly use their motility in stratified waters to assimilate nutrients from below the pycnocline and use them for production in the upper mixed layer. Cyanobacteria can regulate their buoyancy, and diazotroph cyanobacteria can fix nitrogen and are thus less dependent on nitrogen-limitation. However, cyanobacteria generally have lower growth rates and are seldom observed in turbulent waters and waters having salinities above 10. Chlorophytes mostly dominate in brackish water, particularly areas influenced by fluvial inputs from freshwater sources. Thus, our current understanding of phytoplankton community structure is that physical conditions are the primary factors regulating the composition. This is also consistent with *Carstensen & Heiskanen (2007)*, who in a study from the Baltic Sea found that it is not possible to identify so-called indicator species of nutrient enrichment, because all species are well adapted in the competition for nutrient resources.

2.1 Indicators of biomass

The most common measurement of phytoplankton biomass is chlorophyll *a* (chla), which is a pigment in the chloroplasts that is responsible for the photosynthesis. In the previous work during the intercalibration process, the summer chla, defined as the mean concentration between May and September, has been used as the common indicator in the Baltic GIG areas BC6 and BC8, whereas the 90-percentile of the chla distribution from March to September was proposed in the NEA GIG. However, since the NEA 8b intercalibration area is shared between Denmark and Sweden only and for consistency with the neighbouring intercalibration areas BC6 and BC8, the Baltic GIG indicator of biomass will be used in this report.

Monitoring data of chla, TN and TP were extracted from the national monitoring database for coastal water bodies in the intercalibration areas shared with Sweden as well as for a large number of additional water bodies, including estuaries and embayments with a more restricted water exchange. Summer means of chla (May-September) and winter-spring means of TN and TP (Jan-Jun) were computed following the approach from *Carstensen et al. (2008)*, resulting in geometric means. The analysis was restricted to data after 1986, since the quality of earlier data can be questionable. An overview of the data material is provided in *Table 2.1*. The variation in chla and nutrients between water bodies in the intercalibration areas is relatively small and the water bodies are generally characterised by low chla and nutrients.

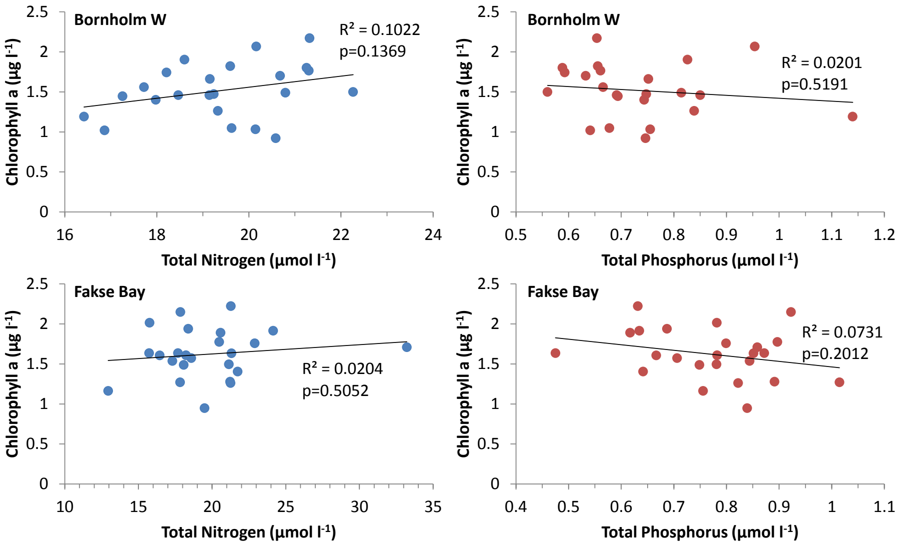
Table 2.1. Overview of water bodies included in the phytoplankton biomass analysis characterised by the period of the time series used, the means of salinity, chla, TN and TP. There are 4 water bodies in intercalibration area BC6, and 7 water bodies in intercalibration area NEA 8b.

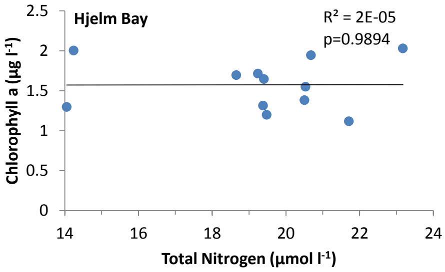
IC area	Water body	Start	End	Salinity (May-Sep)	Chla (May-Sep)	TN (Jan-Jun)	TP (Jan-Jun)
BC6	Bornholm W	1987	2010	7.4	1.52	19.4	0.73
BC6	Fakse Bay	1986	2011	8.5	1.62	19.7	0.77
BC6	Hjelm Bay	1986	2010	8.5	1.76	19.4	0.82
BC6	Køge Bay	1986	2013	9.6	1.44	22.1	0.73
NEA 8b	Hevring Bay	1989	2013	24.6	1.94	21.3	0.78
NEA 8b	Kattegat N	1986	2013	24.0	1.84	23.6	0.73
NEA 8b	Sejerø Bay	1989	2013	16.7	1.36	21.0	0.70
NEA 8b	The Sound N	1987	2013	14.8	1.60	19.4	0.80
NEA 8b	The Sound S	1988	2013	12.8	1.36	21.9	0.70
NEA 8b	Zealand coast N	1988	2010	17.8	1.18	18.8	0.65
NEA 8b	Aarhus Bay	1986	2013	22.1	1.81	18.5	0.75
	Archipelago S Funen	1986	2013	14.3	1.70	25.4	0.76
	Augustenborg Fjord	1987	2013	16.5	4.20	34.4	1.03
	Dybsø Fjord	1988	2012	11.5	1.71	51.5	0.97
	Flensborg Fjord	1986	2013	16.5	4.42	32.7	1.06
	Fyns Hoved / Great Belt	1986	2013	19.0	2.26	20.5	0.73
	Genner Fjord	1987	2004	16.9	3.81	33.9	1.06
	Hirtshals	1986	2013	32.2	2.18	20.5	0.84
	Horsens Fjord	1986	2013	22.2	4.36	39.0	1.14
	Isefjord	1989	2013	18.0	3.17	30.0	0.78
	Kalundborg Fjord	1989	2010	15.6	2.01	23.0	0.79
	Karrebæksminde Bay	1994	2003	11.9	1.21	23.4	0.67
	Kertinge Nor	1987	2013	17.5	6.41	43.0	1.22
	Kolding Fjord	1986	2013	19.8	11.68	52.1	1.40
	Korsør Nor	1989	2010	15.2	4.83	40.7	0.95

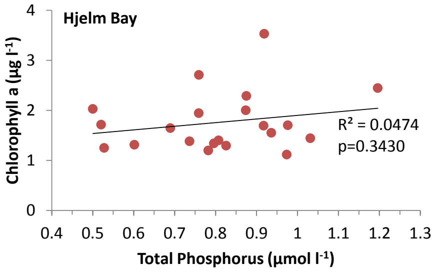
IC area	Water body	Start	End	Salinity (May-Sep)	Chla (May-Sep)	TN (Jan-Jun)	TP (Jan-Jun)
	Limfjorden E	1986	2013	25.1	4.41	61.2	1.46
	Limfjorden S of Mors	1986	2013	29.9	5.58	57.1	1.37
	Limfjorden W of Mors	1986	2013	25.5	7.03	69.1	1.17
	Little Belt N	1986	2013	21.1	3.60	20.6	0.80
	Little Belt S	1986	2013	15.2	2.65	21.6	0.74
	Løgstør Broad	1986	2013	26.8	4.88	57.7	1.04
	Mariager Fjord	1986	2013	15.5	18.99	122.8	2.21
	Nakkebølle Fjord	1989	2013	14.3	3.76	43.1	1.11
	Nissum Broad	1986	2013	31.5	4.59	43.9	1.13
	Nissum Fjord	1986	2013	9.6	29.20	136.5	2.47
	Odense Fjord inner	1986	2009	14.1	6.10	157.1	4.93
	Odense Fjord outer	1986	2013	19.6	4.87	61.0	1.95
	Præstø Fjord	1988	2013	9.2	4.05	46.3	1.09
	Randers Fjord	1989	2013	15.9	6.67	110.5	2.05
	Roskilde Fjord	1986	2013	16.1	4.64	48.3	2.73
	Skive Fjord / Lovns Broad	1986	2013	24.3	10.82	77.2	1.24
	Vejle Fjord	1986	2013	22.2	4.17	29.7	1.03
	Wadden Sea inner	1988	2013	29.4	7.40	64.0	2.27
	Wadden Sea outer	1986	2013	31.2	5.21	45.4	1.24
	Åbenrå Fjord	1986	2013	17.2	3.45	28.0	0.91

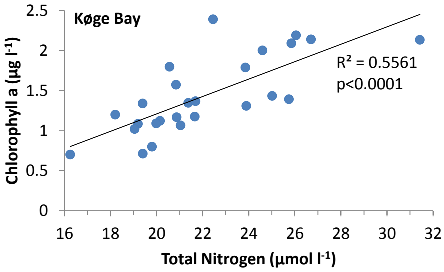
2.1.1 Linking biomass with nutrients

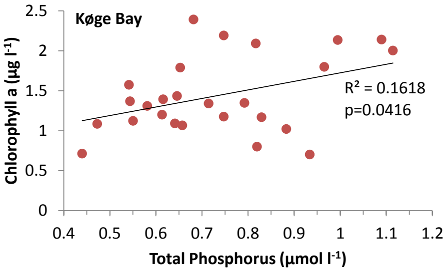
Due to the low span in nutrient concentrations within the different intercalibration areas, only seven of the 11 intercalibration water bodies had significant chla vs. nutrient relationships, and of these, with one exception (The Sound S), the relationship to TN was stronger than to TP (*Figure 2.1*). Overall, this suggests that TN is a better predictor of chla, consistent with the general understanding that phytoplankton growth during the summer period is nitrogen limited in this region. Overall, stronger relationships, correlating most significantly with TN, were found for the other water bodies (see *Table 2.1*; regressions not shown), many of these having a larger span in interannual nutrient levels and chla.

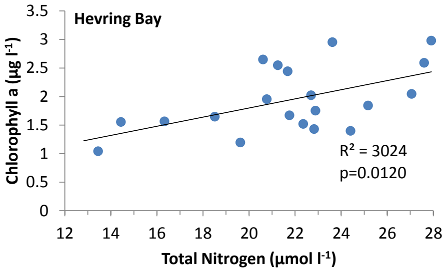


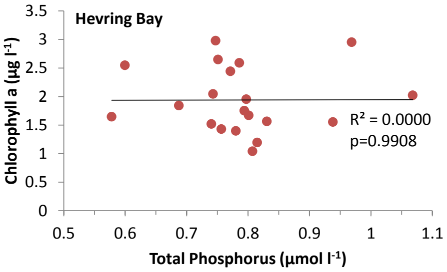

Hjelm Bay
R² = 2E-05
p=0.9894
Chlorophyll a (µg l^{-1})
2.5
2
1.5
1
0.5
0
14
16
18
20
22
24
Total Nitrogen (µmol l^{-1})


Hjelm Bay
R² = 0.0474
p=0.3430
Chlorophyll a (µg l^{-1})
4
3
2
1
0
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
Total Phosphorus (µmol l^{-1})


Køge Bay
R² = 0.5561
p<0.0001
Chlorophyll a (µg l^{-1})
2.5
2
1.5
1
0.5
0
16
18
20
22
24
26
28
30
32
Total Nitrogen (µmol l^{-1})


Køge Bay
R² = 0.1618
p=0.0416
Chlorophyll a (µg l^{-1})
2.5
2
1.5
1
0.5
0
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
Total Phosphorus (µmol l^{-1})


Hevring Bay
R² = 3024
p=0.0120
Chlorophyll a (µg l^{-1})
3.5
3
2.5
2
1.5
1
0.5
0
12
14
16
18
20
22
24
26
28
Total Nitrogen (µmol l^{-1})


Hevring Bay
R² = 0.0000
p=0.9908
Chlorophyll a (µg l^{-1})
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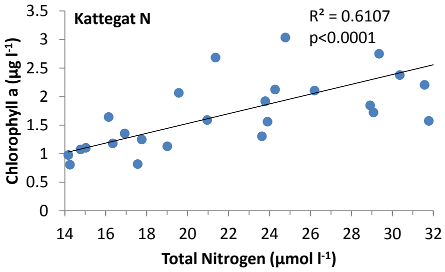
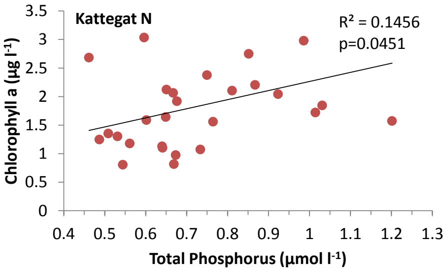
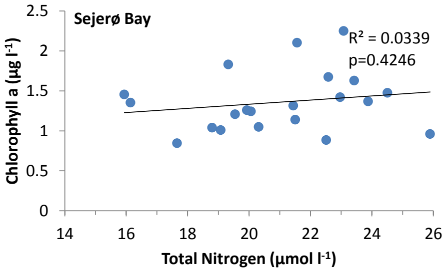
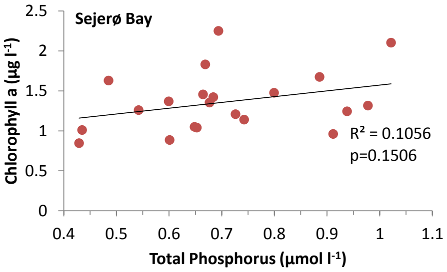
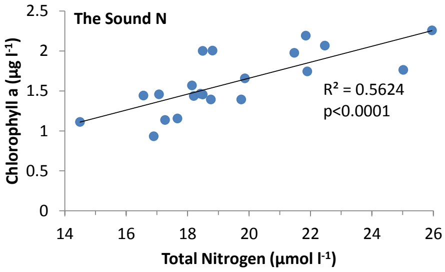
3
2.5
2
1.5
1
0.5
0
0.5
0.6
0.7
0.8
0.9
1
1.1
Total Phosphorus (µmol l^{-1})

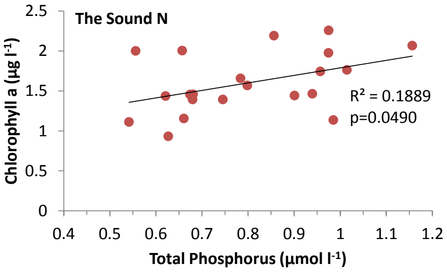

Kattegat N
R² = 0.6107
p<0.0001
Chlorophyll a (µg l^{-1})
3.5
3
2.5
2
1.5
1
0.5
0
14
16
18
20
22
24
26
28
30
32
Total Nitrogen (µmol l^{-1})


Kattegat N
R² = 0.1456
p=0.0451
Chlorophyll a (µg l^{-1})
3.5
3
2.5
2
1.5
1
0.5
0
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
Total Phosphorus (µmol l^{-1})


Sejerø Bay
R² = 0.0339
p=0.4246
Chlorophyll a (µg l^{-1})
2.5
2
1.5
1
0.5
0
14
16
18
20
22
24
26
Total Nitrogen (µmol l^{-1})


Sejerø Bay
R² = 0.1056
p=0.1506
Chlorophyll a (µg l^{-1})
2.5
2
1.5
1
0.5
0
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
Total Phosphorus (µmol l^{-1})


The Sound N
R² = 0.5624
p<0.0001
Chlorophyll a (µg l^{-1})
2.5
2
1.5
1
0.5
0
14
16
18
20
22
24
26
Total Nitrogen (µmol l^{-1})


The Sound N
R² = 0.1889
p=0.0490
Chlorophyll a (µg l^{-1})
2.5
2
1.5
1
0.5
0
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
Total Phosphorus (µmol l^{-1})

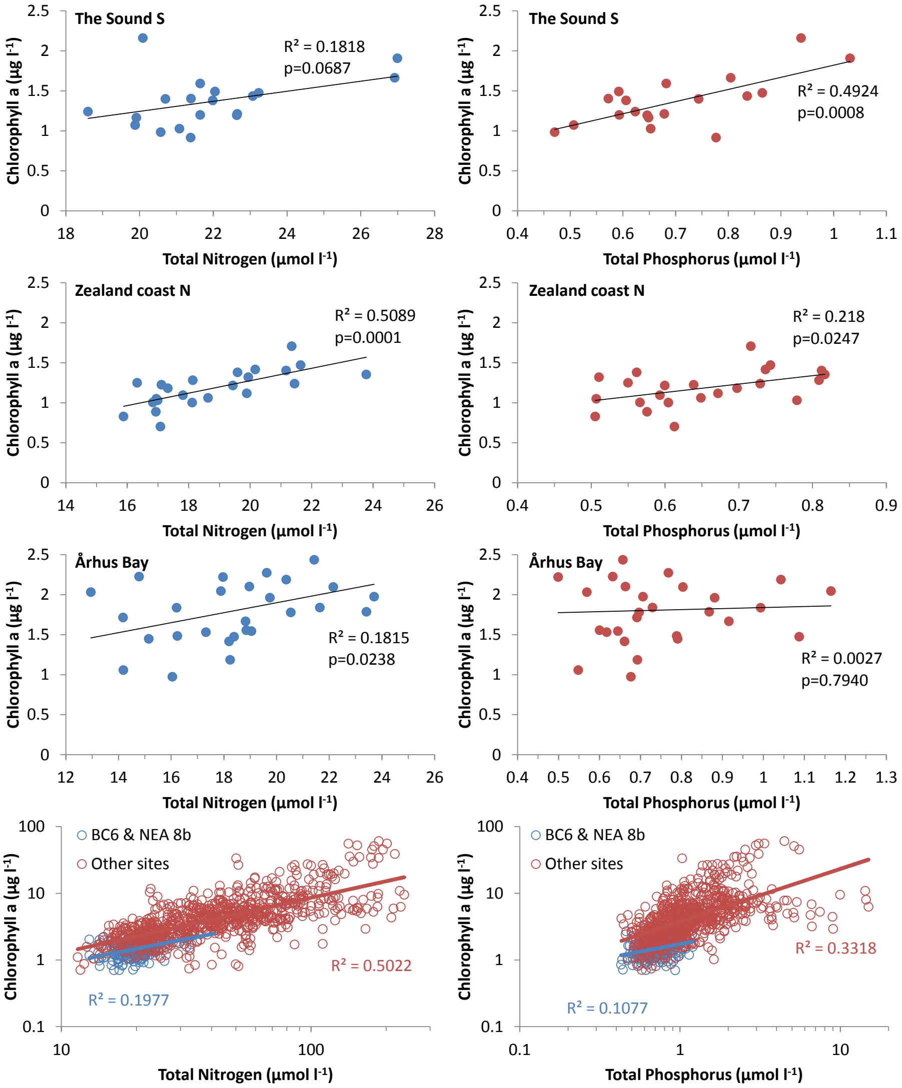


Figure 2.1. Relationships between chla (May-September) and TN/TP (January-June) for the 11 intercalibration water bodies in BC6 and NEA 8b as well as for all water bodies in the study (note the log-scale for displaying the broad ranges of chla and nutrients). Years with data for the regressions are listed in *Table 2.1*. For the regressions the coefficient of determination and significance (p-value) are inserted.

In BC6, only Køge Bay had a significant relationship with TN, whereas Bornholm W, Fakse Bay and Hjelm Bay did not show any significant relationship. The three latter areas are strongly affected by outflows of Baltic Sea water, and relationships between winter-spring nutrients and summer chla would not be expected. The significant relationship for Køge Bay is most likely due to the fact that the water body is shallower (retaining more nutrients through benthic-pelagic coupling) and more influenced by local discharges yielding a broader span in nutrient concentrations.

A better coupling between chla and TN was obtained for the water bodies in NEA 8b with significant relationships for 5 of the 7 water bodies (Hevring Bay, Kattegat N, The Sound N, Zealand coast N, and Aarhus Bay), an almost significant relationship for The Sound S and no relationship in Sejerø Bay. Sejerø Bay is exceptional in the sense that there is no major freshwater influence and the overall conditions are governed by exchanges with the northern

Belt Sea. Thus, using the same argument for Bornholm W, Fakse Bay and Hjelm Bay, which are all affected by outflowing Baltic Sea water, a distinctive relationship between chla and TN would not be expected.

The analyses above support the general understanding that in most marine ecosystems there is a link between TN and the amount of chla, and this link becomes stronger with proximity to land and freshwater sources (*Carstensen et al. 2008; Carstensen & Henriksen 2009*). Similarly, it has been shown that there are strong links between TN concentrations and nitrogen inputs from land, and these links also become stronger with proximity to freshwater sources (*Carstensen & Henriksen 2009*). Thus, the analysis above confirms the existence of a relationship between chla and nitrogen input from land, but it also documents that this relationship disappears in coastal ecosystems influenced more strongly by exchanges with open waters rather than local sources.

2.1.2 Effects of phytoplankton on eelgrass distribution

Nutrient enrichment of coastal waters enhances the growth of phytoplankton, which increases the light attenuation, thereby affecting the depth distribution of both macroalgae and angiosperms. Thus, there is a causal linkage between nutrient inputs, phytoplankton biomass and the depth distribution of benthic vegetation. In Denmark, the depth distribution of eelgrass (*Zostera marina*) is of particular concern, since most bottoms in Danish coastal waters are soft sediments and eelgrass is the dominant plant inhabiting these domains.

The cause-effect relationships from nutrient input to eelgrass depth limits have been explored in *Markager et al. (2010)* and *Carstensen et al. (2013).* There are strong linear relationships between nutrient input from land and nutrient concentrations at the coastal monitoring stations, the strength of these relationships increasing with proximity to land-based sources, e.g. strongest relationships for Randers Fjord and Odense Fjord inner part that receive large amounts of freshwater and weaker relationships for open coastal areas such as north of Zealand, Hjelm Bay, etc.

The relationships between nutrient concentrations and phytoplankton biomass are less subtle, because the relationship between chlorophyll *a* and nutrient level is affected by the bioavailability of the nutrient sources, the potential for nitrogen-fixing cyanobacteria and the presence of benthic grazers, such as mussels and clams capable of suppressing the phytoplankton community *(Petersen et al. 2008).*

Light is attenuated down through the water column by various substances. Particulate organic matter, mostly phytoplankton, absorb and scatter light, whereas dissolved organic matter absorb light, particulate organic matter scatter light and water absorb light. *Markager et al. (2010)* found that particulate organic matter only contributes 22-31 % to the light attenuation in three estuaries strongly affected by nutrient inputs and having relatively high phytoplankton biomass. The contribution of phytoplankton to light attenuation in other coastal ecosystems is presumably less. However, it should be acknowledged that part of the dissolved organic matter, which is the most important light attenuating component, originates from phytoplankton through exudation and cell degradation. Thus, phytoplankton contributes to light attenuation, but other substances are more important and consequently the linkage between phytoplankton and light conditions is not simple.

The depth distribution of eelgrass is mainly related to light conditions, although other factors such as sediment composition and oxygen conditions also play a role. There might also be feedback processes from loss of eelgrass meadows enhancing the resuspension of sediments (*Carstensen et al. 2013*), which complicates the cause-effect relationship between light and eelgrass depth-distribution.

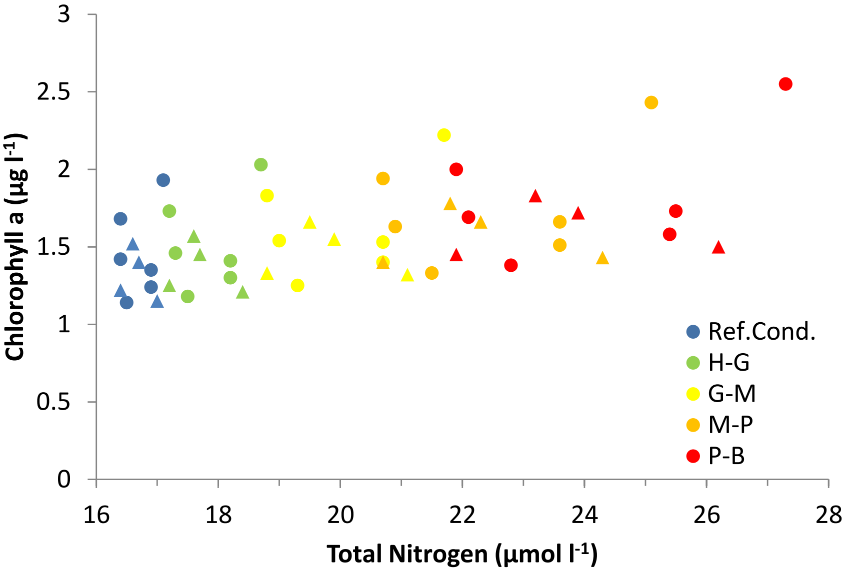
Overall, *Markager et al. (2010)* estimated that a relative change in TN input of 10 % would yield a relative change in eelgrass depth distribution of 0.2-1.1 %, highlighting that the cause-effect relationship from nutrient inputs to eelgrass depth limits is not simple and involves other factors than nutrients alone.

2.1.3 Intercalibration of summer chla with Sweden

In *Carstensen et al. (2008)*, reference conditions and class boundaries were proposed for a wide range of coastal water bodies in Denmark, including all of the 11 intercalibration water bodies analysed above except for The Sound S. Using a reconstructed time series from 1900 and onwards on nitrogen input to Danish coastal waters (connected with the Danish Straits), thresholds of nutrient inputs were proposed to characterise different periods supposedly representative of different pressure levels, i.e. representing different status classes *sensu* the WFD. Boundaries between the nutrient pressure levels were used to establish reference conditions and class boundaries for TN and chla by means of relationships between TN input and TN concentrations and between TN concentrations and chla concentrations.

The proposed TN boundaries ranged from 16 to 27 µmol l^{-1}, whereas chla boundaries ranged from 1.2 to 2.6 µg l^{-1} (*Figure 2.2*). Reference conditions and boundaries for chla were distributed along a band of about 1 µg l^{-1} along the TN range.

Figure 2.2. Proposed reference condition and class boundaries for TN and chla for 10 intercalibration water bodies in BC6 (triangles) and NEA 8b (circles). Data from *Carstensen et al. (2008)*.



Analysing the proposed boundaries between high and good (and similarly for the G-M boundary; data not shown) demonstrated a curvilinear relationship suggesting that chla attains a level of saturation for high levels of nutrient concentrations (*Figure 2.3*).

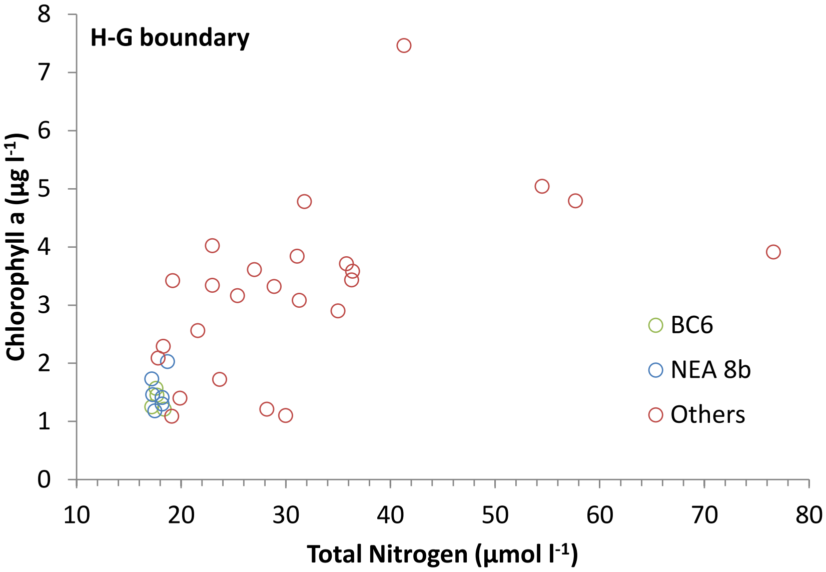


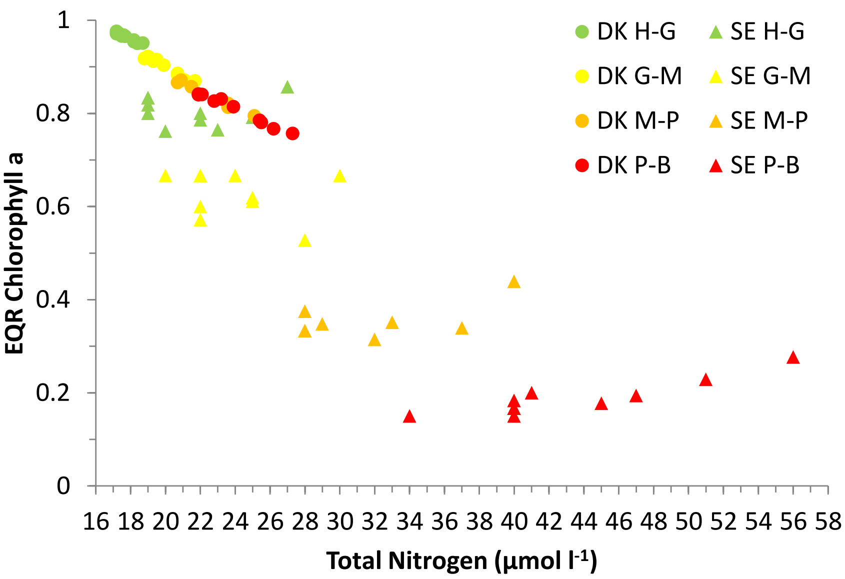
Figure 2.3. Proposed H-G boundaries for TN and chla for the 10 intercalibration water bodies in BC6 (triangles) and NEA 8b (circles) as well as other coastal and estuarine water bodies used in the analysis. Data from *Carstensen et al. (2008)*.

The Danish reference conditions and class boundaries were compared with the Swedish values (*Naturvårdsverket 2007*) for the Swedish type areas in NEA 8b (type 1n, 1s, 2, 3, 25, 4, 5, and 6) and in BC6 (type 7 and 8). The Swedish values are summer chla (Jun-Aug) and winter TN (Dec-Feb), so the assessment methods are similar, although not exactly the same. The Swedish values are based on a different modelling approach.

The established reference conditions for Denmark and Sweden are similar. In NEA 8b, the Danish values average 16.7 µmol l^{-1} (range: 16.4-17.1) for TN and 1.46 µg l^{-1} (range: 1.14-1.93) for chla. For comparison, the Swedish values average 17.0 µmol l^{-1} (range: 17.0-17.0) for TN and 1.13 µg l^{-1} (range: 0.9-1.6) for chla. Similarly, in BC6, the Danish values average 16.7 µmol l^{-1} (range: 16.4-17.0) for TN and 1.32 µg l^{-1} (range: 1.15-1.52) for chla. For comparison, the Swedish values average 18.5 µmol l^{-1} (range: 17-20) for TN and 1.20 µg l^{-1} (range: 1.2-1.2) for chla. Thus, established reference conditions are comparable and generally in good agreement, acknowledging differences in assessment method and regional differences.

However, there are differences in the boundary settings between Denmark and Sweden (*Figure 2.4*). First, Denmark and Sweden employ different levels of the pressure (here TN); Denmark suggesting relatively smaller deviations from the TN reference conditions to characterise an altered nutrient status, whereas Sweden allows a larger span on the TN range. Moreover, the proposed TN levels are calculated differently in the two countries. Second, in the Danish approach the chla response to changing nutrient levels is not as large as suggested in the Swedish approach, resulting in substantially higher chla boundaries for the latter and consequently lower EQR values (*Figure 2.4*).

Figure 2.4. Proposed boundaries for TN versus EQR chla values for the 10 Danish intercalibration water bodies (circles) and the 10 Swedish types (triangles) in BC6 and NEA 8b. Data from *Carstensen et al. (2008)* and *Naturvårdsverket (2007)*.



Differences in the boundary values can be due to differences in the methods applied to derive these values and in the definition of how to characterise the different status classes. These differences between Denmark and Sweden should be settled before the H-G and G-M boundaries can be intercalibrated in the two intercalibration areas.

2.2 Indicators of phytoplankton community

The phytoplankton community is standardly assessed by counting the number of specimens of different species in an integrated surface water sample (either combined from discrete water samples or from a hose sampling) fixed with Lugol using an inverted microscope (*Utermöhl 1958*). From the counts and dimensions of the different species, abundances, biovolumes and carbon biomasses can be calculated. Typically, between 10 and 20 different species are identified in a sample with an increasing tendency for more species identified over time, presumably due to improved taxonomical resolution, i.e. specimens identified on the genus level in earlier years are separated out on different species in later years. To overcome this changing taxonomical resolution and that many species are actually similar in their function and behaviour, the phytoplankton community is described by means of functional groups.

The following functional groups are considered: chlorophytes, cryptophytes, cyanobacteria, diatoms, dinoflagellates and others. The latter includes a diversity of functional groups that are less common in coastal ecosystems such as euglenophytes, prasinophytes, prymnesiophytes and flagellates. Whereas the amount of nutrients and light set the limit for the phytoplankton production and hence biomass, the relative distribution or proportion of the different functional groups could be regulated by physical-chemical factors such as salinity, mixing, water depth and nutrients. In order to investigate the relative distribution of the functional groups across a broad range of coastal ecosystems, the proportion of these functional groups relative to the total biomass of autotrophic and mixotrophic species was calculated. Just as for biomass, these proportions underlie considerable seasonal and interannual variations, which are mostly related to the succession of the phytoplankton community. Therefore, these variations were accounted for using a statistical model with month and year of monitoring as factors to produce an estimate of the annual mean proportion of the different functional groups for each monitoring station (see *Carstensen et al. in prep.* for details).

These station-specific proportions were compared to station-specific means of salinity, TN and TP as well as information on water depth, tidal amplitude and mixing pattern derived from CTD profiles and knowledge of the coastal systems (classified as mixed, intermittently stratified and stratified). The proportions of the functional groups, after logistic transformation, were related to the environmental variables by means of logistic regression using backward elimination, i.e. removing the least significant factor one at a time until all factors were significant ($p < 0.05$), although salinity was maintained in all models even though the p-value slightly exceeded 0.05 for two of the functional group proportions. Since the number of observations in the logistic regression was low, the influence of individual stations on the regression results was assessed and factors were also removed if their significance depended on a single station only.

The results from the logistic regression using Danish data only are described for each functional group below and compared with the Swedish data. However, for many stations in the Swedish monitoring programme only abundances have been calculated, limiting the possibilities for intercalibrating the phytoplankton composition, whereas in the Danish monitoring programme biovolumes and carbon biomasses have been routinely calculated. In fact, there are no Swedish monitoring stations with biovolumes or carbon biomasses in BC6, whereas there are two stations along the Swedish west coast, belonging to NEA 8b, that have sufficient samples with carbon biomasses to characterise the phytoplankton community (N14 Falkenberg and Släggö). Two additional stations have long-term time series of phytoplankton but these data were not downloadable at the public website.

The proportion of chlorophytes was generally low (< 1 %) with the exception of Nissum Fjord and Ringkøbing Fjord, both characterised by low salinities. Salinity was the only significant environmental factor for the proportion of chlorophytes (*Figure 2.5*), which is consistent with the general understanding that chlorophytes are mostly freshwater and brackish water species.

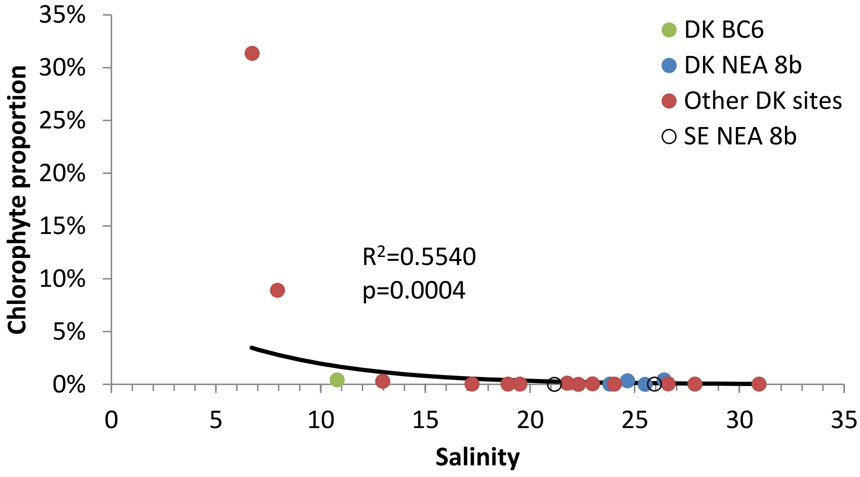
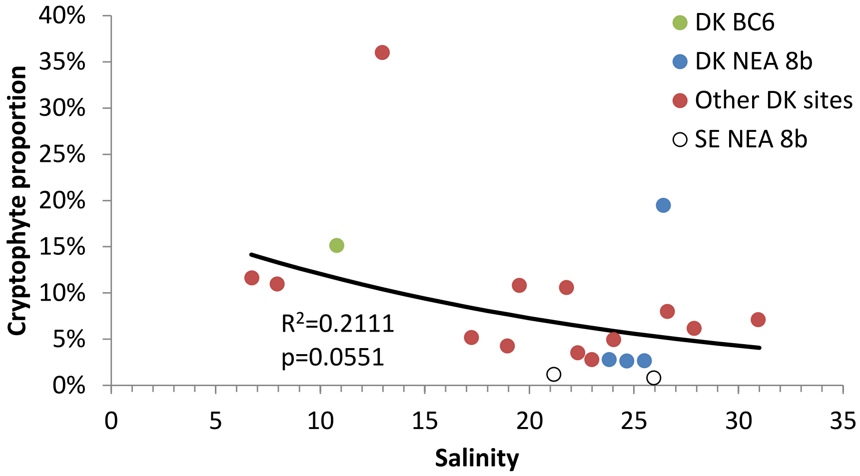


Figure 2.5. Proportion of chlorophytes versus salinity for the different intercalibration areas. The solid line shows the logistic regression with statistics for that regression inserted. Swedish data were not used in the regression.

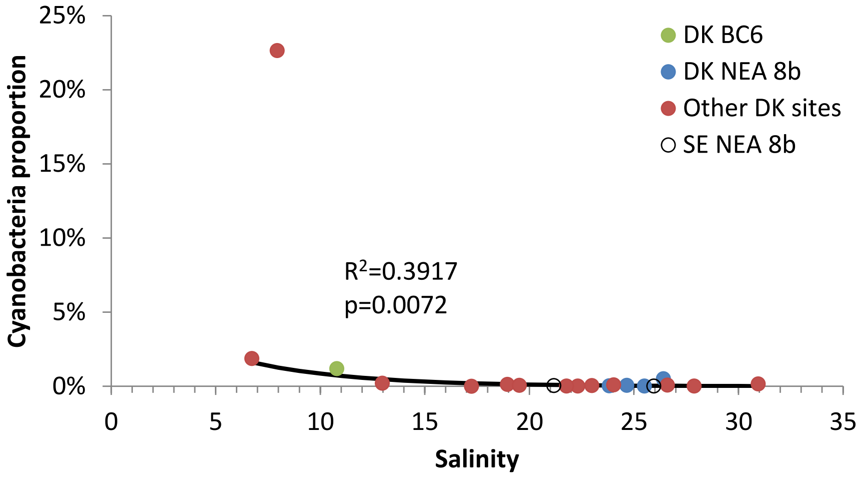
The proportion of cryptophytes was higher than the proportion of chlorophytes and about 10 % for most stations (*Figure 2.6*). The highest proportion was observed in Roskilde Fjord but also the Ven station in The Sound had a relatively high proportion. The proportion of cryptophytes decreased from around 15 % at low salinities to about 5 % for salinities around 30.

Figure 2.6. Proportion of cryptophytes versus salinity for the different intercalibration areas. The solid line shows the logistic regression with statistics for that regression inserted. Swedish data were not used in the regression.



The proportion of cyanobacteria also decreased significantly with salinity and was less than 1 % for stations with salinities > 11 (*Figure 2.7*). The three sites with more than 1 % cyanobacteria were Nissum Fjord, Ringkøbing Fjord and Køge Bay. Among the stations with higher salinities, station 431 (Ven) deviated slightly from the rest by having a distinguishable proportion of cyanobacteria. This station is influenced by outflowing water from the Baltic Sea, most likely containing some cyanobacteria during summer, which explains this deviation.

Figure 2.7. Proportion of cyanobacteria versus salinity for the different intercalibration areas. The solid line shows the logistic regression with statistics for that regression inserted. Swedish data were not used in the regression.



The proportion of diatoms was significantly related to both salinity and TN, displaying increasing tendencies for both (*Figure 2.8*). Diatoms were dominating (more than half of the community) for water bodies having salinity above 15, whereas the proportion of diatoms was generally low in the more brackish systems. On the other hand, the proportion of diatoms increased from about 30 % in those water bodies having low TN values to more than 80 % in systems with high TN values (Wadden Sea inner, Skive Fjord and

Mariager Fjord), when adjusted to an average salinity of 21. Only Roskilde Fjord and Ven appeared to deviate slightly from these overall patterns.

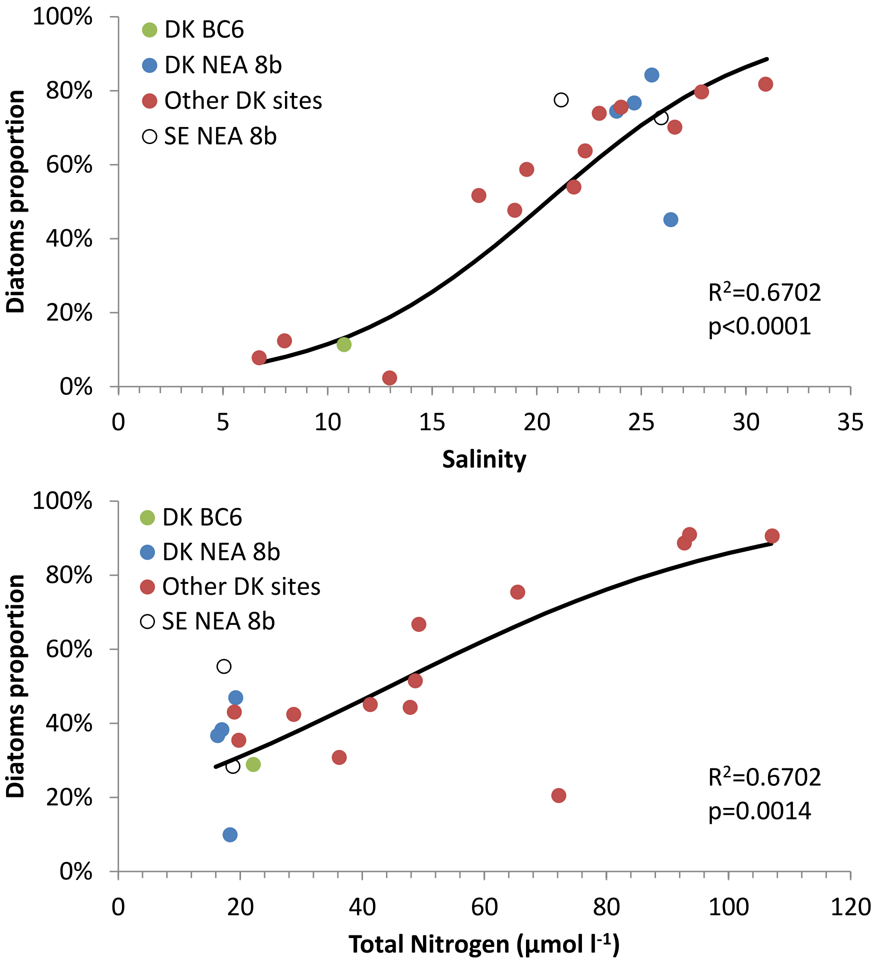
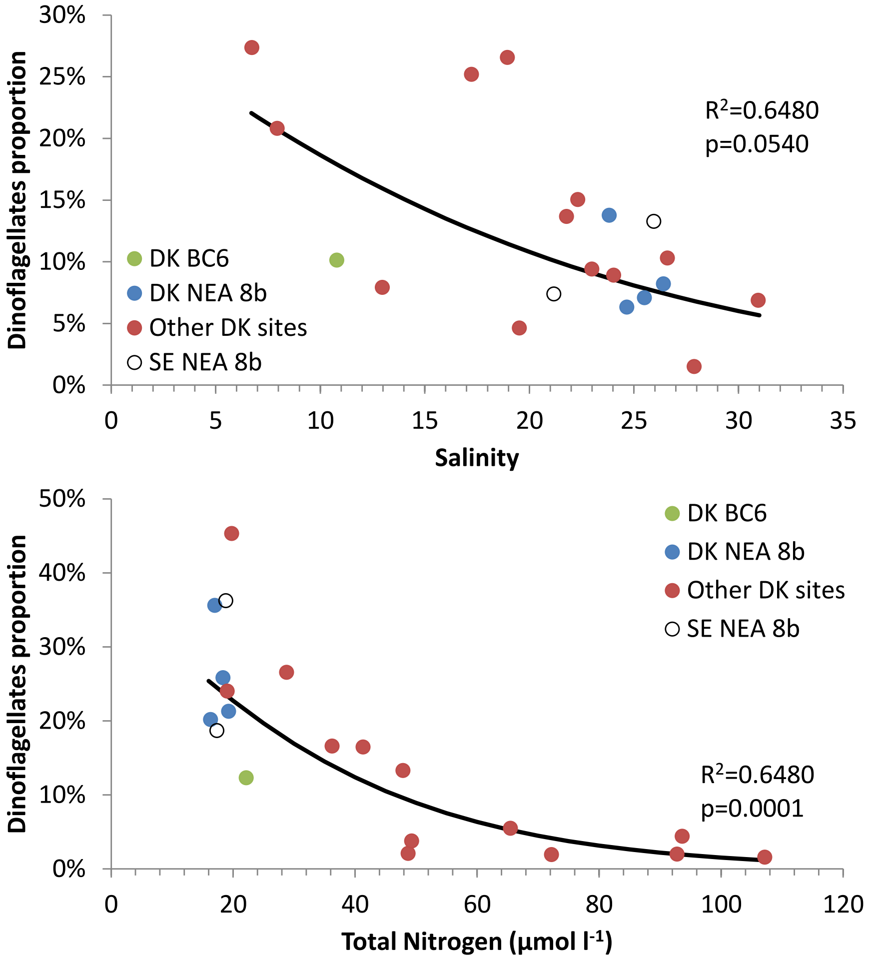


Figure 2.8. Proportion of diatoms versus salinity and TN for the different intercalibration areas shown as marginal relationships, i.e. variations in the other regression variable are accounted for. The solid line shows the logistic regression with statistics for that regression inserted. Swedish data were not used in the regression.

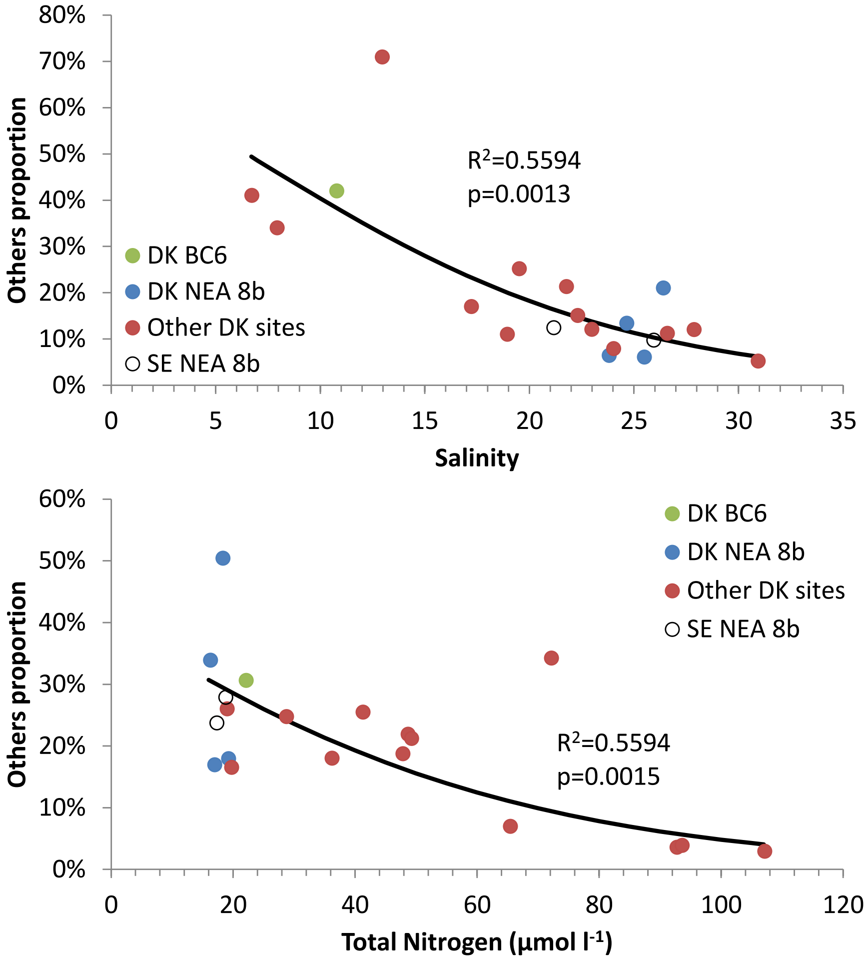
The proportion of dinoflagellates decreased with both salinity and TN (*Figure 2.9*). The relationship to salinity was not strong (borderline significant) showing a decrease from about 20 % for salinity < 10 to about 5 % for salinity > 30, when adjusted to an average TN concentration of 43 µmol l^{-1}. Two sites deviated from this overall pattern with salinity, Mariager Fjord and Little Belt S, both having a dinoflagellate proportion of about 25 % and intermediate salinity. Dinoflagellates were also relatively more abundant at stations with low TN and their proportion decreased to < 10 % for TN above 50 µmol l^{-1}. The intercalibration sites all had a relatively high proportion of dinoflagellates.

Figure 2.9. Proportion of dinoflagellates versus salinity and TN for the different intercalibration areas shown as marginal relationships, i.e. variations in the other regression variable are accounted for. The solid line shows the logistic regression with statistics for that regression inserted. Swedish data were not used in the regression.



Similar to dinoflagellates, the proportion of the other functional group decreased with both salinity and TN (*Figure 2.10*). The proportion of others decreased from ~50 % to ~10 % over the salinity range (predicted for an average TN of 43 µmol l^{-1}) and similarly from ~30 % to ~5 % over the TN range (predicted for an average salinity of 21). Roskilde Fjord deviated from the overall patterns of both relationships having a relatively high proportion of other species. The Ven station in The Sound also had a relatively high proportion of other species compared to the other intercalibration sites, when adjusted to an average salinity. Otherwise, the intercalibration sites appeared to follow the overall tendencies reasonably well.

Figure 2.10. Proportion of other phytoplankton species versus salinity and TN for the different intercalibration areas shown as marginal relationships, i.e. variations in the other regression variable are accounted for. The solid line shows the logistic regression with statistics for that regression inserted. Swedish data were not used in the regression.



In summary, salinity is the single most important factor regulating shifts between functional groups in Danish waters. The proportion of chlorophytes, cryptophytes and cyanobacteria all decreased with salinity and did not have any relationship with other environmental variables. The combination of salinity and nitrogen levels was important for the proportion of diatoms, dinoflagellates and other species such that both increasing salinity and TN favoured diatoms over dinoflagellates and other species. This may suggest, based on the relationship with TN, that the proportion of diatoms could be a useful indicator for the anthropogenic pressure. However, such an indicator should also reflect ecological status, and diatoms are generally considered good for the transfer of energy through the food web and there are no harmful species among diatoms. Thus, even though diatoms increase their proportion with the nitrogen level, an increased proportion of diatoms is considered a healthy sign for the coastal ecosystem. Therefore, the analysis cannot provide any indicator for the phytoplankton community that suggests a deterioration of ecological status with increasing nutrient pressure, and it may be questioned if such indicator does exist.

2.3 Conclusions

- Interannual variations in summer chlorophyll *a* were significantly correlated with TN levels (January-June) for most of the 11 investigated intercalibration areas.

- TN concentrations were better than TP concentrations in describing variations in chlorophyll *a*, confirming the existing knowledge of the coastal areas in Denmark being mainly nitrogen limited.

- Denmark and Sweden have comparable reference conditions for summer chlorophyll *a* in coastal water bodies in the intercalibration areas BC6 and NEA 8b.

- Class boundaries for summer chlorophyll *a* deviate between Denmark and Sweden with Denmark having tighter boundaries.

- Phytoplankton composition is governed by salinity, but increasing TN levels can shift the composition towards stronger diatom dominance. However, diatoms are considered healthy species with an efficient energy transfer and therefore not necessarily an expression of degrading ecological quality.

3 Macroalgae

Eutrophication changes the balance between primary producers by favouring phytoplankton and opportunistic macroalgae at the expense of benthic macroalgae and seagrasses which are shaded (*Duarte 1995, Borum & Sand-Jensen 1996, Cloern 2001*). As eutrophication increases, the distribution and abundance of the benthic macrovegetation thus tend to decline and the composition to change towards dominance of more opportunistic species (*Duarte 1995, Cloern 2001*). Recovery of eutrophied ecosystems demands a reduction in eutrophication pressure but pathways of recovery may differ from those of deterioration and may require long time (*Duarte et al. 2009, Krause-Jensen et al. 2012, Duarte et al. 2014*).

In order for coastal waters to qualify for good ecological status *sensu* the WFD, most disturbance-sensitive macroalgae and angiosperm taxa associated with undisturbed conditions must be present and the level of macroalgae cover and angiosperm abundance must show only slight signs of disturbance. A wide diversity of macroalgae indicators is being used across Europe to characterize the status of macroalgae and seagrass communities (*Birk et al. 2010 and 2012, Marbá et al. 2013*). The EU-programme WISER compiled biological indicators used in European monitoring programmes (http://www.wiser.eu/programme-and-results/dataandguidelines/method-database) and 30 of these include macroalgae (see *Blomqvist et al. 2012*). The macroalgae indicators address distribution, abundance and/or or diversity/composition of the vegetation and, thus, relate more or less directly to the demands of the WFD. ‘Distribution indicators’ include the depth extension of e.g. key macroalgae species and the horizontal extension of e.g. mats of opportunistic macroalgae while ‘abundance indicators’ describe the cover/biomass of the vegetation/key species. ‘Diversity and composition indicators’ are the most frequently used and relate to either the entire community, functional groups as defined by tolerant/opportunistic and sensitive/late-successional species, taxonomic groups represented by the three large classes of macroalgae (green, red and brown algae) and selected key species (see *Blomqvist et al. 2012*). The rationale is that anthropogenic pressure will reduce overall diversity, favouring tolerant species, often dominated by green algae, at the expense of sensitive species, often dominated by perennial brown and red algae and representing key species such as kelp species, *Fucus vesiculosus* and *Furcellaria lumbricalis*.

In our previous work for the Danish EPA, we tested the response of Danish coastal macroalgal communities to eutrophication and found that it to some extent followed the patterns outlined above (*Krause-Jensen et al. 2007a & b, Carstensen et al. 2008*). The abundance (cover) of the macroalgae community as a whole as well as of sensitive and tolerant algae and the number of sensitive species declined significantly along eutrophication gradients while the abundance of tolerant species increased. Our previous studies thus suggested that these macroalgae variables are potential useful indicators of ecological status but they were only described for a subset of coastal Danish water bodies, and our previous studies did not attempt to select a key set of indicators among them and define how to combine their response into a single estimate of ecological status.

3.1 Aim

The overall aim of this project was to improve and refine tools for assessing water quality of Danish coastal areas based on macroalgae and render the indicators ready for intercalibration with neighbouring countries within the Baltic and Northeast Atlantic (NEA) intercalibration groups. More specifically we aimed to:

- Refine existing macroalgae indicators by basing the models on the full spectrum of available Danish monitoring data - ca. 70 areas and 12 years of data (2001-2012)
- Compare uncertainty and sensitivity to eutrophication pressure of the various macroalgae indicators and on this basis choose a set of indicators that fulfils the demands of the WFD
- Define reference levels and class boundaries for indicators in each of the studied water bodies
- Provide suggestion/guidance for assessing ecological status according to the WFD on the basis of the full set of selected macroalgae indicators as well as for combinations between macroalgae and angiosperm indicators.

3.2 Methods

We used data from the Danish National Aquatic Monitoring and Assessment Programme (DNAMAP) and regional monitoring activities collected by the Danish counties and stored centrally in the national database (MADS) located at the Danish Centre for Environment and Energy (DCE). Data represent a total of more than 6000 observations, depending on the specific indicator, distributed along 324 sites each with a number of observations along a depth gradient in each of 59 coastal areas (*Table 3.1*). Some of these areas were subdivided so that the data set contained a total of ca. 70 areas/sub-areas. Out of the 70 areas/sub-areas, 42 were in typology NEA 8b, four were in typology BC6 and 22 were in typology BC8, whereas four open-water areas (stone reefs beyond the WFD baseline) in the Belt Sea area, Kattegat and Skagerrak were also included.

Table 3.1. Overview of sampling areas, depth range and number of sites and observations of the macroalgae variables included in the statistical analyses (see below). Number of observations is indicated in parentheses for the variables 'cumulative cover', and the number of observations used for the other indicators was similar. Sampling period: 2001-2012.

Area	Depth range (m)	No. of sites (No. of obs.)
Weakly exposed areas		
Augustenborg Fjord	1.0-9.3	8 (127)
Flensborg Fjord (inner)	1.0-5.8	5 (118)
Genner Fjord	1.2-3.6	1 (13)
Haderslev Fjord	1.2-2.9	1 (7)
Holbæk Fjord	1.0-6.0	3 (62)
Horsens Fjord (inner)	1.0-5.1	6 (64)
Horsens Fjord (outer)	1.0-4.8	4 (52)
Kolding Fjord (inner)	1.1-1.2	1 (2)
Limfjorden NW of Mors	1.0-5.6	5 (280)
Limfjorden S of Mors	1.0-4.7	5 (272)
Lindelse Nor	1.2-5.3	2 (15)
Lovns Bredning	1.0-4.6	1 (73)
Lunkebugten / Thurø	1.2-6.5	3 (33)

Area	Depth range (m)	No. of sites (No. of obs.)
Løgstør Bredning	1.0-6.2	11 (362)
Nærå Strand	1.0-1.1	1 (2)
Odense Fjord (outer)	1.1-4.9	5 (111)
Riisgaarde Bredning	1.0-5.2	2 (131)
Roskilde Fjord (inner)	1.0-5.7	6 (180)
Roskilde Fjord (outer)	1.0-5.2	4 (63)
Skive Fjord	1.1-3.6	1 (62)
Moderately exposed areas		
Aabenraa Fjord (coastal)	3.0-11.6	3 (83)
Aabenraa Fjord (inner)	3.0-7.8	4 (77)
Aabenraa Fjord (outer)	3.0-7.2	6 (78)
Als Sund	3.0-7.8	3 (84)
Anholt	3.0-5.1	8 (16)
Bøgestrømmen	3.0-11.0	1 (4)
Det Sydfynske Øhav	3.0-9.8	7 (103)
Ebeltoft Vig	3.0-9.0	4 (45)
Guldborgsund	3.0-6.9	3 (14)
Helnæs Bugt	3.0-5.8	1 (5)
Horsens Fjord (outer coast)	3.0-11.1	3 (33)
Inderbredning	3.0-5.0	4 (15)
Jammerland Bugt	3.1-5.1	2 (12)
Kalundborg Fjord (inner)	3.0-9.7	9 (109)
Kalundborg Fjord (outer)	3.0-12.9	10 (156)
Karrebæksminde Bugt	3.0-8.7	8 (127)
Kolding Fjord (outer)	3.0-3.4	1 (3)
Køge Bugt	3.0-8.8	6 (102)
Lillebælt Midt	3.0-11.2	3 (155)
Lillebælt Nord	3.0-12.8	3 (44)
Lillestrand	3.0-3.0	1 (1)
Nakkebølle Fjord	3.5-8.8	2 (25)
Nissum Bredning	3.0-5.7	3 (118)
Nivå Bugt	3.1-6.8	2 (27)
Vejle Fjord (inner)	3.1-3.9	1 (4)
Vejle Fjord (outer)	3.0-11.5	5 (126)
Yderbredning	3.0-5.9	6 (69)
Ålbæk Bugt	3.2-11.8	3 (72)
Aarhus Bugt (inner)	3.0-9.1	4 (58)
Aarhus Bugt (outer)	3.0-11.0	2 (60)
Øresund	3.0-11.4	11 (240)
Highly exposed areas		
Bornholm	5.0-12.9	15 (300)
Endelave	5.0-11.0	7 (38)
Flensborg Fjord (outer)	5.0-12.7	10 (206)
Grenå kyst	5.1-11.9	3 (53)
Hjelm	5.0-12.0	3 (28)
Hjelm Bugt	5.0-12.3	7 (158)
Kattegat Nord	10.0-12.9	2 (6)
Kattegat Syd	5.0-12.1	4 (72)
Lillebælt Syd	5.0-12.9	11 (400)
Læsø	5.0-12.5	4 (86)
Nord for Sjælland	5.0-12.9	3 (113)
Samsø vest	5.0-10.0	5 (27)

Area	Depth range (m)	No. of sites (No. of obs.)
Sejerø Bugt	5.0-11.1	12 (129)
Skagerrak	7.7-12.8	2 (26)
Smålandsfarvandet	5.0-12.9	9 (108)
Storebælt Nord	5.0-12.4	12 (182)
Storebælt Syd	5.0-9.8	1 (21)
Aarhus Bugt (open coast)	5.0-12.0	7 (116)
Øresund Nord	5.0-12.3	3 (99)
Total		324 (6262)

Algal data were collected during summer (May-September) from 2001 to 2012. Although there are macroalgae data from before 2001, we chose to use data from 2001 onwards rather than the entire data set dating back to 1989 because the more recent monitoring data set has been collected according to an improved procedure and are better integrated with the pelagic monitoring programme. The data were collected according to new common guidelines (*Krause-Jensen et al. 2001*), where divers visually recorded the percent of cover of individual erect algal species and the total erect macroalgae community (excluding the crust-forming algae). Algal cover was estimated in percent of the hard substratum at various depths along the depth gradients/sites.

Data sets where the summed cover of algal species constituted < 80 % of the estimated total algal cover were excluded, because we suspected that species identification in these data sets might be incomplete.

All species were allocated to a functional group, using the system of *Steneck & Dethiers* (*1994*, *Table 3.2*). The functional groups 1-3: micro-algae, filamentous algae and single-layered foliose algae are dominated by opportunistic algal species with thin thalli, fast growth rates and ephemeral life forms, while the remaining groups primarily include perennial species with thick, corticated, leathery or calcareous thalli and generally slower growth rates. In the following we therefore refer to group 2, 2.5 and 3 as 'opportunistic macroalgae' while algae belonging to groups 4, 5 and 6 are considered 'late-successional algae'. Microalgae (functional group 1) and crustose algae (functional group 7) were not consistently recorded in the entire data set and were therefore excluded from analysis.

Table 3.2. Overview of functional groups (*Steneck & Dethiers 1994*) and our grouping of late-successional and opportunistic species in the present study. *Microalgae and crustose algae are not represented in the present study and therefore not included in our grouping.

Functional group		Examples of algal genus	Grouping in this study
1.	Microalgae (single cell)*	Cyanobacteria and diatoms	Opportunists
2.	Filamentous algae (uniseriate)	*Cladophora*, *Bangia*	Opportunists
2.5	Filamentous and thinly corticated algae	*Polysiphonia*, *Ceramium*, *Sphacelaria*	Opportunists
3.	Foliose algae (single layer)	*Monostroma*, *Ulva*, *Porphyra*	Opportunists
3.5	Foliose algae (corticated)	*Dictyota*, *Padina*	Late-successionals
4.	Corticated macrophytes	*Chondrus*, *Gigartina*	Late-successionals
5.	Leathery macrophytes	*Laminaria*, *Fucus*, *Halidrys*	Late-successionals
6.	Articulated calcareous algae	*Corallina*, *Halimeda*	Late-successionals
7.	Crustose algae*	*Lithothamnion*, *Peyssonnelia*, *Ralfsia*	Not included

We analysed six algal variables: Total cover represented the diver estimates of total erect macroalgae cover for each sub-sample, with values in the range 0-100 %. Cumulative cover was calculated by summing the cover values of all erect macroalgae species in each sub-sample. Cumulative cover values could surpass 100 %, because algae can grow in several layers. The remaining algal variables to be analysed were related to the composition of the macroalgae community. Cumulative cover of opportunistic algae was calculated as the summed cover of all algal species belonging to functional groups 2-3, and cumulative cover of late-successional algae was calculated as the summed cover of algae belonging to algal groups 3.5-6. Relative cover of opportunistic algae was finally calculated by dividing the cumulative cover of opportunists by the cumulative cover of all species and therefore provided data in the range 0-100 %. Finally, the number of late-successional algal species in each subsample was calculated as the total number of the species belonging to this group and having a cover of at least 1 %.

All algal variables were tested for responses to physico-chemical gradients which is a central demand for indicators of ecological status according to the WFD.

3.2.1 Substratum

Composition of substratum was registered along with the collection of algal data. Divers visually recorded the total cover of suitable hard substratum as well as the cover of various substratum classes: size classes of stones, sand, mud and shells. Data on cover of suitable hard substratum were extracted from the database together with each algal data set.

3.2.2 Physical-chemical variables

Spatial variations in algal variables were related to the physical-chemical variables salinity, nutrient concentrations, and Secchi depth. These data were sampled at sites situated in the vicinity of vegetation sites. The water chemistry sites were typically located centrally in the investigated coastal areas or sub-areas, and generally two or more algal sites/depth gradients were related to the same water chemistry site.

We assumed that mean values from the various algal sites would represent the algae of a given coastal area and that the centrally located water chemistry site would represent the physical conditions and water chemistry of the same coastal area in spite of some distance between macroalgae and water chemistry sites.

Water chemistry data were also collected within the framework of DNAMAP and stored in the national database. Sampling and chemical analysis were performed according to common guidelines (*Andersen et al. 2004*) and typically represented a sampling frequency between weekly and monthly sampling, although some of the areas/sub-areas were less frequently sampled.

3.2.3 Statistical analyses of algal variables

Algal model

We focused the analysis exclusively on algae from the depth range where disturbance was no longer a major controlling factor for cover (*Figure 3.1*). The coastward end of this depth range was estimated as the water depth with the highest algal cover using non-parametric smoothing function (LOESS, *Cleveland 1979*). This adjustment was made separately for each area and showed that the areas could be categorised in weakly exposed areas where maximum cover was located at water depths of ~1 m, moderately exposed areas with maximum cover at water depths of ~3 m and highly exposed areas with maximum cover at water depths of ~5 m (*Carstensen et al. 2005*). Consequently, we restricted the analysis to water depths > 1 m in weakly exposed areas, > 3 m in moderately exposed areas and > 5 m in highly exposed areas. Only relatively few (272) observations represented water depths > 13 m (mainly from around Bornholm, Kattegat, Little Belt, Northern Belt Sea and Hjelm Bugt), and we therefore restricted the analysis to water depths < 13 m.

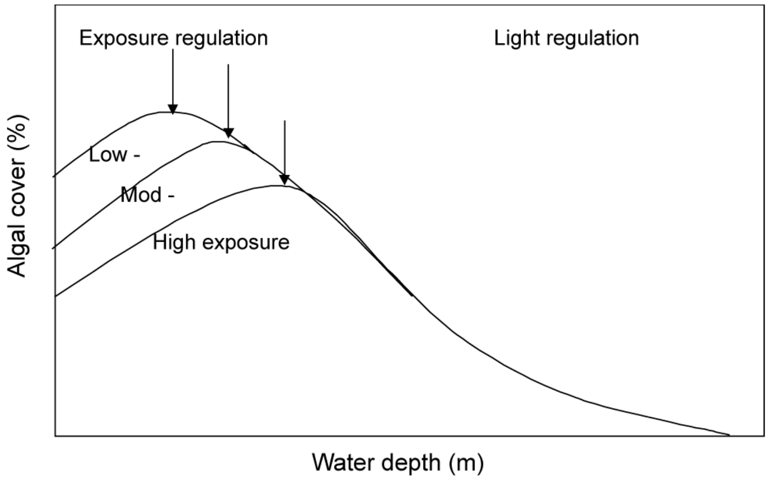


Figure 3.1. Illustration of the hypothesis that algal cover in shallow water is reduced due to physical exposure while from intermediate water depth towards deeper water, algal cover is reduced in parallel to reductions in available irradiance. Consequently, maximum algal cover is found at intermediate water depths and is located deeper in more exposed areas.

Algal cover was estimated as substratum-specific cover, which should imply that cover levels were independent of substratum composition at the sampling sites. A possible dependence on the amount of hard substratum was tested initially using a non-parametric adjustment (LOESS, *Cleveland 1979*) of each of the potential algal indicators to the amount of hard substratum. This analysis led to the formulation of a model, in which the relation between algal cover and hard substratum differed for levels of hard substratum of below and above 50 % (similar to the results in *Carstensen et al. 2008*).

Algal data representing cumulative cover levels were ln transformed before analysis. By contrast, raw values of the algal variables 'total cover' and 'fraction of opportunists' were in the range 0-100 % and greater variation was expected around 50 % than at 0 % and 100 %, so for use in the statistical analyses we employed the angular transformation to these data (p, *Sokal & Rohlf 1981*):

$$x = \arcsin\sqrt{p} \qquad (1)$$

Species number was counted as the total number of perennial macroalgal species, which covered at least 1 % of the sea bottom in a given sub-sample. Data were ln transformed before analysis:

$$x = \ln(p+1)$$

Variations in algal variables (representing either ln transformed or arcsin transformed data, x) were described by the following generic model:

$$
\begin{gathered}
x = \text{area} + \text{sub-area (area)} + \text{site (sub-area)} + \text{year} + \text{month} + \text{depth} + \\
\text{\% hard substratum (0-50 \%) x depth} + \text{\% hard substratum (50-100 \%)} \ast \\
\text{depth} + \text{diver} + \text{area x month} + \text{area x year} + \text{month x year} + e
\end{gathered} \tag{2}
$$

The model is based on the assumption that the observed level of each algal variable depends on coastal area, sub-area (inner or outer parts of estuaries or open coasts), site, water depth in combination with substratum composition, sampling year and month (common to all areas), and diver effects. The random effects included ‘site’ (variation between transects within an area/sub-area), ‘diver’ (variation between divers conducting the monitoring), ‘area × month’ (area-specific seasonal variation), ‘area × year’ (area-specific interannual variation), ‘month × year’ (differences in seasonal variation across years), and the residual effect describing random variation at sites, typically associated with variation around the depth-specific relationship.

‘Depth’ is treated as a continuous variable in the models describing algal cover, since algal cover (transformed) declines linearly with depth. By contrast, ‘depth’ is treated as a categorical variable in the models describing ‘fraction of opportunists’ and ‘species richness’, since these variables are not expected to be linearly related with depth.

The dependence on substratum composition is expressed by a linear relation that differs between depth intervals as well as between levels of hard substratum below and above 50 %.

The model calculates the marginal distributions for the area-specific and depth-specific variations as well as for the year-specific and month-specific variation in algal variables. Marginal distributions describe the variation in a specific factor of the model when variations of all other factors are taken into account. Thus, mean values of each algal variable were calculated for each area, taking into account that monitored depth intervals, substratum composition and sampling year and month could vary among areas. Thereby, the model provided comparable values of algal variables between areas. These marginal means represented expected values corresponding to a water depth of 7 m (average of the depth range 1-13 m included in the analysis), averaged over the sampling years (2001-2012), averaged over the months used in the analysis (May-September), and for a substratum composed of 50 % hard bottom. An example of this data harmonisation procedure is given in *Figure 3.2* for a constructed data set representing sampling stations along three transects in two areas all with different depth distributions.

The variation shown by the marginal means should be interpreted as relative variation and not actual levels as some areas, for instance, may be shallower than 7 m. In principle, the model can also compute site-, depth-, time- and substratum-specific levels of algal cover.

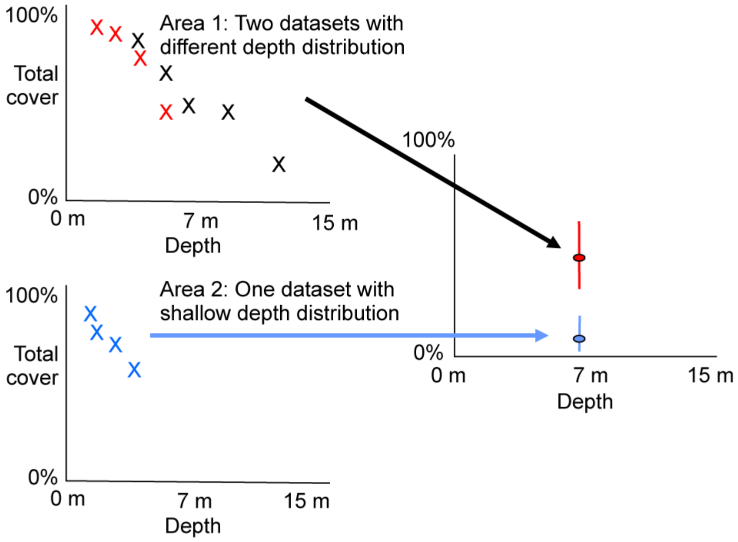


Figure 3.2. Example of the data harmonization procedure resulting in an estimated marginal mean value of the selected indicator with confidence level represented at a water depth of 7 m.

Coupling algal variables to water quality

The variation in water quality variables was initially analysed using a model similar to the algal model. The model describes water quality variables with respect to area-specific variation, site-specific variation, seasonal variation (only from March to September for better causal relationship to macroalgae data) and year-to-year variation. For each water quality variable, we calculated area-specific marginal means.

Algal variables were related to physical-chemical variables through multiple regression analysis using backward elimination. First we introduced all the potential independent variables in the regression, and then excluded variables one by one until only the significant variables remained. The analyses were conducted on a spatial basis to explain differences in algal parameters between various coastal areas/sub-areas.

3.3 Results

Data on the various algal variables were modelled based on fixed variation between areas, sub-areas and sites within each sub-area as well as on variation between depth intervals or depth as a continuous variable, substrate composition in depth intervals, seasonal variation and year-to-year variation (*Table 3.3*). Moreover, the model took into account stochastic variation due to variation between sub-samples, diver effects, differences in the seasonal pattern between areas and years, changes in the interannual variation between areas, and residual variation (*Table 3.4*). The change in indicator level with depth was also taken into account by the model (*Figure 3.3*) and algal variables allocated to the same fixed depth (7 m) before linking them to the physical-chemical status.

Table 3.3. P-values for each model component (fixed and random) for each of the modelled algal variables: total cover (Tot. cov.), cumulative algal cover (Cum. cov.), cumulative cover of opportunists (Cum. opp. cov.), cumulative cover of late-successional species (Cum. late cov.), fraction of opportunists (Frac. opp.) and number of late-successional species (Species no. late). Total cover and fraction of opportunists were arcsin-transformed and the other variables were log-transformed before the analysis. Total number of observations used for estimating each model was 6262.

Model component	**Tot. cov.**	**Cum. cov.**	**Cum. late cov.**	**Cum. opp. cov.**	**Frac. opp.**	**Species no. late**
Fixed effects						
- Area	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
- Sub-area (I, O, C)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
- Depth interval	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
- % hard substratum (0-50) × depth	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.6726	< 0.0001
- % hard substratum (50-100) × depth	0.0098	0.0002	< 0.0001	< 0.0001	0.0003	0.2123
- Month	0.3358	0.6974	0.5487	0.4434	0.7009	0.2591
- Year	0.1181	0.0910	0.1405	0.3417	0.1326	0.0084
Stochastic effects						
- Site	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
- Diver	0.0073	0.0036	0.0092	0.0059	0.0093	0.0154
- Month × Area	0.0046	0.0545	0.0011	0.0056	0.0016	0.0011
- Year × Area	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
- Month × Year	0.0265	0.2069	0.3402	0.0251	0.0465	0.0939
- Residual	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 3.4. Quantification of variances of stochastic effects for the models describing each of the algal variables: Cumulative algal cover (Cum. cov.), total cover (Tot. cov.), cumulative cover of opportunists (Cum. opp. cov.), cumulative cover of late-successional species (Cum. late cov.), fraction of opportunists (Frac. opp.) and number of late-successional species (Species no. late).

Model component	**Tot. cov.**	**Cum. cov.**	**Cum. late cov.**	**Cum. opp. cov.**	**Frac. opp.**	**Species no. late**
- Site	0.0395	0.1371	0.4578	0.1724	0.0223	0.0446
- Diver	0.0123	0.0858	0.0510	0.2154	0.0053	0.0160
- Month × Area	0.0078	0.0104	0.0540	0.0704	0.0054	0.0086
- Year × Area	0.0234	0.0920	0.1086	0.1722	0.0112	0.0187
- Month × Year	0.0036	0.0026	0.0022	0.0540	0.0022	0.0019
- Residual	0.0630	0.2808	0.5056	0.5889	0.0507	0.0924

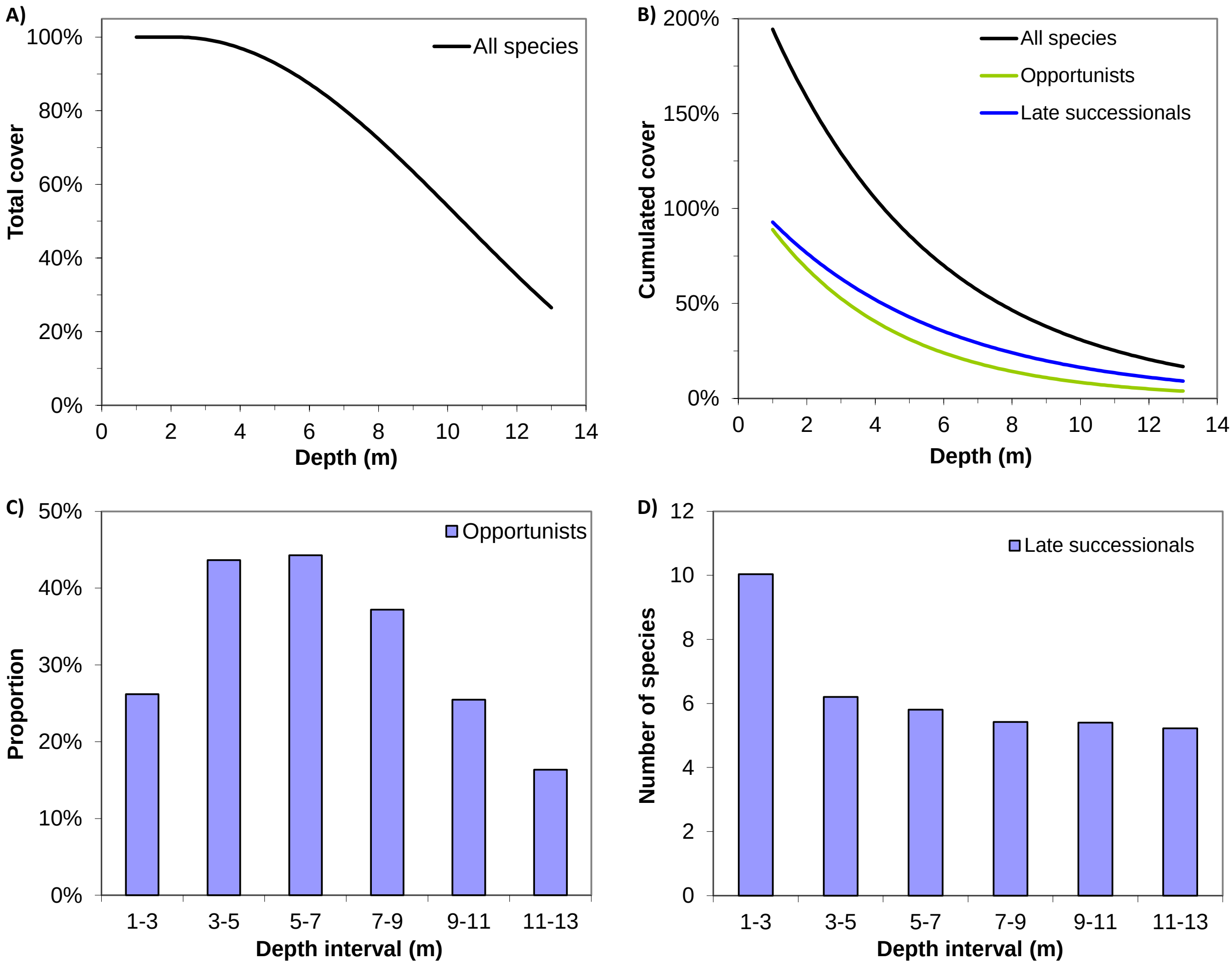


Figure 3.3. Modelled levels of algal variables as a function of water depth: A) total cover, B) cumulative cover, cumulative cover of late-successional species and cumulative cover of opportunistic algae, C) fraction of opportunists, D) number of late-successional algal species. The depth relationships are averages over all sampling areas covering May-September during the period 2001-2012 and for a hard substrate proportion of 50 %.

3.3.1 Indicator: Total cover

Macroalgae total cover varied significantly between areas and between sub-areas within areas as well as with depth and the amount of hard substratum (*Table 3.3*). However, common seasonal and interannual patterns across all areas were not significant relative to the random variation of these components between areas and the random seasonal variation between years. The random factors were all significant with the residual variations being largest followed by variation between sites, area-specific variations in the interannual pattern, and variation between divers (*Table 3.4*). Random variations in the seasonal pattern across areas and years were relatively smaller.

The different areas/sub-areas were compared based on a model of declining cover with depth (*Figure 3.3A*) that is shifted towards shallower or deeper depths for each of the areas/sub-areas (cf. *Figure 3.2*). For the areas in the Baltic GIG, the expected total cover at 7 m varied from 21 % in the inner part of Flensborg Fjord to 100 % in several areas (*Figure 3.4A*). Likewise, for the areas in the NEA GIG, the expected total cover at 7 m varied from 3 % in Lovns Bredning to 100 % in several areas (*Figure 3.4B*). Overall, there was a tendency for increasing mean total cover from inner parts of estuaries towards open coastal waters. The confidences of the mean total cover estimates reflected the amount of data available in the different areas/sub-areas and there was a considerable uncertainty in the estimates.

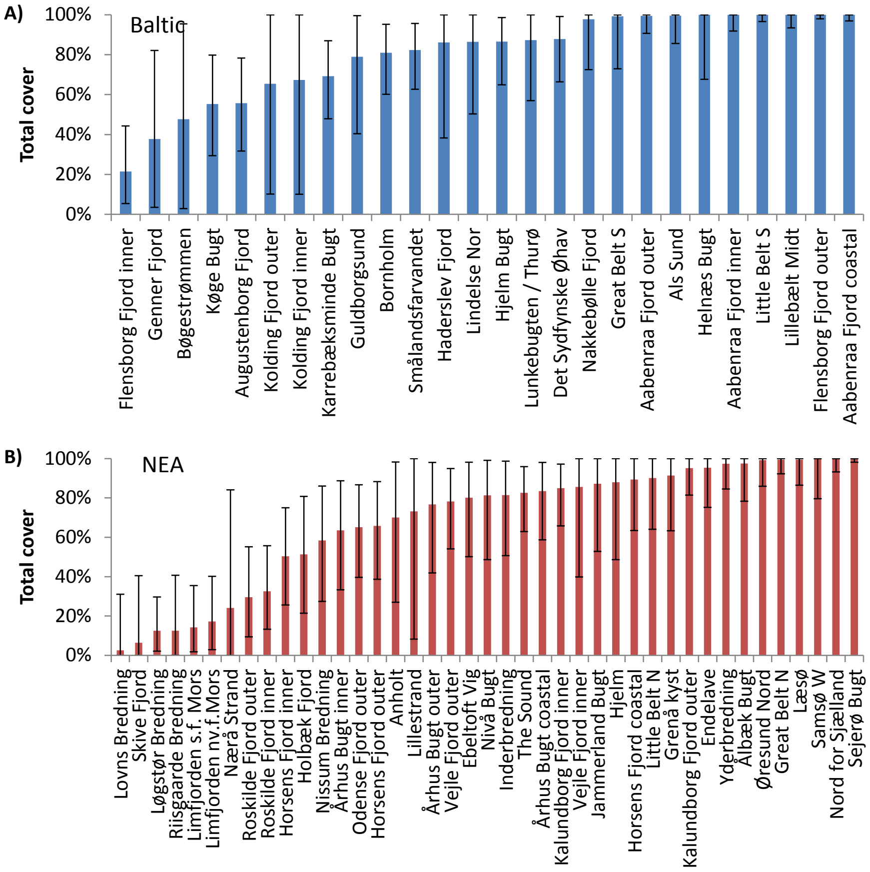


Figure 3.4. Modelled mean level of total cover at a standard depth of 7 m and 50 % hard substrate for areas/sub-areas in the A) Baltic GIG and B) NEA GIG. Area-specific means represent all months (May-September) and years (2001-2012). Error bars show the 95 % confidence intervals of the means.

Differences in total cover across areas/sub-areas could be explained by a combination of salinity and total nitrogen (*Table 3.5*). Total cover decreased with increasing levels of total nitrogen, but the decrease was stronger for high salinities (*Figure 3.5*). The model predicts that for a total nitrogen concentration of 40 µmol l^{-1} there will be no macroalgae for waters with salinity > 30, and for salinities around 10 and 20, the total cover at 7 m is expected to be 67 and 23 %, respectively. For TN concentrations in the lower end of the range (approximately open-water conditions), the algal response did not differ markedly with salinity but at higher N-levels the response intensified as salinity increased.

Table 3.5. Significant parameter estimates (Est.), coefficients of determination (R^2) and levels of significance (p) for relationships between algal variables and physico-chemical factors modelled by linear regression analysis. The following algal variables were analysed: Total cover (Tot. cov.), Cumulative algal cover (Cum. cov.), Cumulative cover of late-successional species (Cum. late cov.), Cumulative cover of opportunists (Cum. opp. cov.), fraction of opportunists (Frac. opp.) and number of late-successional species (No. late).

Variable	**TN**		**Salinity**		**Salinity*TN**		**Intercept**		**R^2**
	Est.	**p**	**Est.**	**p**	**Est.**	**p**	**Est.**	**p**	
Tot. cov. (arcsin)			0.0453	0.0031	-0.0017	< 0.0001	1.4101	< 0.0001	0.5408
Cum. cov. (ln)			0.0511	0.0041	-0.0037	< 0.0001	5.0178	< 0.0001	0.6280
Cum. late cov. (ln)	-0.1432	< 0.0001			0.0022	0.0164	5.9708	< 0.0001	0.5655
Cum. opp. cov. (ln)	0.0396	0.0070			-0.0051	< 0.0001	4.1192	< 0.0001	0.7029
Frac. opp. (arcsin)	0.0391	< 0.0001			-0.0014	< 0.0001	0.3209	0.0001	0.6203
No. late. (ln)			0.0806	< 0.0001	-0.0025	< 0.0001	1.3162	0.0003	0.5839

Whereas the relationship between macroalgae cover and TN confirms our current knowledge (e.g. *Nielsen et al. 2002a,b; Krause-Jensen et al. 2007a,b*), the cause for the relationship with salinity is less evident but may relate to the change in species composition with salinity. Thus, whereas brackish waters typically have relatively high fractions of green algae, of which many are tolerant species with thin tissues, more saline waters have larger fractions of red and brown algae of which many have thicker tissue and are more sensitive to eutrophication (*Nielsen et al. 1995, Steneck & Dethiers 1994*). It may also play a role that several of the areas with high salinity and high TN levels are from Limfjorden, where mussel dredging is intensive and larger substrates are generally absent. Mussel dredging, in combination with historical stone fishing, is believed over time to have affected the substrate size composition by removing larger stones and boulders so that only smaller-sized substrate remains, which makes the attached macroalgae more vulnerable to physical exposure. Another possible explanation is that Limfjorden, despite large reductions in nutrient levels, still has turbid waters due to resuspension of sediments and high concentrations of dissolved organic matter (*Carstensen et al. 2013*).

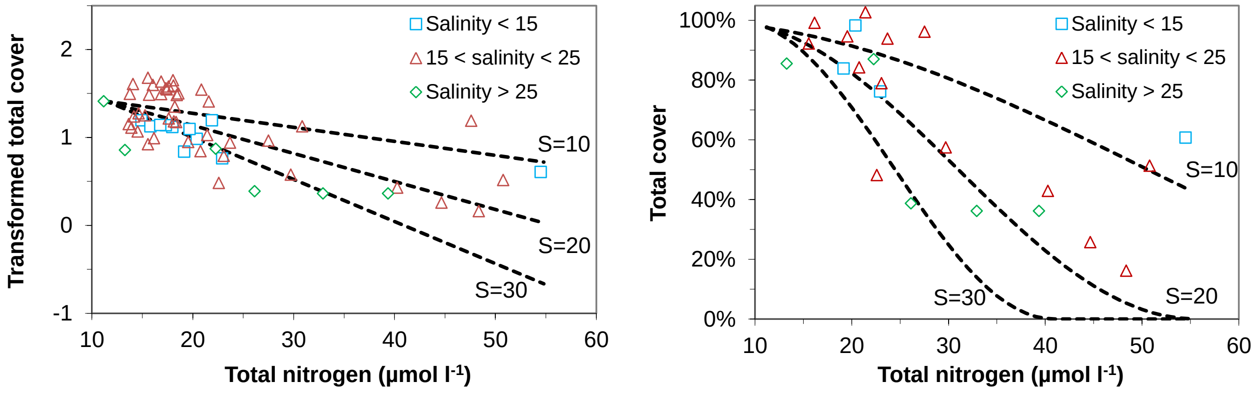


Figure 3.5. Total algal cover versus total nitrogen. Left panel shows transformed data whereas right panel shows back-transformed data. Area-specific means of total algal cover were calculated for a depth of 7 m and hard substratum of 50 %. For visualising the relationship with salinity, bearing in mind that this relationship is continuous, salinity has been stratified in three groups.

3.3.2 Indicator: Cumulative cover

Macroalgae cumulative cover varied significantly between areas and sub-areas within areas as well as with depth and the amount of hard substratum (*Table 3.3*), similar to total cover. The common seasonal and interannual variations, modelled as fixed effects, were not significant. For cumulative cover the seasonal variation among areas/sub-areas and across years, modelled as random effects, were also not significant. This suggests that there are just small differences in cumulative cover over the summer period (May-September) in general and even for individual areas/sub-areas. The largest random variation was between sites, followed by residual variation and changes in the interannual pattern between areas (*Table 3.4*). The variation between divers was significant although relatively small compared to the other sources of random variation.

The different areas/sub-areas were compared using a decreasing depth relationship (*Figure 3.3B*) that is shifted towards shallower or deeper depths for each of the areas/sub-areas (cf. *Figure 3.2*). For the areas in the Baltic GIG, the expected cumulative cover at 7 m varied from 21 % in the inner part of Flensborg Fjord to 244 % in southern Little Belt (*Figure 3.6A*). For the areas in the NEA GIG, the expected cumulative cover at 7 m varied from 10 % in Lovns Bredning to 244 % west of Samsø (*Figure 3.6B*). Similar to total cover, there was a tendency for increasing mean cumulative cover from inner parts of estuaries towards open coastal waters. There was a considerable uncertainty in the estimates due to the large natural variability in the data and the limited amount of observations.

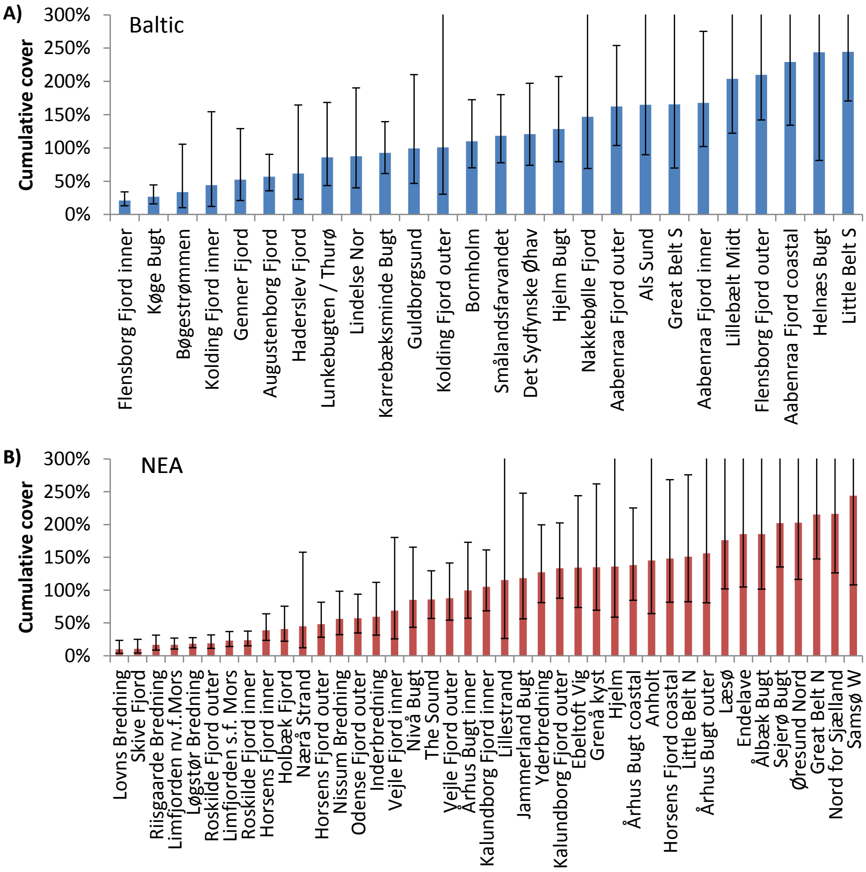


Figure 3.6. Modelled mean level of cumulative cover at a standard depth of 7 m and 50 % hard substrate for areas/sub-areas in the A) Baltic GIG and B) NEA GIG. Area-specific means represent all months (May-September) and years (2001-2012). Error bars show the 95 % confidence intervals of the means.

Differences in cumulative cover across areas/sub-areas could be explained by a combination of salinity and total nitrogen (*Table 3.5*). Cumulative cover decreased as expected with total nitrogen, but the decrease was also related to salinity as also identified for total cover (*Figure 3.7*). The model predicts that for a total nitrogen concentration of 40 µmol l^{-1} there will be 8 % cumulative cover for waters with salinity > 30, and for salinities around 10 and 20 the cumulative cover at 7 m is expected to be 57 % and 18 %, respectively. For TN concentrations in the lower end of the range (approximately open-water conditions), the effect of salinity on the response was not pronounced, but at higher TN-levels the algae responded most markedly at high salinities. The relationship with TN confirms our current knowledge, whereas the relationship with salinity is most likely due to the reasons given for total cover.

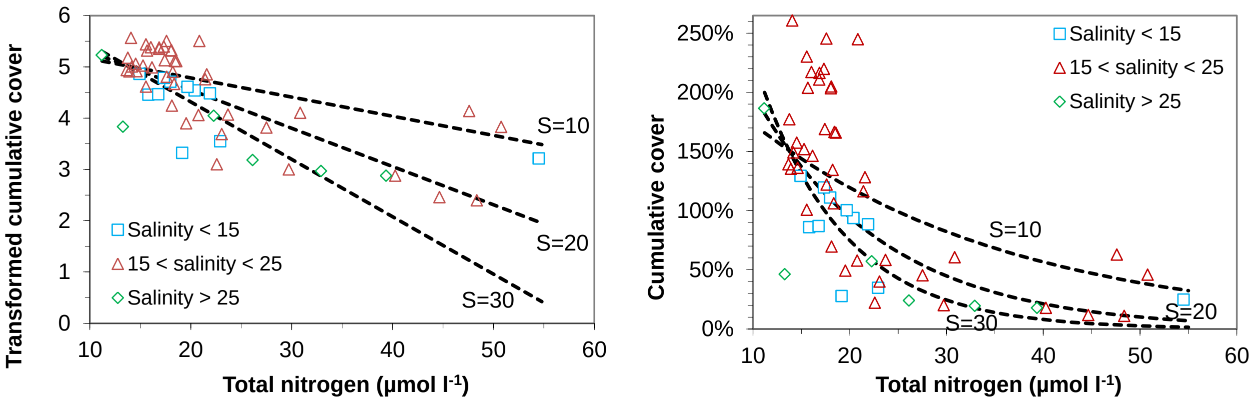


Figure 3.7. Cumulative algal cover versus total nitrogen. Left panel shows transformed data whereas right panel shows back-transformed data. Area-specific means of cumulative algal cover were calculated for a depth of 7 m and hard substratum of 50 %. For visualising the relationship with salinity, bearing in mind that this relationship is continuous, salinity has been stratified in three groups.

3.3.3 Indicator: Cumulative cover of late-successionals

The cumulative cover of late successionals varied significantly between areas and sub-areas within areas as well as with depth and the amount of hard substratum (*Table 3.3*). The common seasonal and interannual variations, modelled as fixed effects, were not significant. The random factors were all significant, except for the interannual changes in the seasonal pattern (May-September). The residual variation and variations between sites were the largest, almost an order of magnitude larger than the other random sources of variation (*Table 3.4*).

The depth relationship for cumulative cover of late successionals was similar to that for total cumulative cover, although displaying approximately 50 % less (*Figure 3.3B*). The cumulative cover of late successionals in the Baltic GIG predicted at 7 m depth varied from 0 % in Bøgestrømmen to 157 % in the middle part of Little Belt (*Figure 3.8A*). In the NEA GIG, the predicted cumulative cover at 7 m depth ranged from 0 % in inner Roskilde Fjord to 170 % west of Samsø (*Figure 3.8B*). The ranking of the areas/sub-areas suggested that cumulative cover of late successionals increased from inner parts of estuaries towards open coastal waters, but there was also a tendency for low salinity areas to have a lower cumulative cover of late successionals.

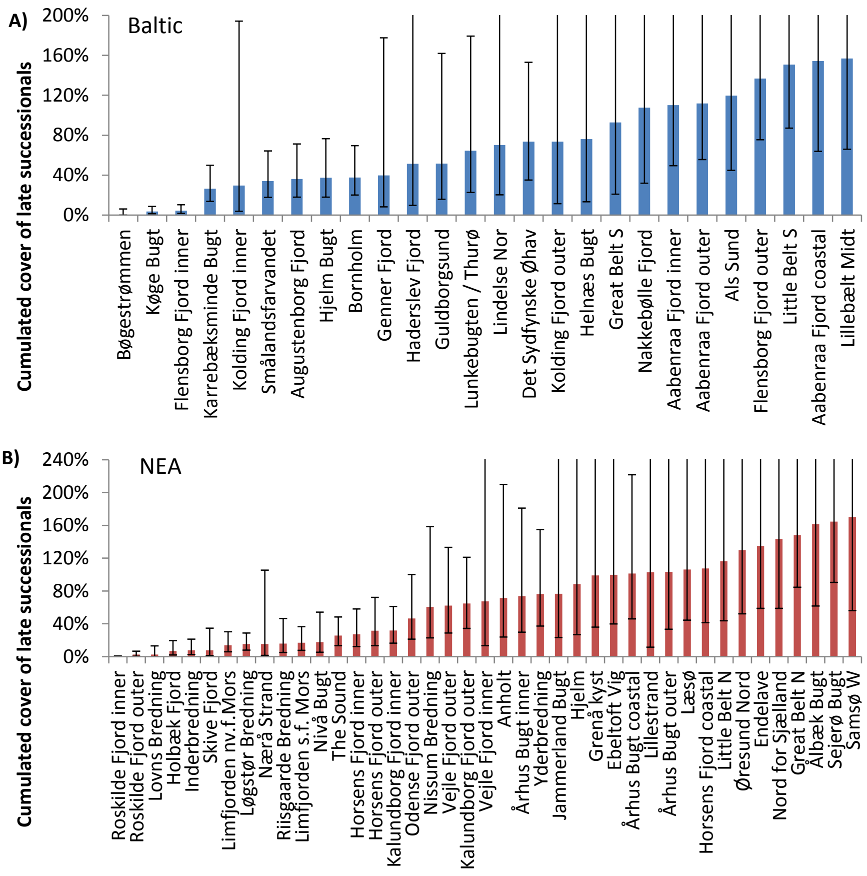


Figure 3.8. Modelled mean level of cumulative cover of late successsionals at a standard depth of 7 m and 50 % hard substrate for areas/sub-areas in the A) Baltic GIG and B) NEA GIG. Area-specific means represent all months (May-September) and years (2001-2012). Error bars show the 95 % confidence intervals of the means.

Differences in cumulative cover of late successionals across areas/sub-areas could be explained by a combination of salinity and total nitrogen (*Table 3.5*), decreasing with TN and increasing with salinity (*Figure 3.9*). It generally decreased from 100-150 % for low TN levels to < 20 % for TN concentrations above 40 µmol l^{-1}. For the low TN levels the cumulative cover of late successionals varied from ~100 % in areas/sub-areas with low salinity to ~150 % in areas with high salinity. Although there were salinity differences in the transformed variable for high TN concentrations, these differences translated into minor variations after back-transformation. It is noteworthy that even though total cumulative cover and cumulative cover of late successionals responded similarly to TN concentrations, the two indicators differed in their response to salinity, i.e. cumulative cover decreasing with salinity and cumulative cover of late successionals increasing with salinity for a given TN level.

The relationship with salinity for the cumulative cover of late successionals is in accordance with our existing knowledge suggesting that more diverse communities in saltier water should also lead to higher cumulative cover. The kelps are also most abundant in salty waters and as they increase habitat diversity and thereby stimulate biodiversity (*Steneck et al. 2002*), they may further contribute to the high levels of cumulative cover.

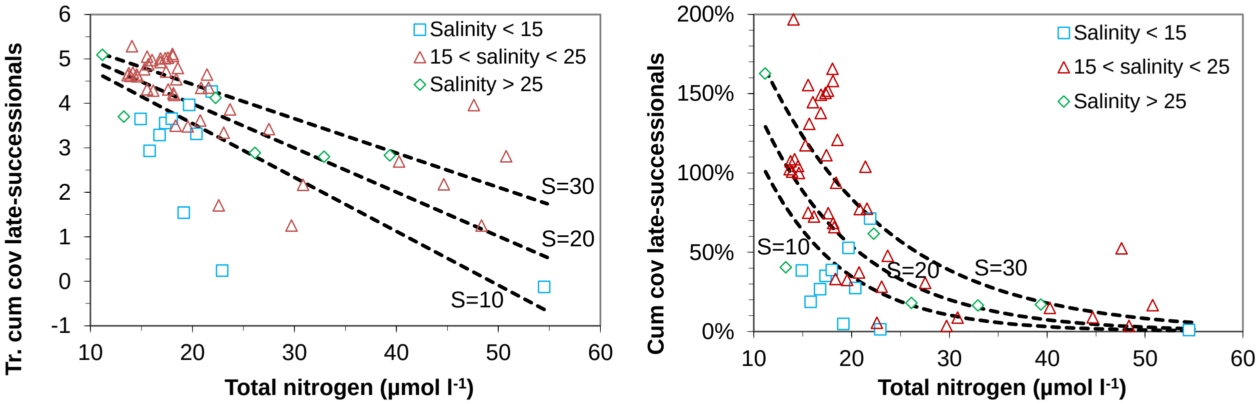


Figure 3.9. Cumulative algal cover of late successionals versus total nitrogen. Left panel shows transformed (Tr.) data whereas right panel shows back-transformed data. Area-specific means of cumulative algal cover were calculated for a depth of 7 m and hard substratum of 50 %. For visualising the relationship with salinity, bearing in mind that this relationship is continuous, salinity has been stratified in three groups.

3.3.4 Indicator: Cumulative cover of opportunistic species

The cumulative cover of opportunistic species varied significantly between areas and sub-areas within areas as well as with depth and the amount of hard substratum (*Table 3.3*). The common seasonal and interannual variations, modelled as fixed effects, were not significant. The random factors were all significant, although the random interannual variations in the seasonal pattern were not highly significant. The residual variation was the largest source of random variation, followed by diver-specific variation, site-specific variation and differences in the interannual variation among areas (*Table 3.4*). The relatively large diver-specific variation, compared with the other cumulative indicators, could indicate that divers have greater difficulties in assessing the cumulative cover of opportunistic species. This is probably because many of the opportunistic species are filamentous and can be interpreted to represent a large cover if basing the estimate on the outer contour or a lower cover if taking the spaces between filaments into account. Also opportunistic species have a more ephemeral occurrence than late-successionnals which increase their variability in space and time.

The depth relationship for cumulative cover of opportunists was similar to that for total cumulative cover, although displaying approximately 50 % less (*Figure 3.3B*). The cumulative cover of opportunists in the Baltic GIG predicted at 7 m depth varied from 3 % in Haderslev Fjord to 52 % in the coastal part of Aabenraa Fjord and with an exceptionally high cumulative cover of 97 % in Helnæs Bugt (*Figure 3.10A*). In the NEA GIG, the predicted cumulative cover at 7 m depth ranged from 0 % in Riisgaarde Bredning to 48 % north of Zealand (*Figure 3.10B*). Similar to the other cumulative indicators, the cumulative cover of opportunistic species increased from inner parts of estuaries towards open coastal waters.

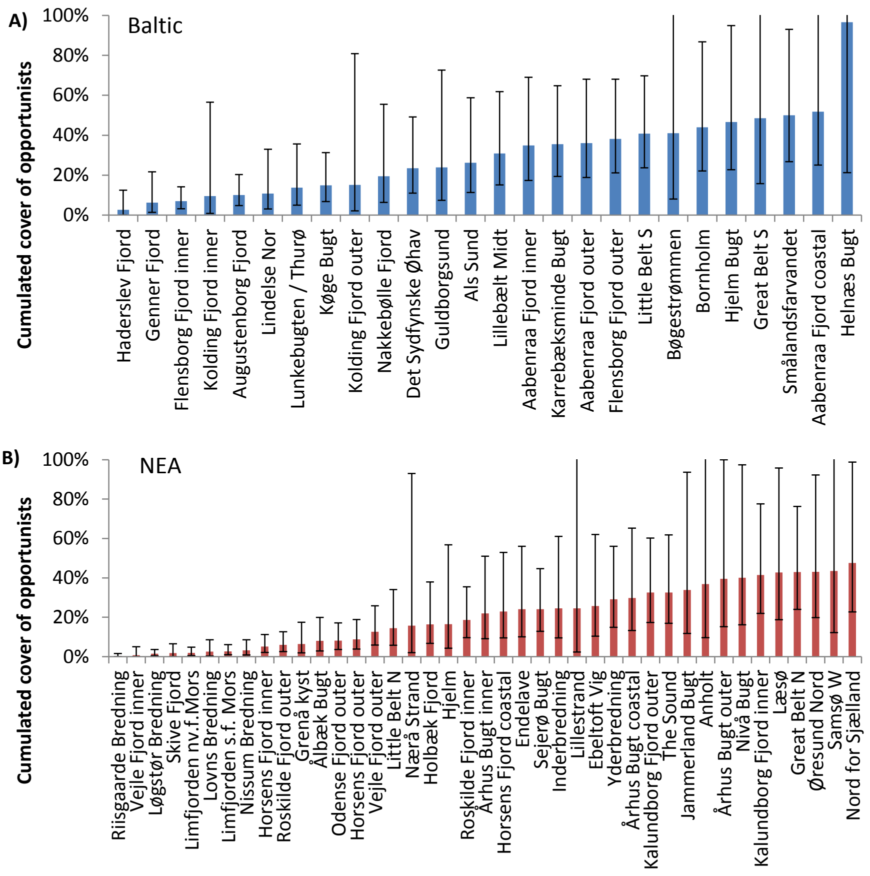


Figure 3.10. Modelled mean level of cumulative cover of opportunists at a standard depth of 7 m and 50 % hard substrate for areas/sub-areas in the A) Baltic GIG and B) NEA GIG. Area-specific means represent all months (May-September) and years (2001-2012). Error bars show the 95 % confidence intervals of the mean.

Differences in cumulative cover of opportunistic species across areas/sub-areas could be explained by a combination of salinity and total nitrogen (*Table 3.5*), decreasing with both TN and salinity (*Figure 3.11*). The decline with TN was less pronounced for low salinities (~10), suggesting a minor decrease from 55 % to 33 % over the entire TN range. For higher salinities the cumulative cover of opportunistic species decreased from 20-30 % at low TN levels to < 10 % for TN levels above 30 µmol l^{-1}. Overall, the cumulative cover of opportunistic algae is expected to decrease with TN, but this decrease can be compensated by opportunistic macroalgae being more competitive for resources (nutrients and light) than late successionals at low salinities.

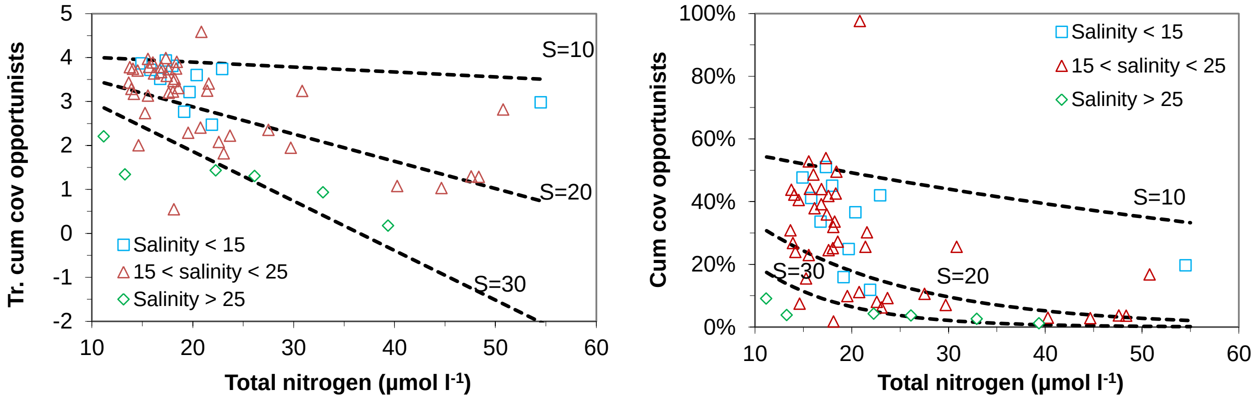


Figure 3.11. Cumulative algal cover of opportunistic species versus total nitrogen. Left panel shows transformed (Tr.) data whereas right panel shows back-transformed data. Area-specific means of cumulative algal cover were calculated for a depth of 7 m and hard substratum of 50 %. For visualising the relationship with salinity, bearing in mind that this relationship is continuous, salinity has been stratified in three groups.

3.3.5 Indicator: Fraction of opportunistic species

The fraction of opportunistic species varied significantly between areas and sub-areas within areas as well as with depth and the amount of hard substratum over 50 % (*Table 3.3*). The common seasonal and interannual variations, modelled as fixed effects, were not significant. The random factors were all significant, although the random interannual variations in the seasonal pattern were marginally significant. The residual variation was the largest source of random variation, followed by differences in the interannual variation among areas (*Table 3.4*). Despite a relatively large variation between divers in assessing the cumulative cover of opportunistic species, this random source of variation was less pronounced when the cumulative cover of opportunistic species was normalised by the cumulative cover of all species.

The fraction of opportunistic species was highest at depths between 3 and 7 m (~45 %) and lower at both shallower and deeper depths (*Figure 3.3C*). The fraction was < 20 % for depths over 11 m. In the Baltic GIG, the fraction of opportunists predicted at 7 m depth varied from 15 % in Nakkebølle Fjord to 72 % in Køge Bugt and with an exceptionally high fraction of 100 % in Bøgestrømmen (*Figure 3.12A*). In the NEA GIG, the predicted fraction of opportunistic species at 7 m depth ranged from 0 % in the inner part of Vejle Fjord to almost complete dominance in Roskilde Fjord (*Figure 3.12B*). The gradient from inner estuaries towards open coastal waters, observed for the other indicators, was not as apparent for the fraction of opportunistic species.

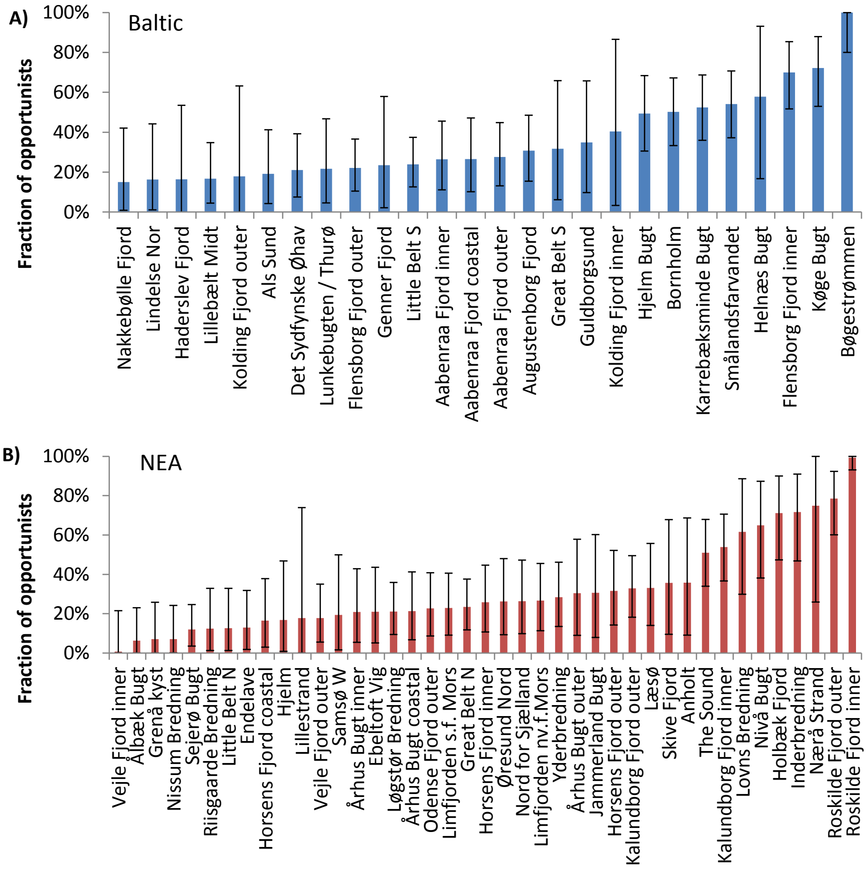


Figure 3.12. Modelled mean level of fraction of opportunistic species at a standard depth of 7 m and 50 % hard substrate for areas/sub-areas in the A) Baltic GIG and B) NEA GIG. Area-specific means represent all months (May-September) and years (2001-2012). Error bars show the 95 % confidence intervals of the means.

Differences in the fraction of opportunistic species across areas/sub-areas could be explained by a combination of salinity and total nitrogen (*Table 3.5*). Whereas the fraction of opportunists remained relatively low for high salinities (~30) across the entire TN range, this fraction increased with TN at lower salinities (*Figure 3.13*). Actually, the model predicted that for salinities around 10 and TN > 40 µmol l^{-1} the macroalgae community would be completely dominated by opportunistic species. The fraction of opportunistic species decreased with salinity at low TN levels as well. These regression results suggest that both TN and salinity have an important role in structuring the macroalgae community.

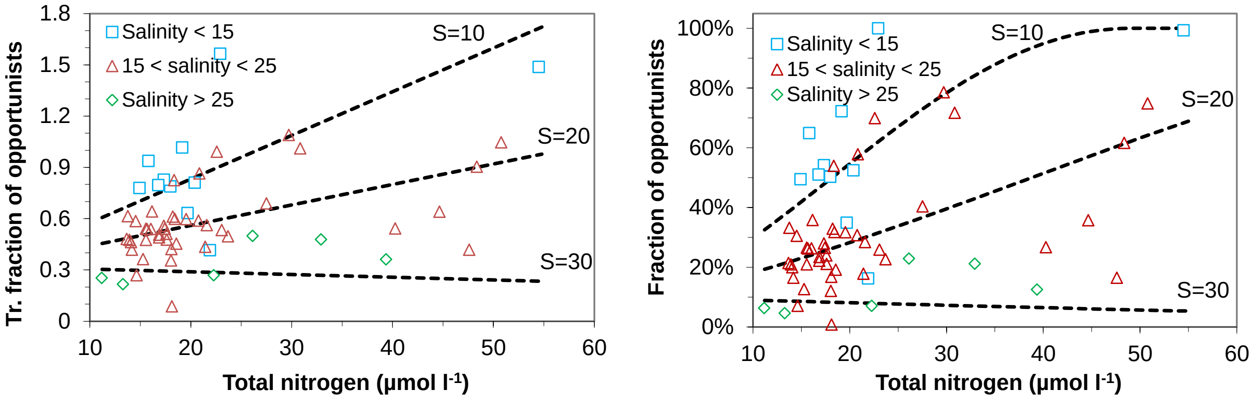


Figure 3.13. Fraction of opportunistic species versus total nitrogen. Left panel shows transformed (Tr.) data whereas right panel shows back-transformed data. Area-specific means of cumulative algal cover were calculated for a depth of 7 m and hard substratum of 50 %. For visualising the relationship with salinity, bearing in mind that this relationship is continuous, salinity has been stratified in three groups.

3.3.6 Indicator: Number of late successionals

The number of late successional species varied significantly between areas and sub-areas within areas as well as with depth and the amount of hard substratum less than 50 % (*Table 3.3*). The common seasonal variation was not significant, whereas there was a significant variation between years. The random factors were all significant, except for the changes in seasonal patterns across years. The residual variation was the largest source of random variation, followed by site-specific variation (*Table 3.4*). The other sources of random variation were relatively small compared to these.

The number of late successional species was much higher at 1-3 m depth compared to deeper depths, and there was a small decline for depth > 3 m (*Figure 3.3C*). The mean number of late successional species at 7 m in the Baltic GIG varied from 1.0 in the inner part of Flensborg Fjord to 10.8 in the southern Great Belt (*Figure 3.14A*). In the NEA GIG, the number of late successional species at 7 m varied from 0.8 in Lovns Bredning to 13.8 around Læsø (*Figure 3.10B*). Many of the areas/sub-areas with low numbers of successional species had a high proportion of opportunistic species, suggesting that only a few late successional species could compete with the opportunistic species in these environments. In both GIG regions, there was also an increasing gradient from inner parts of estuaries towards open coastal waters.

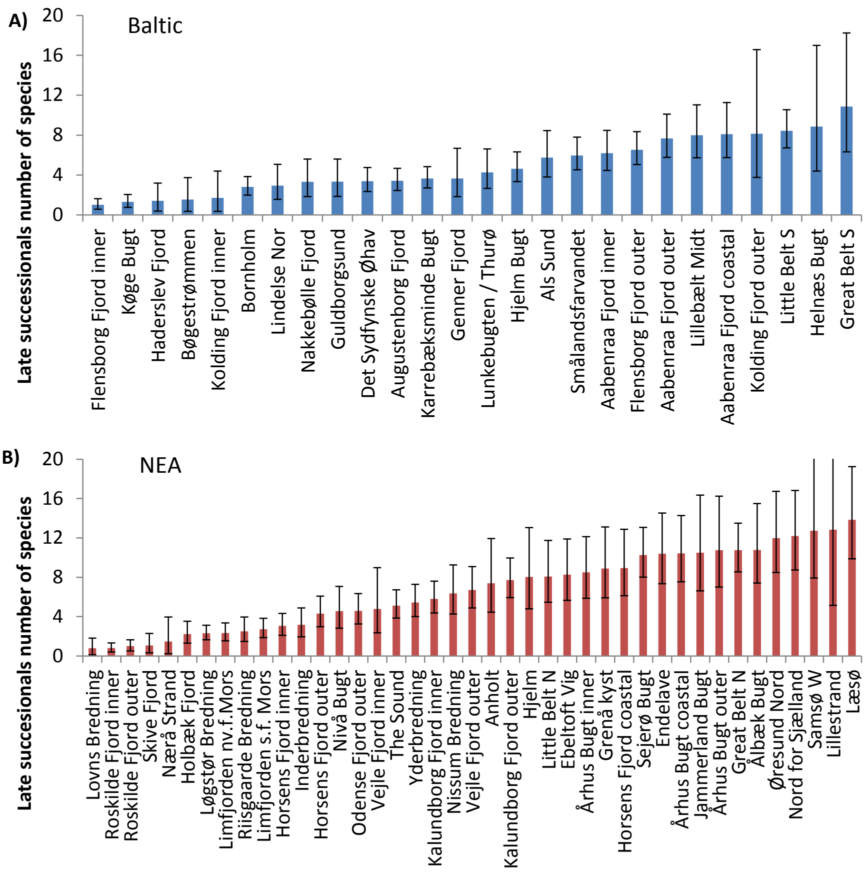


Figure 3.14. Modelled mean number of late successional species at a standard depth of 7 m and 50 % hard substrate for areas/sub-areas in the A) Baltic GIG and B) NEA GIG. Area-specific means represent all months (May-September) and years (2001-2012). Error bars show the 95 % confidence intervals of the means.

Differences in the number of late-successional species across areas/sub-areas could be explained by a combination of salinity and total nitrogen (*Table 3.5*). There were only few late successional species (< 5) for TN levels > 30 µmol l^{-1}, but for lower nutrient levels the expected number of species increased, especially in areas with high salinities reaching more than 15 late successional species in the saline northern Kattegat in this type of investigation. These regression results suggest that both TN and salinity have an important role for the diversity of late successional species, but also for the diversity of the entire macroalgae community.

The maximum of 15 late-successional species recorded in areas of ca. 25 m^2 in this type of monitoring survey is far less than the maximum number of algal species (318 species) which has been identified in western Kattegat through detailed, high-intensity surveys (*Nielsen et al. 1995*). This underlines the importance of clearly defining the level of detail and the size of the areas surveyed when species numbers are to be compared.

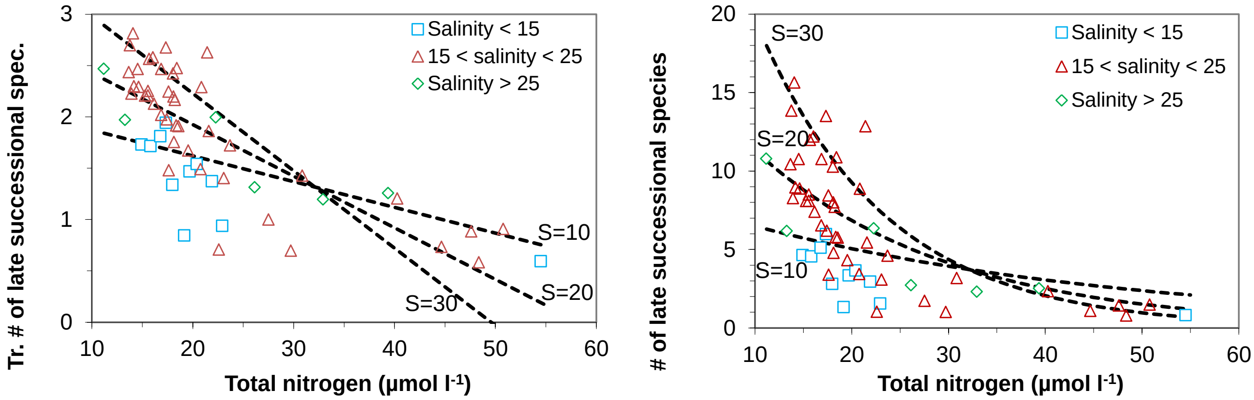


Figure 3.15. Number of late successional species versus total nitrogen. Left panel shows transformed (Tr.) data whereas right panel shows back-transformed data. Area-specific means of cumulative algal cover were calculated for a depth of 7 m and hard substratum of 50 %. For visualising the relationship with salinity, bearing in mind that this relationship is continuous, salinity has been stratified in three groups.

3.4 Boundary setting and assessment of macroalgae status

In order to calculate reference conditions and boundaries for the six different indicators proposed above (*Annex 1-6*), the TN boundary levels proposed in *Carstensen et al. (2008)* and mean salinities (2001-2012) were used in the regressions described above (*Figures 3.5, 3.7, 3.9, 3.11, 3.13 and 3.15; Table 3.5*). The confidence of the reference conditions and class boundaries was also calculated by means of Monte Carlo simulation, taking the uncertainties from the estimated TN boundaries and from the regressions into account. There were large differences in indicator boundaries across the different areas as well as in the confidences used to characterise these boundaries. However, in all cases the estimated confidence intervals for the boundaries had large overlaps, making it difficult to clearly discriminate between quality classes.

3.4.1 Uncertainty of the indicators

In order for an algal variable to be applicable for assessment of ecological status, the class boundaries should be relatively well-defined (i.e. narrow confidence intervals) and the status class bands should be relatively broad. This demand was best fulfilled for the indicators, which were associated with a low degree of stochastic variability and a strong response to changes in TN concentrations.

Different transformations were employed for the different indicators and therefore, the variances cannot implicitly be compared (*Table 3.4*). The three indicators for cumulative cover and the number of late successional species were all log-transformed, which implies that the variances represent a measure of the relative variability of the different sources of random variation. Overall variances for total cumulative cover were smaller than for the cumulative cover of opportunists and late successionals, which had somewhat more comparable variances. The variances for the number of late successional species were even lower, suggesting that this indicator is associated with less uncertainty and thus a relatively precise indicator. For the two indicators where the arcsin-transformation was employed (total cover and fraction of opportunists), the fraction of opportunists had the lowest variances on all random sources (*Table 3.4*). Thus, the indicator for the fraction of opportunists is more precise than the total cover. The uncertainty of these two indicators can be roughly compared to the log-transformed indicators by calculating the standard deviation of a single transformed observation by taking the square root of the sum of the variance components. The relative uncertainty of the two arcsin-transformed indicators was assessed by considering the ratio of this standard deviation to the expected value of the transformed indicator (*cf. Figures 3.5 and 3.13*). Using this approach it is assessed that total cover indicator is more precise than the total cumulative cover, and that the fraction of opportunists is more precise than the cumulative covers of opportunists and late successionals, but less precise than the number of late-successional species.

3.4.2 Sensitivity of the indicators to nutrient level

The response of TN concentration to changes in TN loading in a given area also affects the width of the status class. Thus, areas which demonstrate a strong response in TN upon changes in N-loading, i.e. areas having a large regression slope for the regression of TN-concentration upon TN-input (*Carstensen et al. 2008*) have broader status class bands for TN than areas showing a weak response to TN inputs. Many open areas, such as Bornholm, show weak responses in TN concentrations to changing TN inputs and, therefore, have narrow status class bands for TN, which will similarly affect the class bands for the macroalgae indicators. By contrast, areas with significant nutrient sources and low water exchange with the open boundary, such as inner Odense Fjord, several Limfjord basins, Randers and Nissum Fjords, show strong responses in TN concentration to changes in TN inputs and therefore have broader status class bands. The demand for the indicators to have low stochastic variability and high sensitivity to TN is intensified if the area in question has narrow status class bands for TN.

The indicators ‘total cover’, ‘cumulative cover’, ‘cumulative cover of late successionals’, and ‘number of late-successional species’ generally had the strongest responses to TN concentration across the entire salinity range, whereas ‘cumulative cover of opportunists’ did not show a strong relationship to TN at low salinities. The ‘fraction of opportunists’ had a weak response to changing TN concentrations at high salinities, but responded strongly to TN levels for both mid-range and low salinities.

3.4.3 Status assessment for macroalgae

Our results suggest that the status of macroalgae in a water body should be assessed based on cover and taxonomical composition, where the latter refers to both the overall composition and the presence of disturbance-sensitive species. The late-successional species are believed to constitute such a group of disturbance-sensitive species, as confirmed by the declines in cumulative cover, increases in the fraction of opportunists in low and medium salinity areas, and decreasing number of species with increasing TN levels. Therefore, in order to simplify and reduce the potential redundancy of information contained in the various indicators, we propose to focus on only three of the six proposed macroalgae indicators, which represent both cover and taxonomical composition: 1) cumulative cover of macroalgae, 2) fraction of opportunists, and 3) number of late-successional species. The rationale for selecting these three indicators is described below.

The indicators total cover and cumulative cover both describe the cover of the entire macroalgae community. Both indicators display significant and similar responses to changing TN levels (*Figures 3.5 and 3.7*) and total cover is relatively more precise than cumulative cover. However, total cover is a truncated indicator in the sense that in shallow waters with good environmental conditions all observations are 100 %. This truncation effect could be the reason that this indicator is associated with less uncertainty than cumulative cover, because it is easier for the statistical model to describe by means of the fixed effects. The truncation effect could also pose a problem for using total cover to assess ecological status in shallow areas with good environmental conditions, since there will be no or very little graduation. Essentially, the truncation effect provides less information compared to cumulative cover that also differentiates between a diverse macroalgae community consisting of several layers and a simple macroalgae community just covering the available substrate. Therefore, cumulative cover is preferred as indicator of the macroalgae cover.

The indicators cumulative cover of late successionals and opportunistic species as well as fraction of opportunists and number of late successionals describe the macroalgae community. The two indicators of cumulative cover were associated with relatively high random variation, implying that more observations are needed to achieve a classification with sufficient confidence. The fraction of opportunistic species responded strongly to changes in TN for low salinities, whereas the number of late successional species responded most strongly to TN at high salinities. Since the two indicators express different components of the macroalgae community, both indicators are chosen as indicators of the macroalgae community.

For combining the three proposed indicators to a joint overall ecological assessment for macroalgae, we propose that each indicator is transformed to an EQR-scale having equidistant class boundaries (*Figure 3.16*). The transformation uses linear interpolation between the proposed boundaries for the different indicators. Applying the transformation will produce three indicator values on an EQR-scale and the combined assessment for macroalgae is proposed to be the average of these three values, thereby weighting the three indicators equally.

These EQR-transformations are not linear or inverse as proposed in the CIS guidelines, but nevertheless relatively simple to apply and probably more meaningful if ecological status is to be assessed on a proportional scale for the status classes, i.e. assuming that the perception of a change from high to good is similar to a change from good to moderate, and so forth. Another important aspect of using this proposed EQR-transformation is that the EQR-scales for the different indicators are identical, allowing computing averages (weighted, if needed). The approach is scalable in the sense that additional indicators can be added, provided that reference and boundary conditions are known. This also implies that the ecological status for the biological quality element benthic vegetation can be calculated as the average of the EQR-value for macroalgae (after averaging the EQR of these) and the EQR-value for angiosperms.

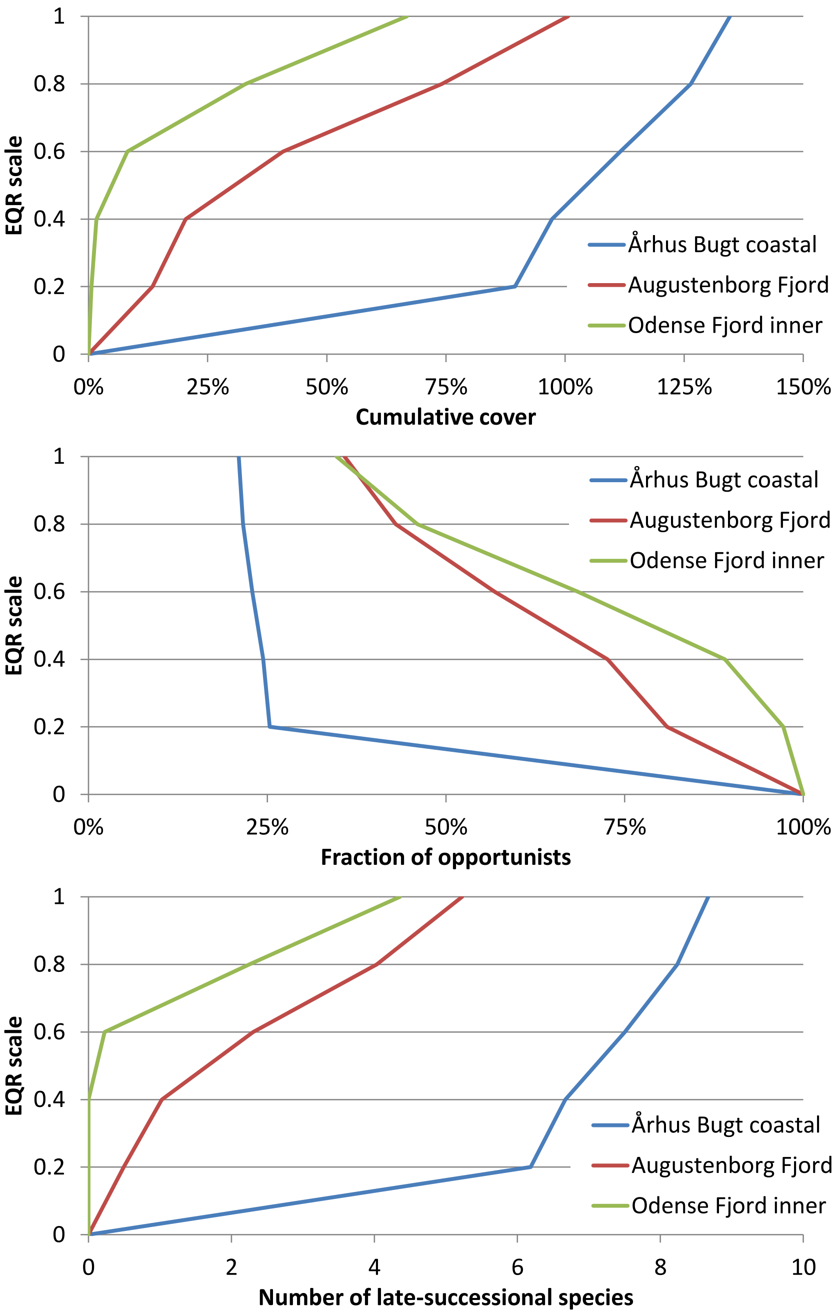


Figure 3.16. Illustration of the EQR-transformation of the three proposed macroalgae indicators for three selected areas. EQR-boundaries are 0.8 (High/Good), 0.6 (Good/Moderate), 0.4 (Moderate/Poor), 0.2 (Poor/Bad) and the indicator boundaries are from Annexes 2, 5 and 6.

Each of the indicator values used in the assessment has an associated uncertainty. In order to assess the distribution of the combined EQR-value for macroalgae the easiest approach is to use Monte Carlo simulations for the various indicators and calculate how these uncertainties propagate to the final macroalgae status assessment. This will provide probabilities of the final macroalgae status assessment belonging to each of the five status classes.

The proposed ecological status classification involves calculations that should be implemented in a standardised and quality-assured statistical software. This software should generate a report that documents the different steps in the classification in a transparent manner (i.e. at the indicator level, at the EQR-scale for each indicator and for the combined EQR-value), as opposed to a composite index where it is not always easy to decipher the actual cause, if good ecological status in not achieved.

3.5 International intercalibration of vegetation indicators

Denmark shares common water body types with Sweden (Type NEA 8b and BC6) and with Germany (BC8) and the benthic vegetation indicators should therefore in principle be intercalibrated with our neighbouring countries. However, neither the Swedish nor the German intercalibration sites fulfil the data acceptance criteria for intercalibration and therefore the intercalibration could not be performed.

The data acceptance criteria of the intercalibration guidelines demand that data must sufficiently cover the geographical area in which the common type area occurs and encompass sampling sites covering the entire gradient of the pressure to be intercalibrated, and hence the complete ecological quality gradient, ranging from high to poor ecological status. It is particularly important that the available data sufficiently cover the GM boundary, i.e. that there is a sufficient number of data points from both good and moderate status.

Almost all Swedish phytobenthic sites in the intercalibration type areas NEA 8b and BC6 show high or good status and therefore do not fulfil the data acceptance criterion regarding an ecological quality gradient in the type areas. Consequently, they do not allow intercalibration.

German data in the intercalibration type area BC8 only cover two status classes (poor, moderate) and therefore also fail fulfilling the data acceptance criterion regarding an ecological quality gradient.

Intercalibration between Denmark and Sweden as well as between Denmark and Germany would have required a so-called 'option 2 intercalibration' since the methods differ among countries and the available data do not allow a direct comparison among the different methods. An option 2 intercalibration involves the use of a 'common indicator' which in our case would have had to be a physico-chemical indicator such as e.g. nutrient concentration or Secchi depth, as no common vegetation indicators are available.

3.6 Conclusions

- Macroalgae monitoring observations depend on depth and amount of substrate and it is necessary to adjust for these covariates when calculating macroalgae indicator values.

- Several sources of random variation contribute to uncertainty in the proposed macroalgae indicators; the most important are spatial variation and variation with depth (residual).

- All macroalgae indicators responded to changes in TN and thereby fulfil an important prerequisite (sensitivity) for use as indicators of water quality.

- All macroalgae indicators also responded to changes in salinity and thereby highlight the need for setting different targets depending on salinity.

- Six different indicators were examined and three of these are proposed to characterise the macroalgae cover and composition. These proposed indicators are: 'cumulative algal cover', 'fraction of opportunists' and 'number of late-successional species.

- Reference and boundary conditions have been calculated for the investigated macroalgae indicators, using TN boundaries from a previous study and translating these to boundaries for the macroalgae indicators by means of established relationships between these and TN and salinity.

- An assessment tool is proposed that scales the indicator values to a common EQR-scale with equidistant status classes. This approach provides a generic framework for combining indicators to assess the ecological status of biological quality elements and sub-elements. This approach is also recommended for combining macroalgae and angiosperm status assessment into a combined assessment for benthic vegetation.

4 Benthic fauna

A revision of the report *Josefson (2008)* was undertaken to include adaptation of the DKI index to be operational in a wider context, and not (as before) only in deeper polysaline areas. The main object was to estimate 'reference' values in coastal waters for components in the Danish Quality Index (DKI) of benthic macrofauna first described in *Borja et al. (2007)* and tested in pollution gradients by *Josefson et al. (2009)*. The chapter consists of three parts:

1) By using data from the *Josefson (2008)* report and some additional data, investigating what natural factors are important for local richness diversity and consequently for the Shannon diversity (H), one major component in the DKI index.

2) Using the results from 1) to evaluate the applicability in coastal and estuarine environments of the salinity corrected version of DKI, the DKIver2, used in the EU Baltic GIG and NEA GIG 8b intercalibration exercises (both approved by the EU Commission).

3) A re-description of the DKIver2 index with some amendments and followed by suggestions of a status determination procedure.

4.1 Background

In order to account for differences in natural factors, methods for assessment of environmental quality according to the EU Water Framework Directive (WFD) should be adapted to the different physical-chemical environments, the so-called typologies. In connection with the EU GIG intercalibration work with benthic macrofauna, one biological quality element in the WFD, there has been developed limits between different quality classes, especially between *Good* and *Moderate* ecological status (GM border) for a number of typologies in Europe. Some of these types, NEA1/26, NEA 8b and Baltic B12 include also parts of the Danish territorial waters, especially the deeper open parts. The shallow (< 15 m) open coastal waters and the more or less closed areas (estuaries, inlets etc.) in Denmark represent other types not yet included in the WFD work. The simple Danish method of assessment, the DKI method (*Borja et al 2007; Josefson et al. 2009*), was developed to measure benthic macrofauna 'quality'. The most important factors in the DKI index are Shannon's H diversity and the AMBI index of species sensitivity.

The aim of the *Josefson (2008)* report was to adjust the DKI method to shallow open coastal areas and estuarine areas in Denmark, and to suggest type specific borders between *Good* and *Moderate* ecological status according to the WFD. Furthermore, the aim was to discuss a possible procedure of assessment of ecological status in water bodies. In the 2008 report, data on benthic macrofauna diversity and composition, amalgamated into DKI values, were collected from 540 stations in ca. 100 areas of the Danish shallow (< 15 m) open coastal and estuarine areas. The total material comprised > 2600 samples, each covering 0.1 m^2, from three decades (1980-2007). For the cases where a smaller sampling gear, the Haps sampler, was used, data were pooled to make up 0.1 m^2. Statistical comparisons indicated that the positive effect of pooling on diversity (H) and consequently on DKI, was minor and if significant, less than 10 %.

The 2008 report resulted in the following conclusions and suggestions:

The development of diversity (H) and DKI in relation to salinity showed that salinity was of high importance in open coastal areas, but less in the most closed areas, where it instead was a negative effect of hydraulic residence time. Based on this information, a new reduced typology was suggested where the open coast was divided into three types based on salinity following the Venice convention, and the closed areas (estuaries etc.) were divided into three types based on a combination of residence time (< or ≥ 20 days) and salinity. Reference for Hmax was determined within each of these six types by taking the 99 % percentile of H values from apparently 'the less disturbed parts' of the (or all values from each) typology. The Good-Moderate border (GM border) in each typology was obtained by the 5 % percentile of H values from what was judged 'less disturbed or undisturbed' parts of the typology.

However, this approach turned out to be operationally complicated and also subject to discussions whether or not the procedure of setting the GM border was valid or not, furthermore, it soon turned out that the AMBImin also was subject to variation depending on environment type (i.e. salinity-open-closed). So it became clear that some other approach was needed for these coastal areas.

Consequently, in connection with the EU Baltic GIG intercalibration work, the DKI index was modified, and because salinity in this area is a major factor influencing H diversity (and AMBI as well), the index was normalized to salinity so that both maximum value of H (Hmax) and minimum value of AMBI (AMBImin) varies with salinity during a part of the salinity range. At the same time, the factor in the index correcting for low species numbers was omitted because species numbers are often naturally low in the Baltic Sea and in fact already included in the Shannon's H. By this modification the DKI could approach a maximum value of 1 irrespective of salinity environment, and therefore the well documented pollution gradient in the Aarhus Bight was used to test the new index and the GM border was determined using the method of *Josefson et al. (2009)* to 0.68. The modified DKI was called DKIver2 and is re-described with a few amendments together with descriptions of border-setting and water body status assessment procedures below.

4.2 Is further normalization of DKI needed in addition to salinity?

The question was raised, if the DKIver2 is applicable in a broader context such as also in the shallow open coastal areas and estuaries of Denmark. Before we could answer this question, we needed to investigate if there are other natural factors for which we need to normalize DKI, in particular in the estuarine environments. Thus, to investigate this, data from the *Josefson (2008)* report, and some additional data, were used to determine what natural factors were important for local diversity and consequently for the Shannon diversity (H), one major component in the DKI index, and for the AMBI part of the index as well.

There are basically three types of factors that may affect community assembly, such as local diversity, which here is species numbers and composition at the scale of 0.1 m^2. A) Niche-based factors such as adaptation to the environment, B) dispersal-based factors such as non-random migration of individuals and propagules from adjacent species pools, and C) ecological drift (*sensu Hubbell 2001*) due to random processes limited by dispersal distance.

Previous work in the Danish waters indicates that dispersal from a species pool into the different water bodies (estuaries) may be an important natural factor affecting local diversity (*Josefson & Hansen 2004*). At the same time it is evident that environmental differences in salinity affect diversity because species show differential adaption to salinity levels (*Bonsdorff & Pearson 1999; Josefson 2009; Bleich et al. 2011*). Other potentially important factors are saltwater flux computed from estuary volume/residence time of the water that may mediate dispersal from the open sea species pool into the estuaries (*Josefson & Hansen 2004*), because invertebrates mostly are passive dispersers (follow the currents). But since residence time estimates are not available from all estuaries, the entrance width (estuary mouth width) of the estuary was used as a predictor of flushing (*Rasmussen & Josefson 2002*). A final natural factor considered was the areal extent (or size) of the water body. A large estuary, for instance, is likely to have a greater variety of habitats and consequently species which could result in a more diverse larval pool inside the estuary.

Here, in an attempt to disentangle the possible effects of the mentioned factors (environmental-based and dispersal-based) on local (0.1 m^2) diversity (alpha and pairwise beta), diversity patterns were examined of three groups of invertebrate species with different dispersivity judging from their mode of reproduction: 1) Highly dispersive species: species with planktotrophic larval development with long pelagic life and exerting high propagule pressure on the settling habitat, 2) low dispersive species: species with direct larval development, having no pelagic larval life and exerting small propagule pressure on the habitat, 3) remaining species with intermediate dispersal ability, mostly with lecithotrophic larval development and short pelagic life. Abundance-occupancy analysis of the same species in Danish waters has verified that species of group 1) are more dispersive than species of group 2) (*Josefson & Göke 2013*). For these three groups, alpha within and among estuaries, pairwise beta among estuaries and between estuaries and open sea pools were analysed in relation to different estuarine environment (salinity, depth, estuary size), in relation to dispersal barriers, i.e. estuary entrance width (mouth width) and distance from entrance in the estuaries, and geographical water-way distance among estuaries.

The test area used, which may be regarded as a metacommunity, i.e. a set of communities connected by dispersal (*sensu Leibold et al. 2004*), contained a marine area with a presumably fairly homogeneous species pool, the Kattegat and Samsø Bælt, fringed by a series of estuaries (17) along the Danish part of the coastline. (*Figure 4.1*, *Table 4.1*). The estuaries are mainly flushed from the sea and have entrance widths (mouth widths) differing over three orders of magnitude, potentially representing a gradient of dispersal barriers between the estuaries and the sea.

The fauna material contained 541 taxa, ca. 340,000 individuals from c. 1,000 van Veen-sized samples collected once or several times from 256 sites (*Figure 4.1*) over two decades (1990s and 2000s). Species with freshwater affinity or terrestrial affinity like insects were omitted from the analysis.

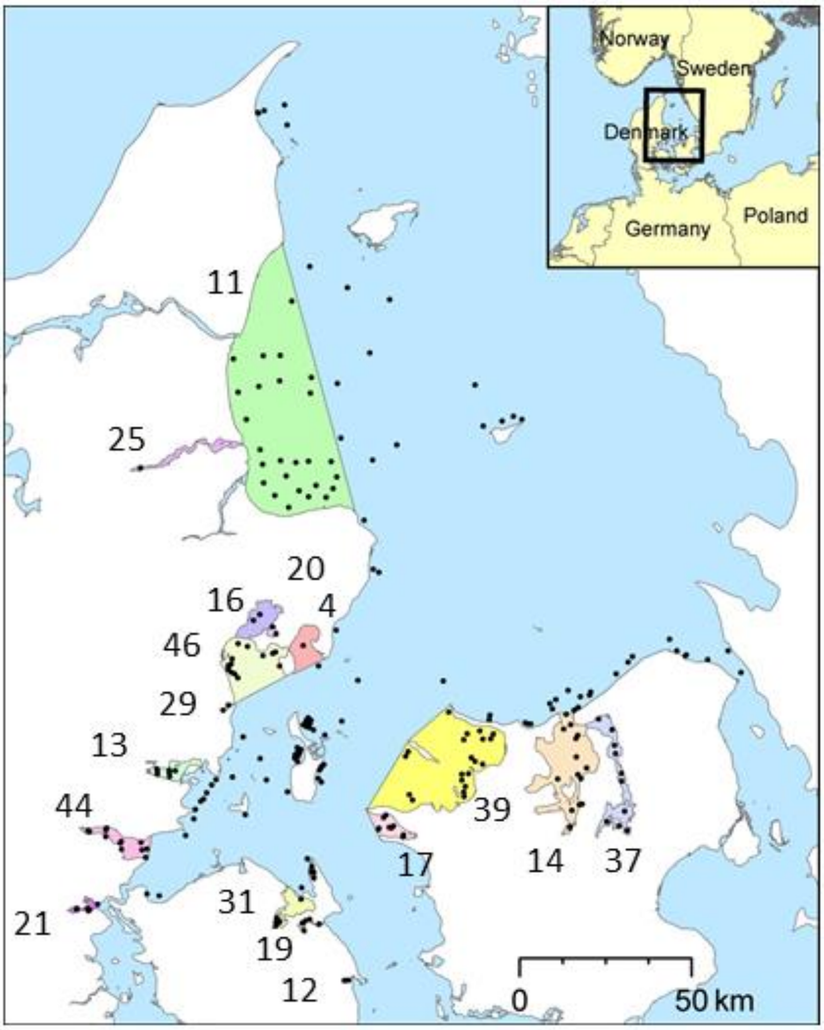


Figure 4.1. Map of investigated areas with the 17 estuaries indicated by numbers and different colours. Estuary names are given in *Table 4.1*. Black dots indicate sampling sites. Map by C. Göke.

Table 4.1. Environmental variables and sampling data for 17 investigated estuaries. Fresh-water index was calculated as: (Open sea salinity – Estuary salinity) / Open sea salinity, a measure of relative freshwater influence in the estuaries.

Estuary	Estuary no.	Area (km^2)	Mouth width (km)	Average salinity	Average salinity outside mouth	Fresh-water index	No. of sites	No. of samples
Holckenhavn Fjord	12	0.5	0.22	9.5	18	0.47	3	10
Norsminde Fjord	29	1.7	0.20	11.5	27	0.57	2	12
Knebel Vig	20	7.7	0.83	23.0	27	0.15	2	2
Kerteminde Fjord and Nor	19	8.7	0.04	17.5	20	0.13	6	6
Kolding Fjord	21	14.9	1.51	18.0	21	0.14	7	58
Horsens Fjord	13	45.7	2.22	22.5	22.5	0.00	7	64
Mariager Fjord	25	45.7	0.67	17.0	28	0.39	1	37
Kalundborg Fjord	17	56.5	9.60	17.5	21	0.17	11	11
Odense Fjord	31	60.8	0.46	17.5	22.2	0.21	6	29
Vejle Fjord	44	73.5	6.65	20.0	22.5	0.11	12	31
Kalø Vig	16	77.9	4.10	27.0	27	0.00	2	24
Ebeltoft Vig	4	84.3	11.15	27.6	27.6	0.00	1	3
Roskilde Fjord	37	124.2	0.86	15.0	22.5	0.33	10	42
Aarhus Bugt	46	233.0	17.14	27.0	27	0.00	14	183
Isefjord	14	315.6	4.78	18.5	22.5	0.18	16	50
Sejerø Bugt	39	674.2	39.39	23.2	23.2	0.00	22	22
Aalborg Bugt, Hevring Bugt	11	1683.5	80.08	27.87	27.87	0.00	27	104
Holckenhavn Fjord	12	0.5	0.22	9.5	18	0.47	3	10

4.2.1 Numerical methods

Alpha diversity is simply the number of species found in a sample covering 0.1 m^2 bottom area.

Beta diversity, i.e. the change of diversity from samples between pairs of estuaries, and between estuaries and sea pools, the pairwise beta, was measured by the Bray-Curtis similarity index (BC index) on presence-absence. The BC similarity index was calculated as:

$$\text{BC similarity} = 100 \times (1 - ((\Sigma \,|\, xij - xik \,|\,) \,/\, (\Sigma\, (xij + xik))) \qquad (1)$$

where j and k are samples (sites) and the summation (Σ) is overall species in both samples. For the case of presence – absence, x was either 1 or 0. Bray-Curtis dissimilarity (the complement of BC similarity) calculated on presence-absence is equivalent to the more commonly used Sørensen dissimilarity index, often considered a suitable measure of species turnover (*Vellend 2001*). However, as argued by *Baselga (2010)*, the Sørensen index contains two additive parts that can be related to different processes: one spatial turnover part where species are substituted by the same number of other species, and one nestedness part influenced by differences in alpha diversity (number of species per 0.1 m^2) possibly relating to loss or gain processes. Here we used a statistical approach to single out effects of true turnover *sensu Baselga (2010)*. Since nested beta is influenced by differences in alpha, alpha difference is included as factor first in the sequence of factors in the DistML models, thus explaining the part of the variation which includes variation due to nested beta. Remaining variation should then include variation due to turnover beta, because when alpha in two samples are equal, then the Sørensen index give true turnover.

Permutational regression

Since similarity index values from similarity matrices are non-independent, we used permutational regression using the distance-based linear model option (DistLM) in PERMANOVA+ for PRIMER (*Anderson et al. 2008*) to assess significance of regressions between similarity (the complement of dissimilarity or beta) and the geographical distance and environmental differences between pairs of estuaries, as well as between alpha and environmental factors. With more than one predictor variable both marginal and sequential estimation were used to evaluate independence of variances. Marginal estimation gives the proportions explained (R2) when predictor variables are fitted alone while sequential estimation (forward selection) gives the contributions to total variation explained after the previous predictor variable(s) has been fitted. All P-values were determined from 999 permutations.

4.3 Results

The total number of species was 541 and of these an equal number (116) occurred only in estuaries or only in the open sea (*Table 4.2*). Thus 57 % of the species were found both in estuarine and open sea environments. The two extremes of dispersivity: planktotrophic and direct developing species had overall similar species numbers, but in the former group far more species occurred both in estuarine and open sea environments (74 %) than in the direct species group (45 %). The corresponding figure for remaining species, i.e. the group with likely intermediate dispersivity, was 54 %. Already these observations indicate that differential dispersal is effective in the system.

Table 4.2. Species number overview in estuarine and open sea areas of all species and of species in groups with different dispersivity.

	Total	In estuaries only	In open sea pools only	Percentage in common estuary-sea
All species	541	116	116	57.1
Planktotrophic	139	17	19	74.1
Direct	128	34	37	44.5
Remaining	274	65	60	54.4
No. samples	961	688	273	

4.3.1 Modelling species richness (alpha) and abundance within and among estuaries

Sequential test (forward selection) of the DistML model '*Alpha = Estuary salinity + site water depth (m) + estuary mouth width Log (km) + distance to estuary mouth (km) + year*' explained 11 to near 40 % of the variation in alpha for the three dispersive groups with the highest degree of explanation for planktotrophic species (40 %) and the lowest for direct species (11 %, *Table 4.3*). Over 25 % of the variation in total alpha was explained by the model. Given the high importance of salinity in the transition area, the variation accounted for by this predictor variable was always put first in the sequence of predictors in the model.

Comparison of the marginal and sequential results indicate co-variation between the three variables salinity, site depth and mouth width, i.e. high saline estuaries have greater depths and wider mouths than low saline estuaries. These three variables co-varied also with the size of the estuaries (area, not used in the analysis). This is particularly clear for alpha of planktotrophic species (and the total alpha as well) where marginal contributions are at the same high level for all three variables and contribution of the first (salinity) totally dominates in the sequential test results. In fact the variation explained by mouth width could almost equally well be explained by salinity and explain an insignificant contribution to the total variation ($p > 0.05$, *Table 4.3*). From *Figure 4.2* it is clear that alpha, mainly due to planktotrophic species, increases with increasing salinity ($p < 0.01$) (or increasing depth or estuary mouth width) and to a smaller degree but also significant ($p < 0.01$) by the remaining species, many with intermediate dispersivity. The trend for direct species is the opposite – a small but significant decline ($p < 0.01$).

Figure 4.2. Change in alpha with increasing estuary mouth width. Cumulative contributions from each of the three species groups with different dispersivity. Open sea bars refer to three subdivisions of the open sea pool area.

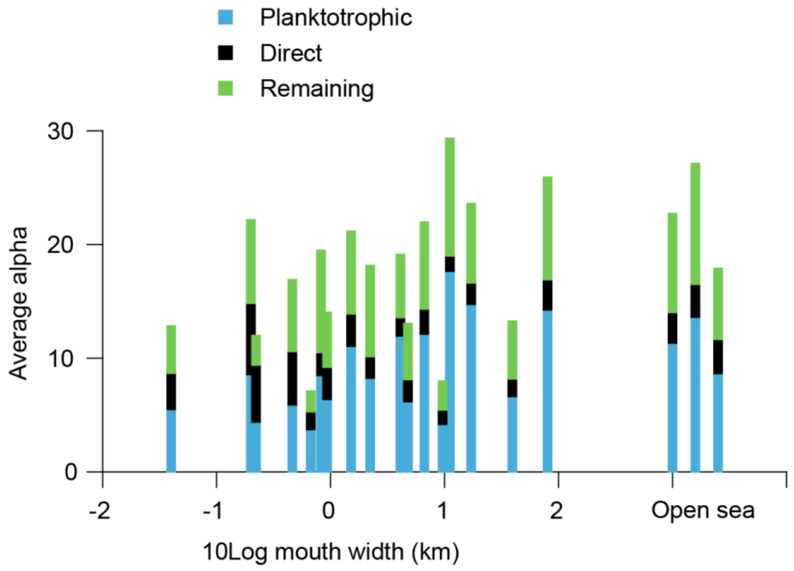


Table 4.3. Results of DistML modelling of alpha (number of species per 0.1 m^2 in the 17 estuaries. R2marginal gives the percentage explained variation if predictor variables were fitted alone, R2sequential (forward selection) gives the percentage explained variation in addition to the variation explained by previous predictor(s). Distance to mouth is the shortest perpendicular distance (in km) from the sampling site to the estuary mouth width distance line.

Model: Alpha = estuary salinity + site water depth (m) + estuary mouth width 10Log (km) + distance to estuary mouth (km) + year.

Predictor	**R2marginal**	**p**	**R2sequential**	**p**	**Df**
Planktotrophic					
Estuary salinity	29.3	0.001	29.3	0.001	686
Site water depth	33.5	0.001	4.2	0.001	685
10Log mouth width	23.1	0.001	0.2	0.208	684
Distance to mouth	6	0.001	3.6	0.001	683
Year	3	0.001	2.0	0.001	682
Total variation explained			39.3		
Direct					
Estuary salinity	6.7	0.001	6.7	0.001	686
Site water depth	8	0.001	2.2	0.001	685
10Log mouth width	5.3	0.001	0.05	0.53	684
Distance to mouth	0.3	0.15	0.8	0.013	683
Year	1.5	0.002	1.4	0.002	682
Total variation explained			11.2		
Remaining					
Estuary salinity	7.5	0.001	7.5	0.001	686
Site water depth	2.1	0.001	0.2	0.186	685
10Log mouth width	6.7	0.001	0.2	0.238	684
Distance to mouth	8.4	0.001	8.1	0.001	683
Year	2.6	0.001	1.6	0.001	682
Total variation explained			17.6		
Total alpha					
Estuary salinity	16.2	0.001	16.2	0.001	686
Site water depth	10.5	0.001	0.6	0.02	685
10Log mouth width	13.2	0.001	0.2	0.258	684
Distance to mouth	8.1	0.001	6.5	0.001	683
Year	3.9	0.001	2.7	0.001	682
Total variation explained			26.1		

In addition to these three overlapping variables, there are two small, but significant ($p < 0.01$), contributions from variables with fairly small overlaps with the variation accounted for by the previous variables. The first one is distance to estuary mouth evident for planktotrophic and remaining species (and total alpha, all $p < 0.01$) but less significant ($p < 0.05$) for direct species. All species categories show a decrease with increasing distance from the estuary mouth, the steepest change for planktotrophs, followed by remaining species and with slight change for direct species (*Figure 4.3*). Interannual variability expressed by the variable year gives significant ($p < 0.01$) contributions 1-3 % to total variation for all species categories.

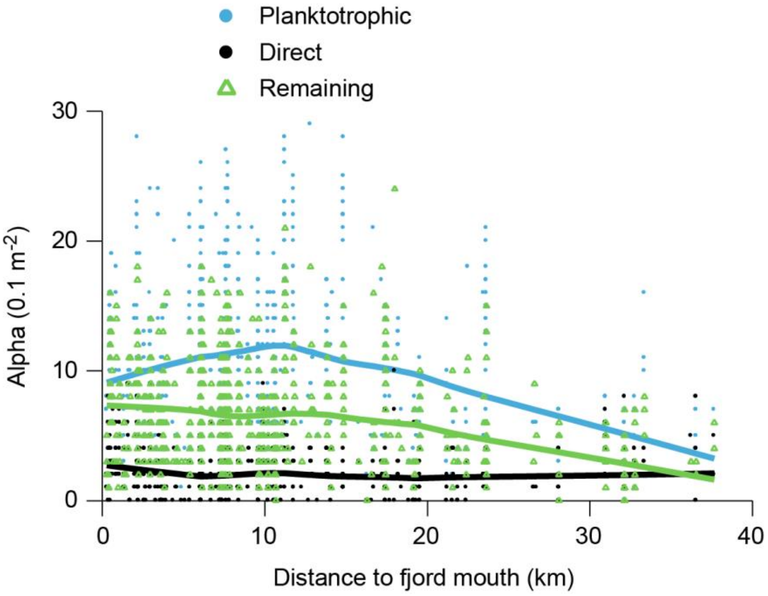


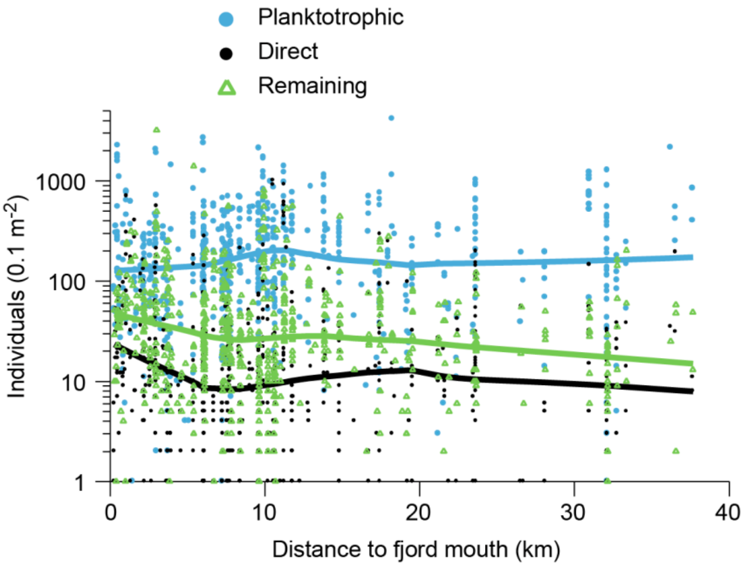
Figure 4.3. Change in alpha with increasing distance from the estuary mouth. Regression lines for each of the species groups with different dispersivity from LOWESS smoothing.

Sequential test of the DistML model 'Number of individuals = Estuary salinity + site water depth (m) + estuary mouth width Log (km) + distance to estuary mouth (km) + year' explained only 3.5 % and 4.7 % of the variation in abundance in the samples of planktotrophic species and total fauna, respectively (*Table 4.4*). While abundance was more or less constant within the estuaries ($p > 0.05$, *Figure 4.4*), there was a decline with increasing salinity among estuaries ($p < 0.01$) and an effect of year ($p < 0.01$), i.e. a small decrease over the 1990s and 2000s.

Table 4.4. Results of DistML modelling of number of individuals in the samples from the 17 estuaries. For further explanation, see *Table 4.3*.

Predictor	**R2marginal**	**p**	**R2sequential**	**p**	**df**
Planktotrophic					
Estuary salinity	1.2	0.002	1.2	0.002	686
Site water depth	0.8	0.018	0.05	0.565	685
10Log mouth width	0.8	0.021	0.01	0.758	684
Distance to mouth	0.2	0.29	0.06	0.517	683
Year	1.7	0.003	2.1	0.001	682
Total variation explained			3.5		
Total number of individuals					
Estuary salinity	2.3	0.001	2.3	0.001	686
Site water depth	2.6	0.001	0.7	0.025	685
10Log mouth width	1.8	0.001	0.03	0.666	684
Distance to mouth	0.02	0.691	0.1	0.366	683
Year	1.6	0.002	1.6	0.002	682
Total variation explained			4.7		

Figure 4.4. Change in number of individuals with increasing distance from the estuary mouth. Regression lines for each of the species groups with different dispersivity from LOWESS smoothing.



4.3.2 Modelling pair-wise beta among estuaries

In order to assess connectivity among the estuary pair-wise, beta among estuary was modelled by the following model:

Similarity estuary-estuary = Estuary salinity difference + Log estuary mouth width difference + inter-estuary log distance (km)

This model explained 27-38 % of the variation in similarity (inverse of pairwise beta) among estuaries, highest for planktotrophic and lowest for direct species (*Table 4.5*). Estuary salinity difference and mouth width difference together explained most of the variation with overlap varying with species group. Similarity decreased (beta increased) with increasing difference of these two variables (*Figure 4.5*). In addition a small part of the variation was explained by distance among estuaries, only significant ($p < 0.01$) for planktotrophic species, where similarity decayed with increasing distance between estuaries.

Table 4.5. Results of DistML modelling of similarity among estuaries (inverse of pair-wise beta). Salinity and mouth width differences are absolute differences and the latter was calculated as the absolute difference of the log-transformed estuary mouth widths (abs ($\log_{10}$(width estuary1) - $\log_{10}$(width estuary2))). Inter-estuary distance is the shortest water way distance (km) between the centres of the mouth width distance lines. Results from 999 permutations are given for the groups of species with different dispersivity.

Model: Similarity estuary-estuary = Estuary salinity difference + Log estuary mouth width difference + inter-estuary log distance (km).

Predictor	R2marginal	p	R2sequential	p	df
Planktotrophic					
Estuary salinity difference	25.0	0.001	25.0	0.001	134
10Log mouth width difference	22.8	0.001	9.3	0.001	133
Inter-estuary10Log distance (km)	4.5	0.014	3.5	0.008	132
Total variation explained			37.9		
Direct					
Estuary salinity difference	20.7	0.001	20.7	0.001	134
10Log mouth width difference	14.0	0.001	4.5	0.008	133
Inter-estuary 10Log distance (km)	2.6	0.068	2.0	0.06	132
Total variation explained			27.2		
Remaining					
Estuary salinity difference	14.1	0.001	14.1	0.001	134
10Log mouth width difference	27.4	0.001	16.7	0.001	133
Inter-estuary 10Log distance (km)	1.6	0.166	1.0	0.169	132
Total variation explained			31.2		

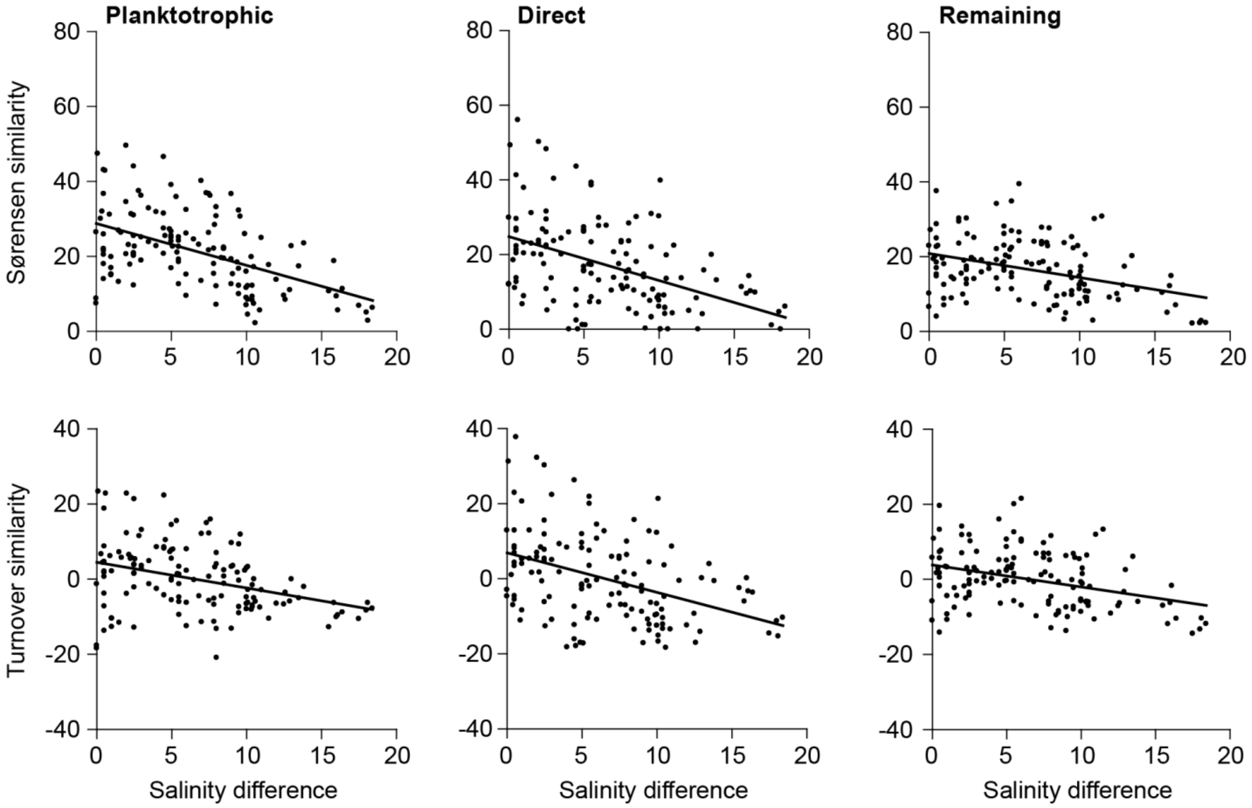


Figure 4.5. Change in similarity (Sørensen similarity, upper panels) between estuaries with increasing difference in salinity regimes. Regression lines are from linear regression and lower panels show the residual variation after removal of variation due to differences in alpha ('Turnover similarity') for each of the species groups with different dispersivity. All changes are significant ($p < 0.01$) with permutational regression (DistML).

In the following model, we account for the variation in similarity due to absolute difference in alpha between samples (averages) and the remaining variation should then contain variation due to turnover beta:

Similarity estuary-estuary = Alpha difference + Estuary salinity difference + Log estuary mouth width difference + inter-estuary log distance (km)

The model with alpha difference as a predictor variable explains more of total variation in similarity for planktotrophic species group but not so much for the other groups (*Table 4.6*). Apparently alpha difference for planktotrophs accounts for a substantial part of the variation due to salinity difference in the previous model without alpha difference. The remaining variation after accounting for alpha difference, which likely includes variation due to true turnover, beta is almost entirely explained by the partly overlapping salinity difference and estuary mouth width difference. Turnover beta increases with increasing salinity difference and mouth width difference among the estuaries (*Figure 4.5*). As in the previous model, a small part of the variation was explained by distance among estuaries, only significant ($p < 0.05$) for planktotrophic species (*Table 4.6*).

Table 4.6. Results of DistML modelling of similarity among estuaries as in *Table 4.4* but with absolute alpha difference added as predictor first in the sequence of predictors in order to separate nested beta effects from turnover beta effects.

Model: Similarity estuary-estuary = Alpha difference + Estuary salinity difference + Log estuary mouth width difference + inter-estuary 10log distance (km).

Predictor	R2marginal	p	R2sequential	p	df
Planktotrophic					
Alpha difference	23.3	0.001	23.3	0.001	134
Estuary salinity difference	25.0	0.001	11.2	0.001	133
10Log mouth width difference	22.8	0.001	9.4	0.001	132
Inter-estuary 10Log distance (km)	4.5	0.014	2.6	0.012	131
Total variation explained			46.4		
Direct					
Alpha difference	1.3	0.204	1.3	0.17	134
Estuary salinity difference	20.7	0.001	20.1	0.001	133
10Log mouth width difference	14.0	0.001	5.1	0.002	132
Inter-estuary 10Log distance (km)	2.6	0.064	1.6	0.095	131
Total variation explained			28.1		
Remaining					
Alpha difference	3.1	0.044	3.1	0.045	134
Estuary salinity difference	14.1	0.001	12.1	0.001	133
10Log mouth width difference	27.4	0.001	17.8	0.001	132
Inter-estuary 10Log distance (km)	1.6	0.127	0.5	0.335	131
Total variation explained			33.5		

4.3.3 Modelling pair-wise beta between estuaries and open sea

In order to assess connection between estuaries and open sea pools of species, beta (similarity) was modelled by:

Similarity estuary-open sea = estuary salinity + 10Log mouth width (km)

A major part of the variation in similarity for planktotrophic and remaining species (60-70 %) was explained by estuary salinity and mouth width, and as seen in previous tests, there was a substantial overlap between them. Similarity increased (beta decreased) with increase in both variables. In contrast, the model did not explain a significant part of the variation in similarity for the direct species group ($p > 0.05$) (*Table 4.7*).

Table 4.7. Results of DistML modelling of similarity between estuary samples and open sea samples. For further information, see *Table 4.2*.

Model: Similarity estuary-open sea = estuary salinity + 10Log mouth width (km).

Predictor	R2marginal	p	R2sequential	p	df
Planktotrophic					
Estuary salinity	65.1	0.002	65.2	0.001	15
10Log mouth width	54	0.002	4.3	0.182	14
Total variation explained			69.5		
Direct					
Estuary salinity	17.9	0.104	17.9	0.073	15
10Log mouth width	17.9	0.096	2.7	0.485	14
Total variation explained			20.6		
Remaining					
Estuary salinity	55.5	0.001	55.5	0.002	15
10Log mouth width	45.7	0.003	3.5	0.28	14
Total variation explained			59		

When adding the absolute alpha difference in the following model:

Similarity estuary-open sea = alpha difference + estuary salinity + 10Log mouth width (km)

the variation remaining after accounting for alpha difference was significantly explained by salinity and estuary mouth difference for planktotrophs and remaining ($p < 0.01$) but not so for direct species ($p > 0.05$) (*Table 4.8*). Turnover beta, thus, decreased (similarity increased) significantly with increase in the two predictor variables (*Figure 4.6*).

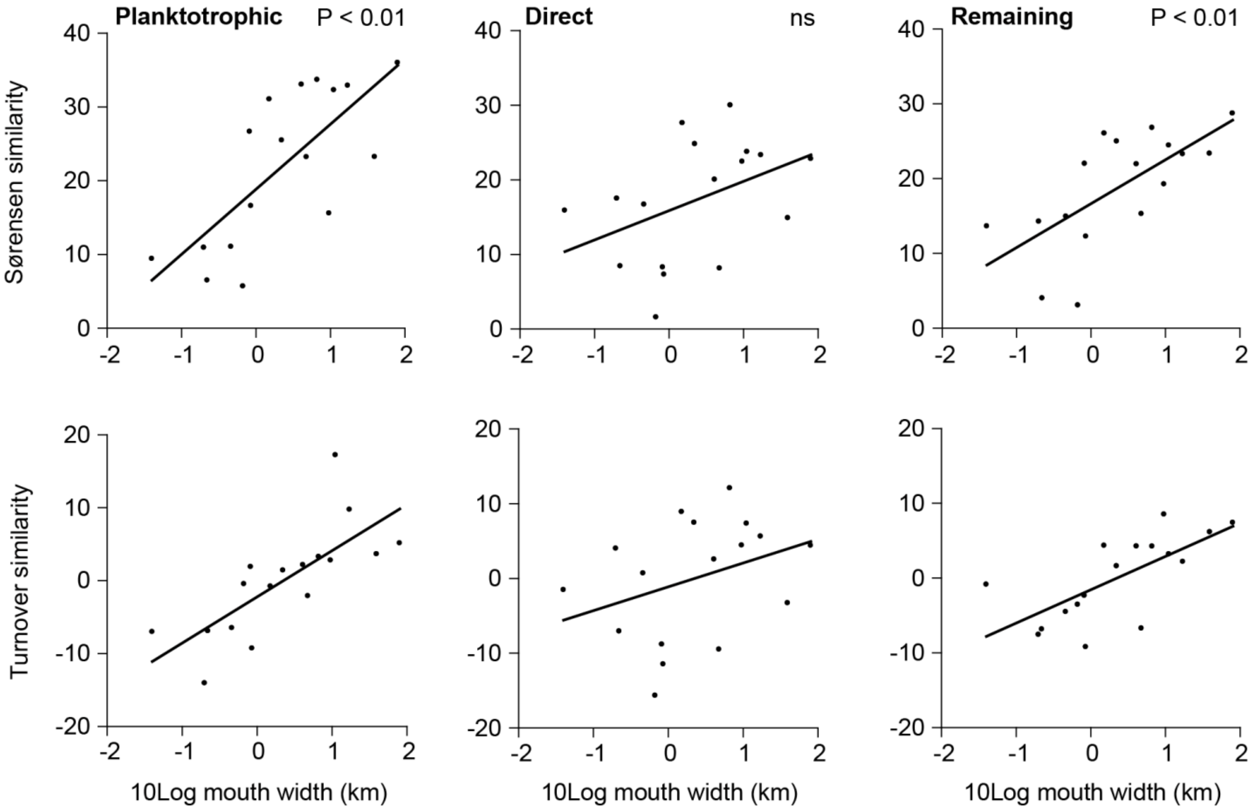


Figure 4.6. Change in similarity (Sørensen similarity, upper panels) between estuaries and open sea pools with increase in estuary mouth width. Regression lines are from linear regression and lower panels show the residual variation after removal of variation due to differences in alpha ('Turnover similarity') for each of the species groups with different dispersivity. P-values refer to permutational regression (DistML).

Table 4.8. Results of DistML modelling of similarity between estuary samples and open sea samples, As in *Table 4.6*, but with absolute alpha difference added as predictor first in the sequence of predictors in order to separate nested beta effects from turnover beta effects.

Model: Similarity estuary-open sea = alpha difference + estuary salinity + 10Log mouth width (km).

Predictor	**R2marginal**	**p**	**R2sequential**	**p**	**df**
Planktotrophic					
Alpha difference	50	0,003	50	0,003	15
Estuary salinity	65,1	0,001	38,3	0,001	14
10Log mouth width	54	0,002	2,6	0,088	13
Total variation explained			90,9		
Direct					
Alpha difference	2,6	0,57	2,6	0,553	15
Estuary salinity	17,9	0,091	15,4	0,118	14
10Log mouth width	17,9	0,102	3,2	0,488	13
Total variation explained			21		
Remaining					
Alpha difference	50	0,006	50	0,005	15
Estuary salinity	55,5	0,001	28,9	0,002	14
10Log mouth width	45,7	0,005	4,3	0,146	13
Total variation explained			78,1		

4.4 Conclusions

Because of co-variation between variables affecting dispersal (estuary mouth width affecting salt water flushing) and variables affecting adaptation or environmental sorting (like salinity), it is not possible to separate effects of these two types of variables based on environmental variables alone. However, by investigating responses of alpha for species groups with different dispersivity it was possible to disentangle effects of dispersal from other effects.

The following findings support the idea of external influence on alpha by dispersive species in the estuaries and that the influence is positively related to the degree of openness of the estuaries:

- Substantial increase in alpha of dispersive species with increasing estuary salinity and estuary mouth width - small increase for less dispersive species or decrease for less dispersive (direct) species.
- Decrease of alpha within estuaries of dispersive species with increasing distance from the mouth - little or no change of alpha of non-dispersive species.
- As much as 73 % of dispersive (planktotrophic) species in common between estuaries and open sea - only 43 % for non-dispersive (direct development) species.
- Turnover beta of dispersive species between estuary and open sea decrease (similarity increase) with increasing mouth width - no significant trend for non-dispersive species.
- Turnover beta among estuaries increase with increasing difference among estuaries with respect to salinity and mouth width for all species categories but with the steepest change for direct species.
- High variation due to nested beta for planktotrophic species also positively related to estuary differences of salinity and mouth width. Nested beta for dispersive species was likely higher than for non-dispersive species.

It is obvious that total alpha in the estuaries and beta between the estuaries are strongly related to differences in the estuarine environments such as salinity regimes. However, at the same time the observations that the alpha of dispersive species increases with increasing salinity, as well as the fact that beta between estuaries and open sea of this category decreases with increasing estuary salinity, suggest that dispersal from the open sea pool also is important. Further support for dispersal is the decrease of alpha of dispersive species in the estuaries in accordance with a source-sink system. Thus, both dispersal and environmental filtering were important in regulating alpha diversity in the estuaries, where salinity filtered primarily dispersive species. Because more species in the open sea areas occur in high salinity conditions than at low salinity, and because only dispersive species can enter the estuaries in a short-term perspective, increasing salinity in the estuaries results in increasing diversity mainly due to addition of dispersive species. So, although dispersal limitation may determine the composition of alpha, the level of alpha is determined by salinity.

4.5 Applicability of DKIver2 in a wider context

Results from the exercise above thus indicate that both the environmental filtering due to salinity changes and dispersal from the outside open sea species pool affect local species richness in the estuaries 'dispersal is subject to environmental filtering'. So, obviously at least the diversity part of DKI should be corrected for salinity. Also, possibly due to differential dispersal within estuaries, the local richness is not constant within estuaries, but decreases with distance from the mouth. However, this variation is small relative to variation due to salinity differences among estuaries and the effect will vary with the size of the estuary and is most important in the largest (longest) estuaries. Therefore, it is suggested that the most operational method although with some uncertainty is to correct only for salinity.

In the report *Josefson (2008,)* Shannon's H was regressed against salinity and mean H increased significantly in open sea areas and in half open areas (residence time < 20 days), while the relation was not significant in the closed areas (residence time ≥ 20 days). However, these were the trends for averages/mean H. Looking at Figure 2 in *Josefson (2008)* there appears to be an increasing trend for H_{max} in all areas.

The distributions of AMBI data over the salinity gradient from the 17 estuaries and all data (estuaries and open areas) from the *Josefson (2008)* report with regression lines from the DKIver2 description superimposed are shown in *Figure 4.7*. The agreement between the data sets of AMBI minimum values and the regression lines seems reasonable over a great part of the salinity interval. Furthermore, the distribution of AMBI groups with changed salinity in the investigated 17 estuaries is in fair agreement with distributions in the open sea data set used when constructing DKIver2 (*Figure 4.8*).

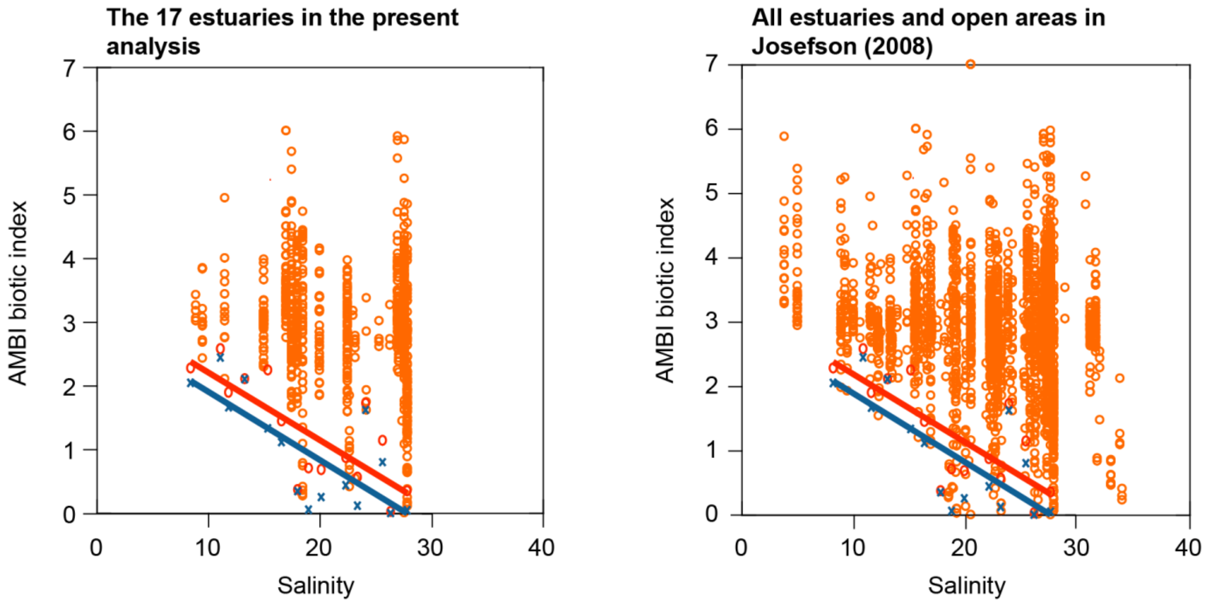


Figure 4.7. AMBI biotic index values versus salinity from the 17 estuaries in the present analysis (left panel) and from all areas in the *Josefson (2008)* report (right panel) with regression lines from the DKIver2 description superimposed. Red line = 5th percentile, blue line = 1st percentile.

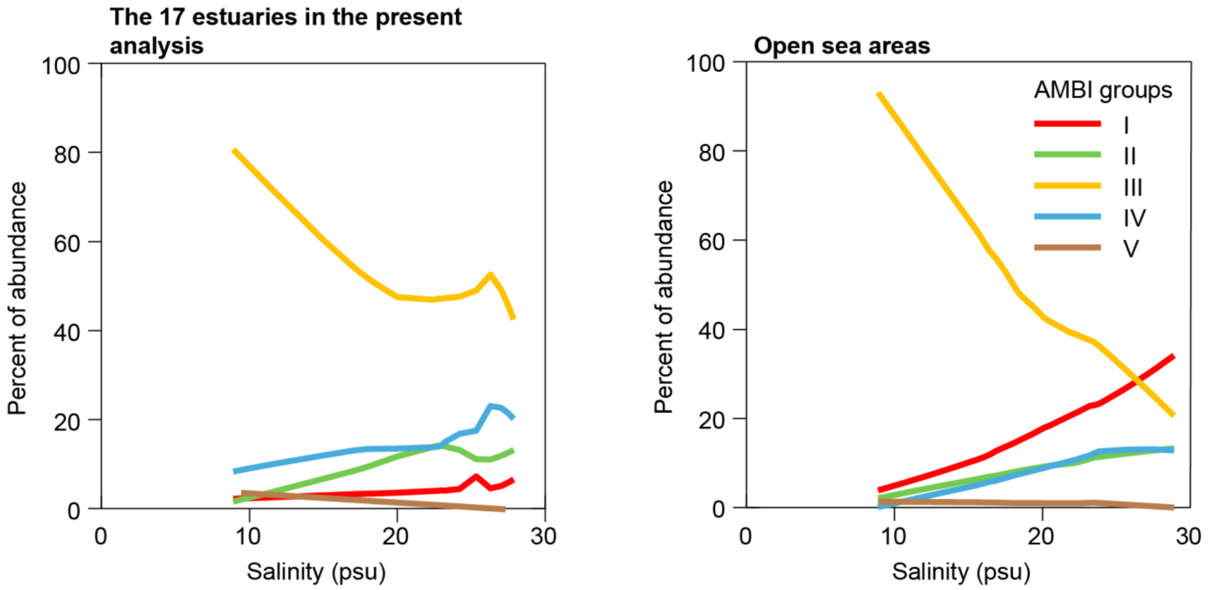


Figure 4.8. Changes in percentages of AMBI sensitivity groups versus salinity from the 17 estuaries in the present analysis (left panel) and from the open sea samples used in the description of DKIver2 (right panel). Regression lines are from LOWESS smoothing (tension = 0.6) and value dots are omitted for clarity. Note the dramatic increase of group III, the 'indifferent species', with decreased salinity in both environments.

The distributions of Shannon's H over the salinity gradient based on the 17 estuaries and the data sets from all areas in the *Josefson (2008)* report are in fair agreement, which is the case also between H_{max} values and the regression lines from the development of DKIver2 in the salinity interval 6-28 psu (*Figure 4.9*). However, maximum values of H over 30 psu seem to fall somewhat lower than would be predicted from the regression. The reason for this is not known but can be due to several reasons in addition to insufficient sampling to capture max values. Data from salinities over 30 come from the Limfjord and from the exposed Skagerrak coast. The Limfjord is a highly anthropogenic, modified water body with restricted connections to adjacent sea areas (long residence time) which may explain a lower diversity. The Skagerrak samples come from highly exposed sandy bottoms which are often species poor due to ineffective sampling. A final possibility is of course that the relationship between H_{max} and salinity is not linear over the whole salinity range but levels off somewhere in the high end of the gradient.

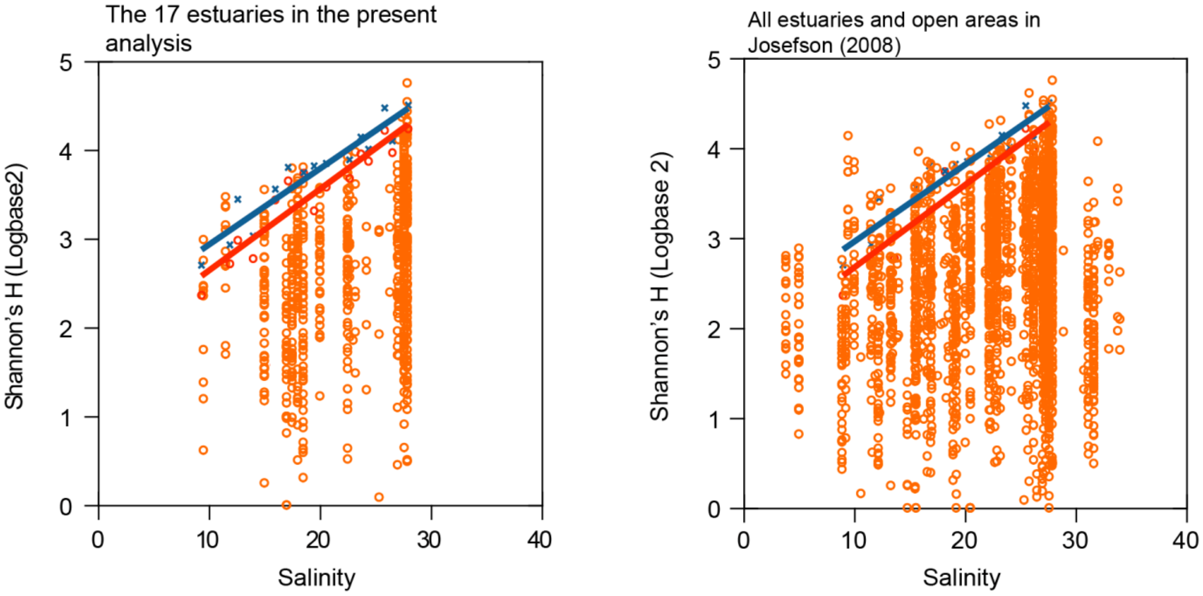


Figure 4.9. Shannon's H values versus salinity from the 17 estuaries in the present analysis (left panel) and from all areas in the *Josefson (2008)* report (right panel) with regression lines from the DKIver2 description superimposed. Red line = 95th percentile, blue line = 99th percentile.

Despite these possible shortcomings (restrictions on generality), it is suggested that DKIver2, as described in *section 4.4*, with two minor amendments, is used generally in Danish waters including coastal areas and estuaries as well as shallow areas off the North Sea coast. The alternative to use the original DKI with a fixed H_{max} is not an option in the environmentally variable transition area along the Danish coasts.

4.6 Re-description of DKIver2

The Danish 'DKI' index of benthic quality was developed for use in poly- to euhaline benthic environments characterised by a relatively high species diversity (*Borja et al. 2007*) and has been used with success in such environments in the Northeast Atlantic area (*Borja et al. 2007, Josefson et al. 2009*). However, when applying DKI on data from low saline and species-poor estuarine areas like the Baltic Sea area, it soon became clear that the range of possible index values was markedly restricted to the lower end of the range in saline areas. This was most likely a result of salinity influence on three of the components in the index, the Shannon-wiener (H) and the number of species (S) components, but also, as we shall see, the AMBI component. It is well known that diversity of species with marine affinity decreases with decreased salinity at several spatial scales, when going from Skagerrak/Kattegat through the Belt Seas into the Baltic and further north and east (e.g. *Remane 1934, Bonsdorff & Pearson 1999, Josefson & Hansen 2004, Villnäs & Norkko 2011*).

The effect of salinity on sensitivity classification such as AMBI, however, has yet to be demonstrated.

In a comprehensive analysis of DKI in Danish waters including species data from 2600 samples of Van Veen size (0.1 m^2) from 540 sampling points (sites), clear salinity effects were demonstrated on Shannon's H (H), species richness (S) and AMBI in open sea areas but not in closed estuaries and lagoons (*Josefson 2008*). Maximum values of H and S decreased, and minimum values of AMBI increased, with decreasing salinity in the salinity range 8-28 psu. In order to resolve the above-mentioned problems with DKI in low saline areas, components in DKI are corrected for salinity as follows:

1) <u>The S factor (1-1/S) which becomes effective at species numbers < 10 has been omitted.</u> This because species numbers per 0.1 m^2 in the Baltic are often below this value also in undisturbed areas, and furthermore S is already included in H.

2) <u>Hmax in the Shannon-wiener factor is determined from a regression between Hmax and bottom water salinity</u> (*Table 4.9*).

The regression was obtained by regressing the 99th percentile of H values from 15, approximately similar sized classes, against salinity (psu) in the interval 8-28 psu (*Figure 4.10*, *Table 4.9*).

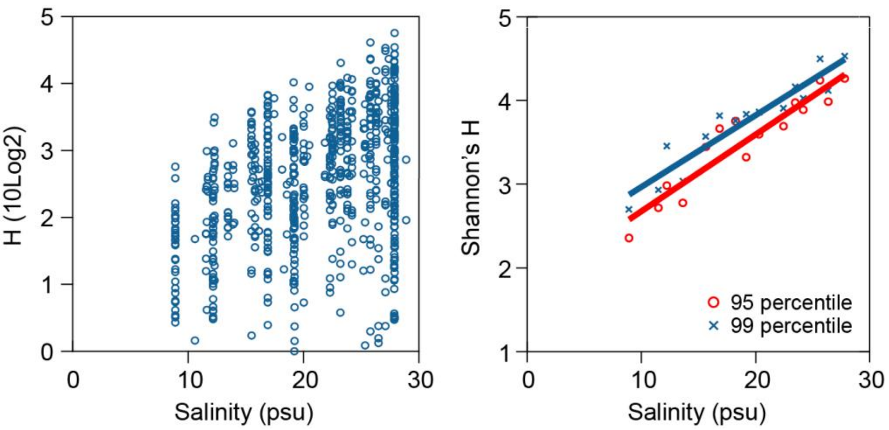


Figure 4.10. Plots of H against salinity (left panel) and regressions of Hmax assessed by 99 or 95th percentiles against salinity (right panel). Data from Van Veen-sized (0.1 m^2) samples from meso- and polyhaline Danish open sea areas (*Josefson 2008*).

Table 4.9. Regressions between Hmax 99 percentile (H99) and salinity, and between AMBImin 1 percentile (AMBI01) and salinity.

Relation	n	R2	P
H99 = 2.117 + 0.086 * Sal (psu) (eq. 1)	15	0.89	0.000
AMBI01 = 3.083 – 0.111 * Sal (psu) (eq. 2)	15	0.57	0.001

3) <u>The minimum value of AMBI is determined from a regression between AMBImin and salinity and subtracted from AMBI in the original formula.</u>

The minimum AMBI ($AMBI_{min}$) decreases with increasing salinity as shown in *Figure 4.11* and the reason behind this is most likely changes in the proportions of different sensitivity groups as shown in the lower panel in *Figure 4.11*. At low salinities AMBI is to a great extent determined by group III (which includes *Macoma balthica*), whereas at high salinities several groups contribute to the index and group I, the 'sensitive species', has the highest proportion of the individuals. $AMBI_{min}$ was assessed by the 1st percentile and regressed against salinity using the same salinity intervals as for H above (*Figure 4.11*, *Table 4.9*).

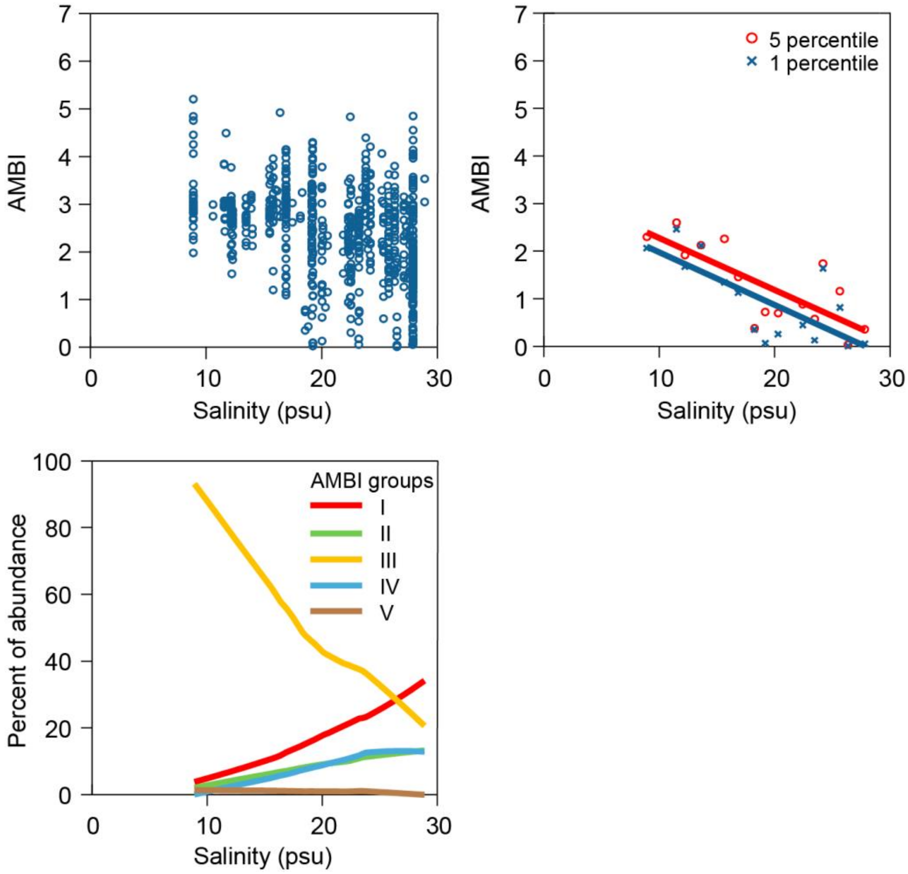


Figure 4.11. Plots of AMBI against salinity (upper left panel) and regressions of AMBImin assessed by 1st or 5th percentiles against salinity (upper right panel). Lower panel shows changes with salinity in proportions of the five AMBI groups of sensitivity (LOWESS lines, data points omitted for clarity). Data from Van Veen-sized (0.1 m^2) samples from meso- and polyhaline Danish open sea areas (*Josefson 2008*).

The resulting formula for DKIver2 now reads:

$$\mathbf{DKI = ((1- ((AMBI-AMBI_{min})/7))+ (H/H_{max}))/2 * (1-(1/N))}$$

where

H_{max} = f (salinity), *(Table 4.9 eq. 1)*

$AMBI_{min}$ = f (salinity), *(Table 4.9 eq. 2)*

N = Number of individuals (as before**)**

With the two amendments: 1) H/H_{max} must never be > 1, if so it should be set to 1. 2) $AMBI_{min}$ must never be negative, if so it should be set to 0, DKI values can now vary between 0 and 1 and may be regarded as EQR values where the 'reference' is the best value we can get at a given salinity.

The DKI is applied on 0.1 m² samples and therefore smaller samples like Haps samples have to be pooled. The index is to be used in areas with a salinity gradient from 5 psu to fully marine and applied on benthic species with marine affinity, which excludes species with freshwater and terrestrial affinity. The salinity values should come from the bottom water where the fauna lives and should be averages of as many reliable measurements as possible.

Boundary setting

Usually, the border between good and moderate EcoQS (GM) is determined as some deviation from a reference situation. Reference data, however, are difficult to find. The GM border for DKI was set by using the discontinuity in the relationship of anthropogenic pressure and the biological response as described in *Josefson et al. (2009)*. The threshold value, where faunal structure deterioration commences, was identified from non-linear regression between DKIver2 and the impact proxy: distance from point source in the Aarhus Bight pollution gradient. Using a bootstrap procedure as described in *Leonardsson et al. (2009)* and *Josefson et al. (2009)* the 5th percentile of the index values from the less impacted side of the threshold was determined. It was assumed that these values represented at least good EcoQS. The 5th percentile of these data was defined as the GM border and attained the value of 0.68. By dividing the ranges 0-0.68 and 0.68-1 with 3 and 2, respectively, the following boundaries were obtained:

Poor-Bad	Moderate-Poor	Good-Moderate	High-Good
0.23	0.45	0.68	0.84

The potential bias due to pooling several small samples (Haps) compared to one Van Veen sample

It is possible or even likely that by pooling several Haps samples some beta diversity is captured and therefore pooling will yield somewhat higher richness (and consequently also Shannon's H) than in one 0.1 m² Van Veen sample. To estimate how large the bias is, one has to use the two methods at the same spot (at the same time) and this has not yet been done. However, both the development of salinity relations for DKIver2 and boundary determination in the Aarhus Bight pollution gradient were mainly based on pooled Haps samples. This probably makes evaluation of data based on Van Veen samples conservative for the benefit of the environment, i.e. if a Van Veen based value falls above the GM border, it is certain that status is at least *Good*.

Water body assessment

Following the 'fail-safe' approach by *Carstensen (2007)* and methods suggested by *Leonardsson et al. (2009)* for Swedish water body assessment, it is suggested to use the 20th percentile of DKI values (that is the lower border of a 80 % confidence interval) when evaluating status of a water body. For instance, when the 20th percentile equals or exceed the border between Good and Moderate status but is below the Good-High border, the status is *Good*, and then the EcoQS of the water body is acceptable.

The method of computing the 20th percentile of index values is described in detail in Box 2 in *Leonardsson et al. (2009)* where the percentile is obtained by bootstrapping mean values of replicates from stations. As argued by *Leonardsson et al. (2009)*, this method is precautionary because the status of a water body is better described by many stations than few stations albeit with many replicates per station, and when the number of stations in a water body is low, the 80 % confidence interval will be wide and consequently the 20th percentile low.

In the Danish monitoring, multiple small Haps samples are taken at point stations and within sampling grids of various areal extensions. First, the Haps samples have to be pooled to correspond to a 0.1 m^2 Van Veen sample. For the case of point stations: if pooled samples or Van Veen samples at a station are more than one, take the mean value of DKI and add the series of station samples. For the case of Haps grids: Because grids normally cover a much wider area than point stations, each pooled sample may be regarded as a station sample, compute DKI for each of them and add them to the series of station sample means. Finally, find the 20th percentile of the station sample means by bootstrapping as described above.

From the above mentioned, and the possible positive relation between area and number of stations, follows that consideration of water body extent is crucial for the reliability and generality of the assessment results. So it is recommended that water bodies to be assessed should be as large as possible, taking the natural environmental variation into account.

5 Conclusions and recommendations

This report concerns three WFD biological quality elements and the conclusions from the three different analyses will be reported separately.

5.1 Phytoplankton

Phytoplankton biomass, estimated by the summer chlorophyll *a* concentration, is the only sub-element that has been intercalibrated (not completed though) in the Baltic GIG and NEA GIG. Changes in summer chlorophyll *a* are tightly coupled to levels of total nitrogen during winter and spring, and therefore it is a sensitive indicator of eutrophication. This has been demonstrated for the coastal areas that are to be intercalibrated with Sweden, and these relationships are even stronger in estuaries. High biomass concentrations lead to adverse effects on other ecosystem components, such as shading out eelgrass.

Denmark and Sweden have identified similar reference conditions for summer chlorophyll *a*, although slightly different seasonal aggregations are employed. However, boundaries between ecological status classes deviate between the two countries with Denmark suggesting more strict boundaries. The boundaries have been suggested based on two different approaches. It is **recommended** that relevant people from authorities in Denmark and Sweden together with scientists harmonise the boundaries between the two countries.

Phytoplankton composition is another biological sub-element, for which assessment tools should be developed. Changes in phytoplankton composition relative to pressures, such as nutrient concentrations, are less subtle than for phytoplankton biomass and the main factor governing the composition of marine phytoplankton is salinity. Nutrient enrichment can precipitate gradual changes from dinoflagellates and other species towards diatom dominance, but this change is not perceived as a degradation of ecological status. As a consequence, there are at present no indicators of phytoplankton community structure expressing decreasing quality of the phytoplankton community in response to nutrient enrichment. Therefore, it is **recommended** to postpone the implementation of this sub-element into an ecological assessment tool for phytoplankton, until there is strong scientific evidence for changes in communities within an operational scale of nutrient level, i.e. realistic to nutrient changes, which coastal ecosystems may experience.

5.2 Macroalgae

Six indicators for the biological quality element macroalgae (two covering macroalgae cover and four covering macroalgae communities) were tested across a broad range of coastal ecosystems in Denmark. The indicators account for differences in depth and available substrate when estimating indicator values from monitoring data. All indicators showed significant responses to salinity and total nitrogen. A subset of three indicators is proposed for assessing the ecological status for macroalgae. These indicators have been selected based on their lower uncertainty and better sensitivity to total nitrogen: 1) cumulative cover of macroalgae, 2) fraction of opportunists, and 3) number of late successional species. The two latter represent the community sub-element, whereas the first represent sub-element macroalgae cover. It is **recommended** that ecological status of macroalgae is based on these three indicators.

Boundary conditions for the macroalgae indicators are proposed based on boundaries for total nitrogen obtained from a previous study (*Carstensen et al. 2008*). A combined assessment of ecological status for macroalgae is suggested which uses the proposed boundaries for scaling the macroalgae indicators to an EQR-scale with equidistant classes. Since the indicators can be implicitly compared on the EQR-scale, a weighted average of the three suggested EQR values (each of the proposed macroalgae indicators translated into the EQR-scale) can be used to assess ecological status. This assessment provides a transparent approach to classification, from the indicator level to the overall assessment of the biological quality element. It is **recommended** that the general approach to status classification could be applied more generally to all biological quality elements, which are to be assessed using several indicators.

5.3 Benthic fauna

A re-analysis of data from 17 estuaries and adjacent open sea areas (*Josefson 2008)* showed that local richness (alpha) diversity in Danish estuaries was influenced both by dispersal from adjacent open coastal areas and by environmental differences among estuaries with respect to salinity.

Because environmental factors were mostly highly co-correlated, effects of dispersal based factors were discriminated by investigating diversity patterns of groups of species with different reproductive traits, i.e. different dispersivity.

Salinity filtered primarily dispersive species. And because more species in the open sea areas occur in high salinity conditions than at low salinity, and because only dispersive species can enter the estuaries in a short-term perspective, increasing salinity in the estuaries results in increasing diversity mainly due to addition of dispersive species.

So although dispersal limitation may determine the composition of alpha, the levels of alpha richness and Shannon' s H are determined mainly by salinity.

Because diversity is an important part of DKI, it is **recommended** to normalize DKI for salinity. This was done in the Baltic GIG intercalibration work where the DKIver2 was constructed. But, are these relations between salinity and H, AMBI applicable in general?

To answer this, the distributions versus salinity of the two major components in the DKI index, the AMBI biotic index and the Shannon H diversity from the investigated estuary material were compared with data from all areas in the *Josefson (2008)* report including the open sea data used when constructing the DKIver2. The agreement among the data sets was high and it was concluded that the original relationships between salinity and $\mathrm{AMBI}_{\mathrm{min}}$ and $\mathrm{H}_{\mathrm{max}}$ could be kept in the 'new' general version of DKIver2.

It is **recommended** to use the DKIver2, as re-described in this report with two minor amendments, in general in Danish waters including the shallow coastal areas and estuaries.

Since the DKIver2 now is normalized for salinity, it can be compared with only one set of borders over the whole salinity gradient. It is **recommended** to use the set with a GM border of 0.68, obtained from a well-known pollution gradient. One exception is, however, the type BC8 in the SW Baltic Sea where the GM border was set to 0.72 as a result of the EU-Baltic GIG intercalibration exercise.

Regarding water body assessment procedure, it is **recommended** to adopt the precautionary 'fail-safe' approach by *Carstensen (2007)* and methods suggested by *Leonardsson et al. (2009),* where the 20th percentile of station means of the index, or index values based on pooled Haps sets for grid data, is determined after bootstrapping.

6 References

Andersen, J., Markager, S.. & Ærtebjerg G. (red.) 2004: Tekniske anvisninger for marin overvågning. National Environmental Research Institute, Denmark. http://bios.au.dk/videnudveksling/til-myndigheder-og-saerligt-interesserede/fagdatacentre/fdcmarintny/ tekniskeanvisningerno-vana20042010/

Anderson, M.J., Gorley, R.N. & Clarke, K.R. 2008: PERMANOVA + for PRIMER: Guide to software and Statistical Methods. PRIMER-E: Plymouth, UK.

Baselga, A. 2010: Partitioning the turnover and nestedness components of beta diversity. - Global Ecology and Biogeography 19: 134-143.

Birk, S., Strackbein, J. & Hering, D. 2010: WISER methods database. Version: October 2010. Available at http://www.wiser.eu/programme/data-and-guidelines/method-database/.

Birk, S., Bonne, W., Borja, A., Brucet, S., Courrat, A., Poikane, S., Soliminie, A., van de Bund W., Zampoukas N. & Hering D. 2012: Three hundred ways to assess Europe's surface waters: An almost complete overview of biological methods to implement the Water Framework Directive. - Ecological Indicators 18: 31-41.

Bleich, S., Powilleit, M., Seifert, T. & Graf, G. 2011: β-diversity as a measure of species turnover along the salinity gradient in the Baltic Sea, and its consistency with the Venice System. - Marine Ecology Progress Series 436: 101-118.

Blomqvist, M., Krause-Jensen, D., Olsson, P., Qvarfordt, S. & Wikström, S. A. 2012: Potential eutrophication indicators based on Swedish coastal macrophytes. Deliverable 3.2-1, WATERS Report no. 2012:2. Havsmiljöinstitutet, Sweden. http://www.waters.gu.se/publikationer/rapporter/.

Bonsdorff, E. & Pearson, T.H. 1999: Variation in the sublittoral macrozoobenthos of the Baltic Sea along environmental gradients: A functional group approach. - Australian Journal of Ecology 24: 312-326.

Borja, A., Josefson, A.B., Miles, A., Muxika, I., Olsgaard, F., Phillips, G., Rodríguez, J.G. & Rygg, B. 2007: An approach to the intercalibration of benthic ecological status assessment in the North Atlantic ecoregion, according to the European Water Framework Directive. - Marine Pollution Bulletin 55: 42-52.

Borum, J. & Sand-Jensen, K. 1996: Is total primary production in shallow coastal marine waters stimulated by nitrogen load? - Oikos 76: 406-410.

Carstensen, J., Krause-Jensen, D., Dahl. K. & Middelboe, A.L. 2005: Development of macroalgal indicators of water quality. - In: Petersen, J.K., Hansen, O.S., Henriksen, P., Carstensen, J., Krause-Jensen, D., Dahl, K., Josefson, A.B., Hansen, J.L.S., Middelboe, A.L. & Andersen, J.H: Scientific and technical background for intercalibration of Danish coastal waters. National Environmental Research Institute, Denmark. - NERI Technical Report No. 563, Ch. 3, pp. 27-58. Available at http://www2.dmu.dk/1_viden/2_Publikationer/3_fagrapporter/rapporter/FR563.PDF

Carstensen, J. 2007: Statistical principles for ecological status classification of Water Framework Directive monitoring data. - Marine Pollution Bulletin 55: 3-15.

Carstensen, J. & Heiskanen, A.-S. 2007: Phytoplankton responses to nutrient status: application of a screening method to the northern Baltic Sea. - Marine Ecology Progress Series 336: 29-42.

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. Available at http://www2.dmu.dk/Pub/FR683.pdf

Carstensen, J. & Henriksen, P. 2009: Phytoplankton biomass response to nitrogen inputs: a method for WFD boundary setting applied to Danish coastal waters. - Hydrobiologia 633: 137-149.

Carstensen, J., Krause-Jensen, D., Markager, S., Timmermann, K. & Windolf, J. 2013: Water clarity and eelgrass responses to nitrogen reductions in the eutrophic Skive Fjord, Denmark. -Hydrobiologia 704: 293-309.

Carstensen, J., Cloern, J. & Paerl, H. *In prep*. Phytoplankton seasonal successions across the world's coastal ecosystems.

Cleveland, W.S. 1979: Robust locally weighted regression and smooting scatterplots. - Journal of the American Statistical Association 74: 829-836.

Cloern, J.E. 2001: Our evolving conceptual model of the coastal eutrophication problem. -Marine Ecology Progress Series 210: 223-253.

Duarte, C.M. 1995: Submerged aquatic vegetation in relation to different nutrient regimes. - Ophelia 41: 87-112.

Duarte, C.M., Conley, D.J., Carstensen J. & Sánchez-Camacho, M. 2009: Return to Neverland: Shifting baselines affect eutrophication restoration targets. - Estuaries and Coasts 32: 29-36.

Duarte, C., Borja, A., Carstensen, J., Elliot, M., Krause-Jensen, D. & Marba, N. 2014: Paradigms in the recovery of estuarine and coastal ecosystems. Estuaries and Coasts. DOI 10.1007/s12237-013-9750-9.

Hubbell, S. P. 2001: The unified neutral theory of biodiversity and biogeography. Princeton University Press.

Josefson, A.B. & Hansen, J.L.S. 2004: Species richness of benthic macrofauna in Danish estuaries and coastal areas. - Global Ecology and Biogeography 13: 273-288.

Josefson, A.B. 2008: DKI beregninger for danske lavvandede og lukkede områder. Report to BLST June 2008 (in Danish).

Josefson A.B. 2009: Additive partitioning of estuarine benthic macroinvertebrate diversity across multiple spatial scales. - Marine Ecology Progress Series 396: 283-292.

Josefson, A.B., Blomkvist, M., Hansen, J.L.S., Rosenberg, R. & Rygg, B. 2009: Assessment of marine benthic quality change in gradients of disturbance: comparison of multi-metric indices. - Marine Pollution Bulletin 58: 1263-1277.

Josefson, A.B. & Göke, C. 2013: Disentangling the effects of dispersal and salinity on beta diversity in estuarine benthic invertebrate assemblages. - Journal of Biogeography 40: 1000-1009.

Krause-Jensen, D., Laursen, J.S., Middelboe, A.L. & Stjernholm, M. 2001: NOVA – Teknisk anvisning for marin overvågning. Ch. 12. National Environmental Research Institute. Ministry of Environment and Energy, Denmark. (In Danish).

Krause-Jensen, D., Carstensen, J. & Dahl, K. 2007a: Total and opportunistic algal cover in relation to environmental variables. - Marine Pollution Bulletin 55: 114-125.

Krause-Jensen, D., Middelboe, A.L., Carstensen, J. & Dahl, K. 2007b: Spatial patterns of macroalgal abundance in relation to eutrophication. - Marine Biology 152: 25-36.

Krause-Jensen, D., Markager, S. & Dalsgaard, T. 2012: Benthic and pelagic primary production in different nutrient regimes. - Estuaries and Coasts 35: 527-545.

Leibold, M.A., Holyoak, M., Mouquet, N., Amasekare, P., Chase, J.M., Hoopes, M.F., Holt, R.D., Shurin, J.B., Law, R., Tilman, D., Loreau, M. & Gonzalez, A. 2004: The metacommunity concept: a framework for multi-scale community ecology. - Ecology Letters 7: 601-613.

Leonardsson, K., Blomqvist, M. & Rosenberg, R. 2009: Theoretical and practical aspects on benthic quality assessment according to the EU-Water Framework Directive – examples from Swedish waters. - Marine Pollution Bulletin 58: 1286-1296.

Marbá, N., Krause-Jensen, D., Alcoverro, T., Birk, S., Pedersen, A., Neto, J.M., Orfanidis, S., Garmendia, J.M., Muxika, I., Borja, A, Dencheva, K. & Duarte C.M. 2013: Diversity of European seagrass indicators - Patterns within and across regions. - Hydrobiologia 704: 265-278. DOI 10.1007/s10750-012-1403-7.

Markager, S.M., Carstensen, J., Krause-Jensen, D., Windolf, J. & Timmermann, K. 2010: Environmental effects of increasing nutrient inputs in Danish estuaries. National Environmental Research Institute, University of Aarhus. 54 pp. - NERI Technical Report No. 787. (*Report in Danish*: Effekter af øgede kvælstoftilførsler på miljøet i danske fjorde). Available at http://www2.dmu.dk/Pub/FR787.pdf

Naturvårdsverket 2007: Status, potential och kvalitetskrav för sjöar, vattendrag, kustvatten och vatten i övergångszon. En handbok om hur kvalitetskrav i ytvattenförekomster kan bestämmas och följas upp. 414 pp. Naturvårdsverket, Stockholm. Available at http://www.naturvardsverket.se/Documents/publikationer/620-0147-6.pdf

Nielsen, R., Kristiansen, A., Mathiesen, L. & Mathiesen, H. 1995: Distributional index of the benthic macroalgae of the Baltic Sea area. - Acta Botanica Fennica 155: 1-51.

Nielsen, S.L., Sand-Jensen, K., Borum, J. & Geertz-Hansen, O. 2002a: Phytoplankton, nutrients and transparency in Danish coastal waters. - Estuaries 25: 930-937.

Nielsen, S.L., Sand-Jensen, K., Borum, J. & Geertz-Hansen, O. 2002b: Depth colonisation of eelgrass (*Zostera marina*) and macroalgae as determined by water transparency in Danish coastal waters. - Estuaries 25: 1025-1032.

Petersen, J.K., Hansen, J.W., Laursen, M.B., Clausen, P., Carstensen, J., & Conley, D.J. 2008: Regime shift in a coastal marine ecosystem. - Ecological Applications 18: 497-510.

Rasmussen, B. & Josefson, A.B. 2002: Consistent estimates for the residence time of micro-tidal estuaries. - Estuarine Coastal and Shelf Science 54: 65-73.

Remane A 1934: Die Brackwasserfauna (Mit besonderer Beruecksichtigung der Ostsee). - Zoologischer Anzeiger 7 (Supplementband): 34-74.

Sokal, R.R. & Rohlf, F.J. 1981: Biometry (second edition). Freeman, San Francisco.

Steneck, R.S. & Dethiers, M.N. 1994: A functional group approach to the structure of algal-dominated communities. - Oikos 69: 476-498.

Steneck, R.S., Graham, M.H., Bourq, B.J., Corbett, D. & Erlandson, J.M. 2002: Kelp forest ecosystems: biodiversity, stability resilience and future. - Environmental Conservation 29: 436-459.

Utermöhl, H. 1958: Zur Vervollkommung der quantitativen phytoplankton-methodik. Mitt. Internat. Verein. - Limnol. 9: 1-38.

Vellend, M. 2001: Do commonly-used indices of beta diversity measure species turnover? - Journal of Vegetation Science 12: 545-552.

Villnäs, A. & Norkko, A. 2011: Benthic diversity gradients and shifting baselines: implications for assessing environmental status. - Ecological Applications 21: 2172–2186.

Annexes

Annex 1. Reference levels and status class boundaries modelled for the algal variable 'total cover' at a depth of 7 metre in various estuaries/coastal areas defined as inner- (I) or outer estuaries (O) or open coasts (C). Data represent means and 95 % confidence limits.

Locality	Type	Reference	H/G	G/M	M/P	P/B
Aabenraa Fjord inner	I	87.9 (78.2-95.3)	79.9 (67.3-89.9)	61.0 (38.9-77.9)	36.8 (9.5-63.3)	22.3 (0.5-54.2)
Augustenborg Fjord	I	85.8 (76.0-93.8)	75.5 (60.8-86.6)	50.1 (25.2-70.8)	21.7 (0.6-52.3)	8.3 (0.0-40.7)
Bornholm	C	92.6 (78.8-99.5)	91.9 (77.4-99.3)	90.5 (74.7-99.1)	88.9 (70.9-98.7)	87.7 (67.9-98.5)
Flensborg Fjord inner	I	84.1 (74.4-92.2)	71.1 (56.6-82.5)	40.7 (18.0-61.8)	10.6 (0.0-37.4)	1.1 (0.0-24.9)
Flensborg Fjord outer	O	90.9 (82.1-97.2)	87.0 (75.7-95.4)	77.9 (58.6-92.1)	65.4 (34.5-88.5)	57.7 (21.0-85.8)
Hjelm Bugt	C	92.9 (80.0-99.3)	92.4 (79.5-99.3)	91.5 (76.5-99.1)	90.3 (73.6-99.2)	89.5 (70.1-99.2)
Horsens Fjord inner	I	77.9 (63.8-89.7)	55.5 (35.9-72.2)	12.8 (0.0-39.0)	0.0 (0.0-11.0)	0.0 (0.0-0.8)
Horsens Fjord outer	O	85.7 (71.3-96.1)	73.9 (56.2-88.9)	45.9 (19.4-69.6)	15.7 (0.0-48.0)	3.9 (0.0-35.7)
Inderbredning	I	80.8 (68.8-90.2)	62.7 (44.6-77.4)	23.9 (3.1-49.5)	0.2 (0.0-22.3)	0.0 (0.0-9.9)
Kalundborg Fjord inner	I	91.0 (82.6-97.4)	87.1 (77.0-95.3)	78.2 (62.2-90.9)	66.2 (40.6-85.1)	57.6 (26.8-81.6)
Kalundborg Fjord outer	O	91.6 (82.6-97.9)	88.4 (78.0-96.4)	81.2 (65.0-93.3)	70.9 (45.8-89.3)	64.1 (33.4-87.5)
Karrebæksminde Bugt	O	92.0 (82.4-98.2)	89.9 (78.4-97.5)	85.8 (68.1-96.9)	80.1 (51.9-96.8)	76.1 (41.1-96.5)
Kattegat Syd	C	93.6 (83.4-99.6)	92.6 (77.5-100.0)	90.6 (59.8-100.0)	88.5 (36.6-100.0)	86.7 (23.2-100.0)
Kolding Fjord inner	I	76.6 (62.2-88.2)	52.6 (29.7-72.4)	9.8 (0.0-41.3)	0.0 (0.0-10.2)	0.0 (0.0-1.5)
Køge Bugt	C	91.9 (79.6-98.8)	90.1 (77.1-98.1)	86.3 (71.1-96.3)	81.0 (61.8-94.3)	77.6 (56.2-92.5)
Lillebælt Midt	C	92.2 (84.1-97.9)	89.7 (81.2-96.3)	84.3 (74.5-92.4)	76.9 (64.9-86.9)	71.8 (57.3-83.1)
Lillebælt Nord	C	92.2 (80.9-98.9)	89.2 (77.4-97.4)	82.0 (68.9-92.6)	72.6 (57.3-85.5)	65.6 (48.3-80.5)
Lillebælt Syd	C	92.2 (84.2-97.9)	90.0 (81.6-96.4)	84.8 (75.3-92.5)	77.8 (65.8-87.4)	73.1 (59.4-83.9)
Limfjorden s.f. Mors	O	69.1 (43.5-90.3)	34.8 (11.7-62.1)	0.0 (0.0-12.6)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Løgstør Bredning	O	64.7 (42.7-83.7)	27.0 (7.0-51.0)	0.0 (0.0-6.5)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Nissum Bredning	O	70.7 (40.0-93.6)	37.0 (10.5-67.4)	0.0 (0.0-16.5)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Nivå Bugt	C	92.8 (84.5-98.4)	91.8 (82.4-97.8)	89.1 (76.5-97.4)	86.1 (68.5-96.9)	83.9 (62.5-96.7)
Nord for Sjælland	C	93.7 (85.4-99.1)	93.0 (83.9-98.9)	91.3 (80.0-98.8)	89.2 (73.0-99.0)	87.8 (68.5-99.2)
Odense Fjord outer	O	71.0 (57.9-82.3)	40.7 (21.6-58.8)	0.6 (0.0-20.2)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Roskilde Fjord inner	I	68.0 (51.7-81.7)	35.6 (14.1-59.8)	0.0 (0.0-19.8)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Roskilde Fjord outer	O	83.8 (74.0-91.9)	70.0 (56.7-81.3)	38.7 (18.4-58.1)	8.5 (0.0-33.5)	0.4 (0.0-20.6)
Sejerø Bugt	C	92.1 (83.8-98.1)	89.9 (80.3-96.9)	84.6 (71.3-94.7)	77.5 (55.5-92.3)	72.8 (46.5-91.0)
Skive Fjord	I	49.7 (30.4-68.1)	6.3 (0.0-28.5)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Storebælt N	C	92.7 (84.7-98.2)	90.8 (81.8-97.5)	86.7 (74.0-96.2)	81.3 (61.7-94.7)	77.6 (53.3-94.4)
Vejle Fjord inner	I	86.3 (71.6-97.2)	75.2 (56.9-90.3)	48.8 (22.3-72.6)	19.4 (0.1-51.1)	6.1 (0.0-38.5)
Yderbredning	O	87.7 (77.7-95.4)	78.9 (67.1-88.9)	58.5 (37.7-74.7)	32.6 (8.4-57.3)	18.4 (0.3-47.1)
Aarhus Bugt coastal	C	93.6 (81.7-99.7)	92.0 (79.8-99.2)	88.9 (75.1-98.1)	84.6 (68.2-96.6)	81.7 (62.1-95.7)
Øresund	C	92.9 (84.9-98.2)	91.5 (83.1-97.4)	88.4 (79.0-95.6)	84.6 (73.3-93.3)	81.9 (68.6-91.7)

Annex 2. Reference levels and status class boundaries modelled for the algal variable 'cumulative cover' at a depth of 7 metre in various estuaries/coastal areas defined as inner- (I) or outer estuaries (O) or open coasts (C). Data represent means and 95 % confidence limits.

Locality	**Type**	**Reference**	**H/G**	**G/M**	**M/P**	**P/B**
Aabenraa Fjord inner	I	107.2 (82.2-143.0)	84.0 (62.1-113.8)	52.0 (32.4-78.3)	29.9 (14.5-53.1)	21.3 (8.9-43.6)
Augustenborg Fjord	I	100.6 (77.4-132.2)	74.2 (53.5-100.6)	40.8 (23.6-65.1)	20.4 (8.6-40.8)	13.4 (4.7-31.2)
Bornholm	C	126.7 (84.1-190.1)	123.3 (81.5-186.6)	117.0 (76.0-180.1)	109.7 (68.3-173.6)	105.1 (63.8-169.5)
Flensborg Fjord inner	I	95.0 (73.8-123.1)	66.6 (48.8-88.5)	33.0 (19.6-51.5)	14.7 (6.5-29.3)	8.8 (3.2-20.7)
Flensborg Fjord outer	O	119.6 (91.2-161.3)	103.9 (76.6-142.9)	79.2 (50.4-122.7)	58.1 (29.4-107.3)	47.6 (20.4-100.6)
Hjelm Bugt	C	128.7 (87.6-190.4)	126.0 (84.6-187.5)	120.7 (79.0-181.9)	115.0 (71.4-181.7)	112.1 (66.6-184.0)
Horsens Fjord inner	I	80.1 (57.7-111.8)	46.4 (30.7-68.0)	16.0 (7.3-30.2)	4.5 (1.4-12.8)	2.1 (0.5-7.6)
Horsens Fjord outer	O	100.2 (69.4-148.0)	71.8 (48.3-107.5)	37.3 (20.1-63.8)	17.4 (6.7-37.2)	10.8 (3.4-28.2)
Inderbredning	I	86.1 (65.0-115.7)	54.3 (37.1-76.2)	22.1 (11.1-39.4)	7.7 (2.5-19.6)	4.0 (1.0-12.5)
Kalundborg Fjord inner	I	120.4 (92.0-162.2)	105.0 (79.3-142.6)	80.4 (54.9-116.2)	58.7 (33.8-96.6)	48.3 (24.6-86.6)
Kalundborg Fjord outer	O	123.6 (93.5-168.1)	109.3 (81.3-152.7)	86.7 (58.4-129.6)	66.0 (37.2-112.8)	56.0 (28.6-104.7)
Karrebæksminde Bugt	O	124.0 (91.4-169.6)	115.2 (81.8-163.1)	99.0 (62.5-154.9)	83.5 (43.2-152.4)	74.9 (34.5-154.7)
Kattegat Syd	C	135.0 (96.0-202.8)	129.6 (80.8-223.6)	120.0 (52.3-302.6)	110.4 (29.5-443.1)	104.8 (21.8-525.9)
Kolding Fjord inner	I	76.9 (55.2-106.6)	43.6 (26.2-67.9)	14.2 (5.4-31.0)	3.9 (0.8-13.4)	1.7 (0.2-8.2)
Køge Bugt	C	123.0 (86.5-173.9)	114.8 (79.4-164.8)	99.9 (67.7-146.7)	85.2 (55.3-129.5)	77.5 (48.0-122.7)
Lillebælt Midt	C	126.0 (97.2-169.1)	114.7 (89.0-151.5)	95.5 (73.9-124.3)	77.2 (58.4-101.2)	67.7 (50.1-89.4)
Lillebælt Nord	C	126.4 (90.9-183.1)	112.5 (81.6-159.2)	89.7 (66.1-125.3)	69.0 (49.4-96.6)	58.7 (40.9-83.1)
Lillebælt Syd	C	125.8 (97.5-167.0)	115.0 (89.2-150.7)	96.7 (75.7-125.3)	79.3 (59.7-102.9)	69.6 (51.2-92.5)
Limfjorden s.f. Mors	O	64.6 (38.1-111.6)	29.3 (16.3-50.8)	6.2 (2.4-14.5)	1.0 (0.2-4.0)	0.3 (0.1-1.8)
Løgstør Bredning	O	57.8 (37.3-88.9)	24.1 (13.8-39.5)	4.3 (1.5-10.9)	0.6 (0.1-2.7)	0.2 (0.0-1.1)
Nissum Bredning	O	66.5 (36.1-125.9)	31.1 (16.3-57.7)	6.8 (2.6-16.8)	1.2 (0.3-4.6)	0.4 (0.1-2.0)
Nivå Bugt	C	129.3 (97.9-171.8)	123.2 (92.3-166.1)	111.5 (77.7-159.7)	100.0 (63.2-158.2)	93.0 (54.8-158.0)
Nord for Sjælland	C	135.2 (101.7-185.8)	131.0 (96.6-183.8)	121.1 (85.7-182.9)	112.5 (71.5-186.0)	108.0 (63.1-188.9)
Odense Fjord outer	O	66.8 (49.6-88.5)	33.0 (21.6-48.2)	8.2 (3.6-17.6)	1.6 (0.4-5.8)	0.6 (0.1-2.8)
Roskilde Fjord inner	I	61.0 (43.9-84.5)	29.2 (17.5-47.8)	6.9 (2.7-17.0)	1.3 (0.3-5.1)	0.4 (0.1-2.6)
Roskilde Fjord outer	O	94.5 (73.6-122.0)	65.1 (48.7-84.7)	31.5 (19.5-47.3)	13.5 (6.5-26.0)	8.0 (3.1-18.0)
Sejerø Bugt	C	126.4 (96.9-168.2)	114.9 (86.9-156.4)	95.6 (67.2-139.1)	78.0 (47.9-125.7)	69.4 (38.0-119.3)
Skive Fjord	I	40.6 (27.2-59.3)	12.3 (6.4-22.5)	1.2 (0.3-4.5)	0.1 (0.0-0.7)	0.0 (0.0-0.2)
Storebælt N	C	128.6 (98.0-171.8)	119.1 (90.8-163.2)	103.6 (73.1-149.9)	87.8 (54.0-140.5)	79.2 (45.1-136.6)
Vejle Fjord inner	I	102.9 (70.1-155.9)	74.9 (49.8-114.3)	40.3 (22.6-68.3)	19.6 (8.0-40.6)	12.3 (4.1-30.2)
Yderbredning	O	106.5 (81.2-142.5)	81.7 (61.0-110.2)	49.0 (32.0-71.3)	27.3 (14.1-46.6)	18.7 (8.4-36.0)
Aarhus Bugt coastal	C	134.6 (92.6-202.8)	126.4 (88.1-190.8)	111.6 (76.4-172.3)	97.3 (63.3-153.4)	89.5 (55.8-149.7)
Øresund	C	128.9 (99.3-169.4)	122.1 (94.3-160.3)	109.1 (83.3-144.3)	95.9 (70.9-129.0)	88.0 (63.6-120.6)

Annex 3. Reference levels and status class boundaries modelled for the algal variable 'cumulative cover of late successional algae' at a depth of 7 metre in various estuaries/coastal areas defined as inner- (I) or outer fjord (O) or open coasts (C). Data represent means and 95 % confidence limits.

Locality	Type	Reference	H/G	G/M	M/P	P/B
Aabenraa Fjord inner	I	60.5 (40.2-93.8)	40.2 (25.1-65.5)	18.0 (8.4-34.3)	7.2 (2.3-18.7)	3.9 (0.9-12.9)
Augustenborg Fjord	I	50.6 (33.6-77.8)	30.8 (18.4-50.1)	11.3 (4.7-24.1)	3.6 (0.9-11.0)	1.8 (0.3-7.1)
Bornholm	C	28.1 (15.8-50.5)	26.8 (14.9-48.2)	24.4 (13.0-45.7)	22.0 (10.9-42.6)	20.6 (9.8-42.5)
Flensborg Fjord inner	I	46.4 (31.1-69.9)	25.6 (15.5-39.9)	8.0 (3.6-16.0)	2.1 (0.6-5.9)	0.9 (0.2-3.4)
Flensborg Fjord outer	O	65.1 (43.1-102.2)	51.8 (31.7-85.7)	32.9 (15.6-67.7)	19.3 (6.2-53.0)	13.9 (3.5-48.5)
Hjelm Bugt	C	30.9 (18.2-53.5)	30.2 (16.9-53.5)	28.1 (15.2-51.3)	26.1 (12.8-52.8)	24.8 (11.2-53.2)
Horsens Fjord inner	I	54.1 (32.4-91.9)	22.1 (11.4-39.7)	3.7 (1.1-10.3)	0.5 (0.1-2.4)	0.1 (0.0-0.9)
Horsens Fjord outer	O	88.0 (49.9-160.7)	50.3 (26.9-95.4)	17.0 (6.2-39.7)	4.9 (1.0-16.7)	2.2 (0.3-9.9)
Inderbredning	I	48.9 (30.9-78.1)	22.7 (12.4-39.2)	5.0 (1.6-12.5)	0.9 (0.1-3.8)	0.3 (0.0-1.8)
Kalundborg Fjord inner	I	70.8 (46.7-112.1)	56.1 (35.7-90.8)	36.0 (19.1-66.6)	21.3 (8.7-48.1)	15.6 (5.0-40.3)
Kalundborg Fjord outer	O	80.2 (52.0-130.3)	65.7 (40.7-111.0)	44.6 (23.5-85.0)	28.1 (11.4-68.2)	21.5 (7.2-61.2)
Karrebæksminde Bugt	O	43.5 (28.0-68.9)	38.5 (23.0-65.3)	30.1 (14.8-62.1)	22.3 (7.8-60.6)	18.7 (5.3-61.4)
Kattegat Syd	C	95.8 (55.1-180.9)	90.2 (40.8-221.3)	80.4 (20.9-349.9)	70.1 (7.8-652.4)	63.3 (4.6-946.3)
Kolding Fjord inner	I	44.4 (26.6-75.3)	17.3 (7.6-35.5)	2.6 (0.6-9.4)	0.3 (0.0-2.4)	0.1 (0.0-1.0)
Køge Bugt	C	32.4 (19.5-53.7)	28.9 (17.1-48.9)	23.0 (12.9-40.1)	17.8 (9.1-32.7)	15.1 (7.1-29.6)
Lillebælt Midt	C	73.6 (49.0-113.7)	63.0 (43.0-96.0)	46.4 (31.1-70.0)	32.6 (20.9-49.5)	26.0 (16.0-41.1)
Lillebælt Nord	C	112.4 (65.9-197.4)	91.9 (56.0-158.2)	63.7 (38.7-107.1)	40.9 (23.9-69.4)	31.2 (17.2-54.4)
Lillebælt Syd	C	67.8 (45.6-103.2)	58.0 (39.8-87.8)	43.5 (29.4-64.6)	31.1 (20.1-46.6)	25.2 (15.6-39.2)
Limfjorden s.f. Mors	O	79.9 (35.2-181.9)	21.6 (8.8-50.8)	1.6 (0.4-6.1)	0.1 (0.0-0.7)	0.0 (0.0-0.2)
Løgstør Bredning	O	49.5 (25.2-97.6)	11.5 (4.8-25.4)	0.7 (0.1-2.7)	0.0 (0.0-0.2)	0.0 (0.0-0.1)
Nissum Bredning	O	108.3 (42.3-285.3)	30.2 (11.2-77.7)	2.4 (0.5-9.5)	0.1 (0.0-1.0)	0.0 (0.0-0.3)
Nivå Bugt	C	51.9 (34.3-79.7)	47.8 (30.8-75.8)	40.5 (23.5-72.0)	33.7 (16.3-69.5)	30.4 (12.8-70.8)
Nord for Sjælland	C	91.1 (58.5-151.0)	86.2 (53.7-144.3)	76.9 (43.8-144.6)	67.0 (31.7-151.4)	62.5 (25.9-158.5)
Odense Fjord outer	O	34.6 (21.7-53.2)	10.7 (5.5-19.3)	1.1 (0.3-3.5)	0.1 (0.0-0.5)	0.0 (0.0-0.2)
Roskilde Fjord inner	I	14.2 (8.5-22.8)	4.1 (1.9-8.4)	0.4 (0.1-1.5)	0.0 (0.0-0.2)	0.0 (0.0-0.1)
Roskilde Fjord outer	O	48.5 (32.5-72.9)	26.2 (16.6-39.6)	7.8 (3.8-14.7)	1.9 (0.6-5.3)	0.8 (0.2-2.8)
Sejerø Bugt	C	72.5 (48.2-114.0)	61.9 (39.9-100.9)	45.8 (26.1-82.8)	32.8 (14.5-72.4)	26.3 (9.8-66.6)
Skive Fjord	I	22.2 (11.6-40.1)	3.0 (1.1-7.7)	0.1 (0.0-0.4)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Storebælt N	C	73.4 (48.6-115.4)	65.0 (42.0-104.1)	51.1 (29.2-94.3)	38.5 (17.8-83.6)	32.8 (12.8-80.1)
Vejle Fjord inner	I	101.6 (56.7-194.9)	59.5 (30.8-116.1)	20.9 (8.1-50.2)	6.2 (1.5-21.0)	2.9 (0.5-12.9)
Yderbredning	O	66.4 (43.5-104.5)	43.0 (27.1-68.3)	18.1 (9.4-33.3)	6.7 (2.4-16.4)	3.7 (1.0-10.3)
Aarhus Bugt coastal	C	136.4 (77.3-256.7)	122.2 (69.5-229.2)	100.3 (55.4-196.0)	78.7 (40.1-166.3)	67.7 (31.8-151.5)
Øresund	C	59.9 (40.9-90.9)	54.6 (37.1-81.8)	45.3 (30.0-69.0)	36.6 (23.1-57.9)	32.0 (19.0-52.4)

Annex 4. Reference levels and status class boundaries modelled for the algal variable 'cumulative cover of opportunistic algae' at a depth of 7 metre in various estuaries/coastal areas defined as inner- (I) or outer fjords (O) or open coasts (C). Data represent means and 95 % confidence limits.

Locality	Type	Reference	H/G	G/M	M/P	P/B
Aabenraa Fjord inner	I	23.1 (18.4-28.9)	19.7 (15.5-24.9)	14.6 (10.2-19.6)	10.3 (6.1-15.8)	8.2 (4.1-13.8)
Augustenborg Fjord	I	23.3 (18.8-29.0)	19.3 (15.1-24.4)	13.4 (8.8-18.9)	8.8 (4.6-14.7)	6.8 (3.0-12.7)
Bornholm	C	58.8 (42.6-81.7)	58.7 (42.5-81.4)	58.6 (41.8-83.0)	58.6 (40.7-84.6)	58.5 (39.6-86.3)
Flensborg Fjord inner	I	22.5 (18.2-27.8)	18.0 (14.0-22.6)	11.7 (7.7-16.7)	7.1 (3.6-12.4)	5.2 (2.3-10.5)
Flensborg Fjord outer	O	26.8 (21.4-34.1)	24.7 (19.5-31.8)	21.0 (15.2-28.2)	17.4 (10.7-26.0)	15.7 (8.7-24.7)
Hjelm Bugt	C	55.4 (40.8-75.2)	55.2 (40.4-75.5)	54.8 (39.5-75.6)	54.7 (39.0-75.7)	54.3 (37.9-77.1)
Horsens Fjord inner	I	13.5 (10.3-17.6)	9.2 (6.4-12.5)	4.3 (2.4-7.2)	1.8 (0.7-3.9)	1.0 (0.3-2.7)
Horsens Fjord outer	O	14.6 (10.9-19.4)	11.5 (8.2-15.6)	7.1 (4.3-10.9)	4.1 (2.0-7.4)	2.9 (1.2-6.0)
Inderbredning	I	17.5 (13.8-22.2)	12.9 (9.6-16.9)	7.1 (4.2-11.1)	3.6 (1.5-7.2)	2.3 (0.8-5.5)
Kalundborg Fjord inner	I	25.5 (20.4-32.5)	23.3 (18.7-29.5)	19.8 (14.9-26.2)	16.4 (11.0-22.9)	14.6 (8.9-21.7)
Kalundborg Fjord outer	O	24.1 (19.2-31.2)	22.3 (17.7-28.8)	19.3 (14.6-25.8)	16.2 (10.8-23.3)	14.6 (8.9-22.1)
Karrebæksminde Bugt	O	39.9 (30.9-51.9)	38.6 (29.7-50.2)	36.2 (26.2-48.4)	33.8 (21.6-47.5)	32.4 (19.3-47.5)
Kattegat Syd	C	24.8 (19.1-33.6)	24.1 (17.1-35.6)	23.0 (13.0-41.7)	22.0 (9.4-53.5)	20.9 (7.3-61.8)
Kolding Fjord inner	I	14.9 (11.3-19.3)	10.1 (6.8-14.1)	4.7 (2.3-8.4)	1.9 (0.6-4.9)	1.1 (0.3-3.5)
Køge Bugt	C	50.1 (37.4-66.9)	49.3 (36.6-66.3)	47.6 (34.3-65.3)	45.8 (31.0-65.8)	44.9 (29.1-67.6)
Lillebælt Midt	C	27.0 (21.7-34.0)	25.4 (20.6-32.1)	22.7 (18.4-28.3)	20.0 (16.0-25.0)	18.4 (14.3-23.2)
Lillebælt Nord	C	19.4 (15.1-25.6)	17.9 (13.9-23.3)	15.3 (11.9-19.8)	12.7 (9.5-16.6)	11.3 (8.3-15.0)
Lillebælt Syd	C	28.9 (23.2-36.4)	27.4 (22.1-34.4)	24.7 (20.1-30.5)	21.9 (17.4-27.2)	20.4 (15.8-25.6)
Limfjorden s.f. Mors	O	6.0 (3.8-9.3)	3.2 (1.8-5.5)	0.9 (0.4-2.0)	0.2 (0.1-0.7)	0.1 (0.0-0.3)
Løgstør Bredning	O	7.1 (4.8-10.3)	3.6 (2.1-5.8)	1.0 (0.4-2.1)	0.2 (0.1-0.7)	0.1 (0.0-0.3)
Nissum Bredning	O	5.0 (2.9-8.1)	2.6 (1.4-4.8)	0.7 (0.3-1.8)	0.2 (0.0-0.6)	0.1 (0.0-0.3)
Nivå Bugt	C	37.2 (29.2-47.8)	36.3 (28.5-46.7)	34.7 (26.7-45.2)	33.0 (24.2-44.5)	31.9 (22.8-43.8)
Nord for Sjælland	C	26.1 (20.6-33.8)	25.5 (20.0-33.2)	24.4 (18.8-33.3)	23.2 (16.8-33.4)	22.5 (15.6-33.8)
Odense Fjord outer	O	13.7 (10.7-17.4)	8.5 (6.0-11.7)	3.3 (1.7-6.1)	1.1 (0.4-2.9)	0.6 (0.2-1.9)
Roskilde Fjord inner	I	27.8 (19.6-38.6)	19.9 (10.9-35.8)	10.3 (3.2-31.8)	4.8 (0.8-29.6)	2.9 (0.3-28.4)
Roskilde Fjord outer	O	21.1 (17.1-26.1)	16.7 (13.1-20.9)	10.6 (7.2-14.9)	6.2 (3.4-10.8)	4.5 (2.1-8.9)
Sejerø Bugt	C	27.4 (21.9-35.1)	25.9 (20.6-33.2)	23.2 (18.0-30.4)	20.5 (14.6-28.5)	19.0 (12.6-27.3)
Skive Fjord	I	6.7 (4.6-9.6)	2.8 (1.6-4.8)	0.5 (0.2-1.3)	0.1 (0.0-0.3)	0.0 (0.0-0.1)
Storebælt N	C	28.1 (22.3-35.8)	26.9 (21.3-34.4)	24.6 (19.0-32.6)	22.3 (16.0-30.9)	20.9 (14.3-30.2)
Vejle Fjord inner	I	13.6 (10.0-18.5)	10.8 (7.7-15.0)	6.8 (4.1-10.4)	4.0 (1.9-7.3)	2.8 (1.2-5.7)
Yderbredning	O	20.9 (16.7-26.5)	17.6 (13.9-22.3)	12.6 (9.0-16.8)	8.5 (5.2-12.9)	6.7 (3.6-11.1)
Arhus Bugt coastal	C	18.9 (14.3-25.1)	18.1 (13.7-24.1)	16.5 (12.3-22.4)	14.9 (10.7-20.9)	14.0 (9.7-20.2)
Øresund	C	33.3 (26.5-42.4)	32.2 (25.5-40.8)	30.4 (24.4-38.7)	28.4 (22.4-36.0)	27.2 (21.1-34.7)

Annex 5. Reference levels and status class boundaries modelled for the algal variable 'fraction of opportunists' at a depth of 7 metre in various estuaries/coastal areas defined as inner- (I) or outer fjords (O) or open coasts (C). Data represent means and 95 % confidence limits.

Locality	**Type**	**Reference**	**H/G**	**G/M**	**M/P**	**P/B**
Aabenraa Fjord inner	I	32.7 (26.5-39.1)	38.0 (31.2-45.0)	48.4 (38.9-59.7)	60.5 (46.7-76.1)	67.9 (51.5-85.4)
Augustenborg Fjord	I	35.8 (29.4-42.2)	43.0 (35.4-51.2)	56.8 (45.5-70.1)	72.6 (56.3-89.3)	80.9 (62.4-96.7)
Bornholm	C	49.2 (38.5-60.3)	51.7 (39.7-64.0)	56.7 (40.9-72.8)	62.0 (41.3-82.6)	65.3 (41.3-88.0)
Flensborg Fjord inner	I	37.1 (31.0-43.3)	45.3 (38.4-53.0)	62.0 (50.9-74.2)	79.2 (63.6-93.1)	88.0 (71.2-98.9)
Flensborg Fjord outer	O	32.8 (26.0-39.4)	36.1 (28.3-43.8)	42.8 (31.8-54.5)	50.6 (35.6-67.8)	55.5 (37.2-75.5)
Hjelm Bugt	C	46.7 (36.1-57.4)	48.4 (36.2-61.0)	51.6 (35.3-68.6)	55.5 (33.2-77.4)	58.0 (31.4-82.8)
Horsens Fjord inner	I	27.7 (21.9-34.0)	34.4 (27.0-43.1)	48.1 (35.0-63.1)	64.6 (44.1-85.5)	73.9 (49.2-94.1)
Horsens Fjord outer	O	22.9 (17.3-29.0)	26.4 (20.1-33.5)	33.2 (24.6-44.0)	41.6 (28.8-57.8)	46.9 (31.2-66.8)
Inderbredning	I	33.1 (27.1-39.3)	41.1 (33.9-49.8)	57.4 (44.7-72.1)	75.2 (56.5-92.1)	84.7 (62.9-98.9)
Kalundborg Fjord inner	I	31.2 (24.9-37.5)	34.1 (27.2-41.0)	40.1 (31.3-49.4)	47.1 (35.6-60.6)	51.7 (38.1-67.8)
Kalundborg Fjord outer	O	29.1 (22.8-35.1)	31.3 (24.7-37.8)	35.8 (27.8-44.2)	41.2 (30.1-53.6)	44.6 (31.8-59.9)
Karrebæksminde Bugt	O	41.0 (32.1-50.1)	44.5 (33.5-55.9)	51.1 (34.1-68.7)	58.7 (33.8-82.4)	63.2 (33.0-90.1)
Kattegat Syd	C	26.7 (19.4-33.7)	27.5 (18.0-36.8)	28.9 (13.9-45.3)	30.5 (9.4-56.3)	31.5 (6.8-62.9)
Kolding Fjord inner	I	32.1 (26.0-38.8)	40.8 (32.5-51.1)	58.6 (43.2-76.9)	77.6 (54.6-96.9)	87.3 (61.8-100.0)
Køge Bugt	C	47.7 (37.9-57.4)	52.2 (41.8-62.8)	60.9 (48.1-74.0)	70.9 (54.5-86.3)	76.5 (57.5-92.3)
Lillebælt Midt	C	30.9 (24.5-37.2)	33.0 (26.7-39.2)	37.2 (30.9-43.6)	42.2 (35.2-49.3)	45.3 (38.1-53.1)
Lillebælt Nord	C	23.2 (17.7-29.2)	24.6 (19.1-30.5)	27.5 (21.8-33.4)	30.8 (24.8-37.5)	33.0 (26.4-40.5)
Lillebælt Syd	C	32.6 (25.9-39.2)	34.7 (28.3-41.1)	39.3 (32.6-45.8)	44.6 (37.6-52.0)	48.0 (40.6-56.2)
Limfjorden s.f. Mors	O	12.3 (6.0-20.5)	14.0 (5.7-25.2)	17.2 (4.5-36.0)	21.4 (3.1-50.5)	24.1 (2.3-60.3)
Løgstør Bredning	O	18.9 (12.2-27.0)	23.5 (13.8-35.3)	33.4 (15.1-54.7)	45.7 (16.2-77.9)	53.5 (16.8-88.1)
Nissum Bredning	O	8.1 (2.6-16.2)	8.2 (1.8-18.8)	8.6 (0.5-25.5)	9.1 (0.0-34.6)	9.4 (0.0-40.8)
Nivå Bugt	C	37.7 (29.6-45.6)	39.5 (30.5-48.0)	43.2 (31.8-54.8)	47.3 (32.0-63.4)	50.0 (32.1-68.5)
Nord for Sjælland	C	27.6 (21.3-33.9)	28.4 (21.7-34.8)	29.7 (21.9-37.2)	31.1 (21.3-41.0)	32.2 (20.9-43.5)
Odense Fjord outer	O	34.7 (28.7-41.0)	46.0 (37.1-55.6)	68.5 (51.2-83.9)	89.0 (66.5-99.8)	97.2 (75.8-100.0)
Roskilde Fjord inner	I	69.3 (59.5-78.7)	92.4 (81.4-98.9)	100.0 (100.0-100.0)	100.0 (100.0-100.0)	100.0 (100.0-100.0)
Roskilde Fjord outer	O	35.5 (29.6-41.6)	43.5 (37.1-50.7)	59.5 (49.3-70.5)	76.6 (62.0-89.7)	85.6 (69.3-97.2)
Sejerø Bugt	C	31.2 (24.5-37.6)	33.3 (26.3-40.1)	37.5 (28.8-46.5)	42.4 (31.2-54.8)	45.6 (32.2-60.3)
Skive Fjord	I	27.9 (20.0-36.6)	38.4 (24.8-53.2)	60.4 (33.0-85.1)	83.5 (42.8-100.0)	93.3 (46.5-100.0)
Storebælt N	C	31.1 (24.7-37.5)	32.8 (25.7-39.8)	36.3 (27.5-45.3)	40.3 (28.8-52.2)	42.8 (29.3-57.4)
Vejle Fjord inner	I	20.6 (15.0-26.8)	23.2 (17.1-30.3)	28.6 (20.5-38.5)	35.3 (23.9-50.5)	39.3 (25.3-58.6)
Yderbredning	O	30.5 (24.7-36.4)	35.3 (29.1-41.8)	44.8 (36.8-54.5)	56.3 (44.5-70.1)	63.2 (49.0-79.0)
Aarhus Bugt coastal	C	21.0 (15.4-26.9)	21.6 (15.9-27.7)	22.9 (16.9-29.2)	24.4 (17.9-31.1)	25.3 (18.7-32.7)
Øresund	C	34.8 (27.6-42.0)	36.7 (29.4-43.8)	40.2 (32.4-47.7)	44.3 (35.9-53.3)	46.9 (37.5-56.5)

Annex 6. Reference levels and status class boundaries modelled for the algal variable ‘number of late successional algal species’ at a depth of 7 metre in various estuaries/coastal areas defined as inner- (I) or outer estuaries (O) or open coasts (C). Data represent means and 95 % confidence limits.

Locality	**Type**	**Reference**	**H/G**	**G/M**	**M/P**	**P/B**
Aabenraa Fjord inner	I	5.7 (4.6-7.2)	4.6 (3.6-6.0)	3.0 (1.9-4.3)	1.7 (0.6-3.1)	1.1 (0.1-2.5)
Augustenborg Fjord	I	5.2 (4.2-6.5)	4.0 (3.0-5.2)	2.3 (1.3-3.6)	1.0 (0.1-2.3)	0.5 (0.0-1.7)
Bornholm	C	3.7 (2.6-5.1)	3.6 (2.5-5.0)	3.4 (2.3-4.9)	3.2 (2.1-4.7)	3.1 (1.9-4.7)
Flensborg Fjord inner	I	5.0 (4.0-6.2)	3.7 (2.8-4.7)	1.8 (1.0-2.9)	0.6 (0.0-1.6)	0.1 (0.0-1.0)
Flensborg Fjord outer	O	5.9 (4.8-7.4)	5.3 (4.1-6.9)	4.2 (2.7-6.1)	3.1 (1.5-5.4)	2.6 (1.0-5.1)
Hjelm Bugt	C	3.9 (2.8-5.4)	3.8 (2.7-5.3)	3.7 (2.5-5.2)	3.5 (2.3-5.2)	3.4 (2.1-5.3)
Horsens Fjord inner	I	5.5 (4.2-7.2)	3.4 (2.3-4.7)	1.1 (0.2-2.2)	0.0 (0.0-0.8)	0.0 (0.0-0.2)
Horsens Fjord outer	O	7.1 (5.3-9.5)	5.3 (3.8-7.4)	3.0 (1.6-4.7)	1.3 (0.2-2.9)	0.7 (0.0-2.2)
Inderbredning	I	5.2 (4.1-6.5)	3.5 (2.4-4.6)	1.4 (0.4-2.5)	0.1 (0.0-1.1)	0.0 (0.0-0.5)
Kalundborg Fjord inner	I	6.2 (5.0-7.8)	5.5 (4.4-7.0)	4.4 (3.1-6.0)	3.3 (1.9-5.2)	2.7 (1.3-4.7)
Kalundborg Fjord outer	O	6.6 (5.3-8.4)	6.0 (4.7-7.7)	4.9 (3.5-6.9)	3.9 (2.3-6.1)	3.3 (1.7-5.7)
Karrebæksminde Bugt	O	4.8 (3.7-6.0)	4.4 (3.3-5.9)	3.9 (2.5-5.7)	3.3 (1.7-5.7)	3.0 (1.3-5.7)
Kattegat Syd	C	7.2 (5.5-9.9)	7.0 (4.7-10.7)	6.5 (3.2-13.3)	6.1 (1.9-17.3)	5.9 (1.2-21.2)
Kolding Fjord inner	I	5.0 (3.7-6.5)	3.0 (1.8-4.4)	0.8 (0.0-2.1)	0.0 (0.0-0.7)	0.0 (0.0-0.2)
Køge Bugt	C	4.0 (2.9-5.3)	3.8 (2.7-5.1)	3.3 (2.3-4.6)	2.9 (1.9-4.1)	2.6 (1.6-3.9)
Lillebælt Midt	C	6.3 (5.1-7.9)	5.8 (4.7-7.2)	5.0 (4.0-6.2)	4.2 (3.2-5.2)	3.7 (2.8-4.7)
Lillebælt Nord	C	7.8 (6.1-10.4)	7.1 (5.5-9.4)	5.9 (4.6-7.7)	4.8 (3.6-6.3)	4.1 (3.0-5.5)
Lillebælt Syd	C	6.0 (4.9-7.5)	5.6 (4.6-6.9)	4.8 (3.9-5.9)	4.0 (3.2-5.1)	3.6 (2.7-4.6)
Limfjorden s.f. Mors	O	6.9 (4.4-10.4)	3.5 (2.0-5.6)	0.5 (0.0-1.7)	0.0 (0.0-0.1)	0.0 (0.0-0.0)
Løgstør Bredning	O	5.4 (3.7-7.7)	2.4 (1.3-3.9)	0.0 (0.0-0.9)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Nissum Bredning	O	8.2 (4.9-13.1)	4.3 (2.4-7.1)	0.8 (0.0-2.3)	0.0 (0.0-0.3)	0.0 (0.0-0.0)
Nivå Bugt	C	5.2 (4.1-6.6)	5.0 (3.9-6.4)	4.6 (3.4-6.1)	4.2 (2.8-6.1)	3.9 (2.4-6.1)
Nord for Sjælland	C	7.0 (5.6-9.0)	6.8 (5.4-8.9)	6.4 (4.8-8.9)	6.0 (4.1-9.0)	5.8 (3.7-9.3)
Odense Fjord outer	O	4.4 (3.4-5.5)	2.2 (1.4-3.2)	0.2 (0.0-1.1)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Roskilde Fjord inner	I	2.6 (1.8-3.4)	1.1 (0.5-1.9)	0.0 (0.0-0.4)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Roskilde Fjord outer	O	5.1 (4.2-6.3)	3.7 (2.8-4.7)	1.8 (1.1-2.7)	0.6 (0.0-1.4)	0.1 (0.0-0.9)
Sejerø Bugt	C	6.2 (5.0-7.9)	5.8 (4.6-7.4)	5.0 (3.7-6.7)	4.2 (2.6-6.2)	3.7 (2.1-5.9)
Skive Fjord	I	3.5 (2.4-4.8)	0.9 (0.2-1.9)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Storebælt N	C	6.3 (5.1-7.9)	5.9 (4.7-7.5)	5.2 (3.9-7.1)	4.5 (3.0-6.8)	4.2 (2.4-6.5)
Vejle Fjord inner	I	7.6 (5.6-10.5)	5.8 (4.1-8.2)	3.3 (1.9-5.3)	1.6 (0.4-3.4)	0.9 (0.0-2.6)
Yderbredning	O	6.0 (4.8-7.6)	4.8 (3.8-6.2)	3.1 (2.0-4.3)	1.7 (0.7-2.9)	1.0 (0.2-2.3)
Århus Bugt coastal	C	8.7 (6.5-11.8)	8.2 (6.2-11.2)	7.5 (5.5-10.3)	6.7 (4.7-9.6)	6.2 (4.2-9.3)
Øresund	C	5.6 (4.6-7.0)	5.4 (4.3-6.6)	4.9 (3.9-6.1)	4.4 (3.4-5.6)	4.1 (3.1-5.3)

DEVELOPMENT AND TESTING OF TOOLS FOR INTERCALIBRATION OF PHYTOPLANKTON, MACROVEGETATION AND BENTHIC FAUNA IN DANISH COASTAL AREAS

This report contributes to the development of indicators and assessment tools for ecological status classification according to the European Water Framework Directive as well as the intercalibration of the phytoplankton biomass indicator with Sweden and Germany. For the open coastal waters in the Kattegat and the Sound Denmark and Sweden have similar reference conditions for summer chlorophyll, whereas class boundaries are more strict in Denmark. Different indicators for phytoplankton composition, based on the distribution of functional groups, have been tested and none of these indicators was found operational for assessing ecological status. Six indicators for macroalgae cover and composition have also been tested and three of these are suggested as the basis for an overall status assessment tool for macroalgae. The proposed assessment tool is modular and sufficiently flexible such that it can be readily adapted to other indicators and biological quality elements, for the WFD and other directives as well. The DKI indicator for benthic macrofauna integrates species sensitivity and diversity. Salinity is an important governing factor for diversity and therefore a salinity normalisation of the DKI is proposed.

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