Type-specific biological reference conditions in Danish coastal waters

Analysis of the '*Composition and abundance of other aquatic flora*' biological quality element of the Water Framework Directive (2000/60/EC)

Joao G. Ferreira

New University of Lisbon

February 2021

Contents

Executive summary

This report analyses the approach taken by Denmark in setting reference conditions for the Water Framework Directive (2000/60/EC) Biological Quality Element (BQE) *composition and abundance of other aquatic flora*, and the Danish proposal for achieving Good/High Ecological Quality Status through the definition of nitrogen emission targets for land-based loading.

We review the spatial scope of the issue, the definition of reference conditions for eelgrass, the factors influencing eelgrass distribution and abundance, the indicators chosen to link pressure and state, and the criteria for setting target nitrogen loads.

We conclude that the use of historical data for eelgrass distribution as a reference condition is appropriate, although nothing can be stated concerning abundance. This and other gaps make it challenging for historical data on distribution *per se* to be considered satisfactory to define the BQE.

The only indicator chosen for assessment is '*Transparency*', which goes against the spirit of the WFD, since the directive emphasises the use of *biological* elements rather than Supporting Quality Elements (SQE). There are shortcomings in the use of this indicator only to deal with eutrophication and its relation to nutrient loading. The addition of the *structure and substrate of the coastal bed* SQE would be helpful in dealing with the additional pressure of mussel dredging.

Finally, the analysis of the adequacy and consistency of the proposed measures (reduction in nitrogen loading from land) raises a number of questions. These include the lack of a holistic management approach, which seems key to successful eelgrass restoration, and a proposal for source control which does not match the historical basis for definition of the reference conditions, and for which no additional justification is provided.

Introduction and objectives

The Water Framework Directive (WFD, 2000/60/EC) mandates that EU Member States define reference conditions for Biological Quality Elements (BQE), with additional reference conditions for Hydromorphological Elements (HME) and Supporting Quality Elements (SQE), as relevant.

These BQE include both pelagic and benthic components—for coastal systems, the flora is addressed through (a) *phytoplankton abundance, biomass, and composition*, for the pelagic elements; and (b) *composition and abundance of other aquatic flora*, for the benthic elements.

The latter BQE could potentially include halophytes (saltmarsh plants), which are at the interface of land and sea, marine angiosperms (seagrasses), macrophyte algae (seaweeds), and microphytobenthos, but the WFD limits the BQE only to seagrasses and seaweeds.

For the BQE *composition and abundance of other aquatic flora*, Denmark has chosen the seagrass *Zostera marina* (eelgrass), and as required by the WFD, has defined the reference condition for this indicator.

The WFD stipulates that, in the case where the condition of *Good* or *High* Ecological Quality Status (EQS) is not met, the competent authorities will implement a programme of measures to ensure that the condition will be met. If a BQE is below *Good* Status (i.e. at *Bad*, *Poor*, or *Moderate* status) these measures must ensure that the BQE moves to at least *Good* status. If a BQE is at *High* status, it must not be allowed to change category to *Good* status.

The objectives of this study are to:

1. Analyse the approach proposed by Denmark in its definition of reference conditions for eelgrass;
2. Evaluate the relevance of the indicators chosen for assessment of the status of this BQE;
3. Examine the adequacy[1] and consistency[2] of the measures proposed to enable the coastal systems to meet the BQE reference condition for eelgrass.

Points 1 and 3 are further contextualised by a review of the pressures from land on the coastal ecosystem (Oenema, 2021), in particular the loading of nitrogen from land, and a review of the legal framework under which this approach has been proposed (Van Calster et al, 2021).

Spatial domain

The WFD is applicable to all waters, which include *Transitional Waters* (*sensu* WFD) and *Coastal Waters*.

Denmark has chosen not to define any *Transitional Waters*, so all applicable brackish and fully saline waters, i.e. estuarine (fjords) and coastal regions are defined as *Coastal Waters*. An immediate consequence of this definition is that it conditions the set of BQE, HME, and SQE for which ranges should be set and on which quality assessment is based.

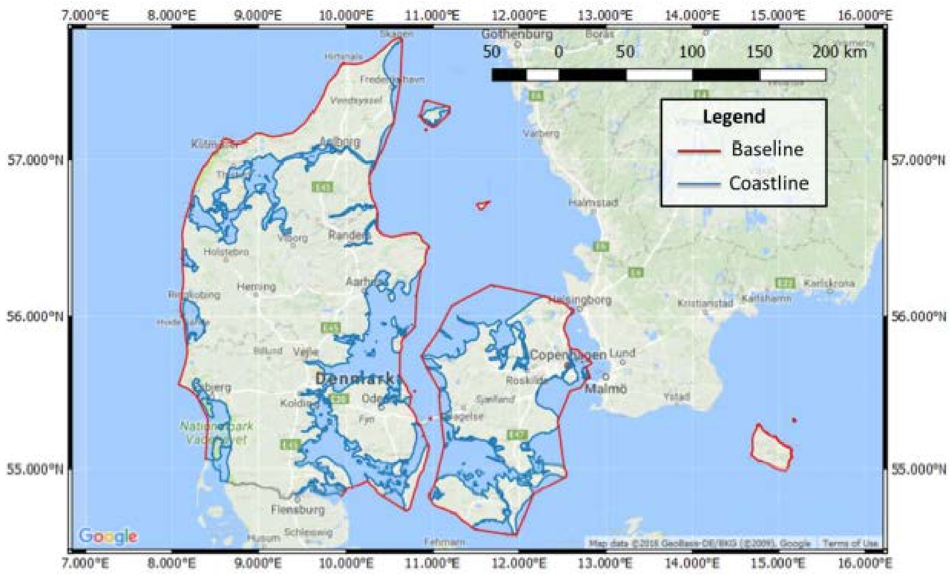

Fig. 1. Baseline of Danish territorial waters (EMODnet, 2018).

Although the WFD defines the coastal area as a narrow strip, one nautical mile offshore of the landward limit of territorial waters,[3] Denmark has adopted, like many other nations, a set of straight

[1] Adequacy of measures means they will succeed in meeting the stated objectives for a desired ecological status.
[2] Consistency of measures means that robust criteria are used in their definition, in the context of the objectives the measures are designed to fulfil.
[3] 'Coastal water' means surface water on the landward side of a line, every point of which is at a distance of one nautical mile on the seaward side from the nearest point of the baseline from which the breadth of territorial waters is measured, extending where appropriate up to the outer limit of transitional waters.

baselines (Fig. 1). As a consequence, the marine area defined as '*Coastal Waters*' (*sensu* WFD) is large, and to this area the fjords must also be added, since they have been defined as coastal waters.

The red lines in Fig. 1 represent the coastal baseline, to which a further one nautical mile must be added.

Definition of eelgrass reference conditions

Denmark has access to a unique resource to determine the reference condition—a historical register of eelgrass distribution dating back to a comprehensive survey from the early 1900s (Ostenfeld, 1908), followed by work by Petersen (1914). On the basis of the bathymetry at the sampling stations, together with the presence of eelgrass at a time when the coastal system was considered to be undisturbed (*sensu* WFD), eelgrass depth limits can be set.

Benthic photosynthesis is limited by both surface irradiance and the attenuation of light in the water column. This attenuation, calculated by means of the light extinction coefficient K_d, is due to both suspended particles and dissolved substances; Dennison (1987) and Duarte (1991) suggest that the limit for growth is on average 11% of the surface irradiance. Fig. 2 illustrates the consequences of different Secchi disk depths typical for Danish waters (Henriksen et al, 2014) for eelgrass growth. For instance, for a location with a depth of 7 m, a Secchi reading of 5.4 m would be required to provide sufficient light energy at the benthic boundary for eelgrass growth. A small correction may be required to account for the length of eelgrass leaves—there is anecdotal evidence that these were up to 2 m in length in the early XXth century (Krause-Jensen, pers. com.), whereas present populations of *Zostera* tend to have shorter leaves.

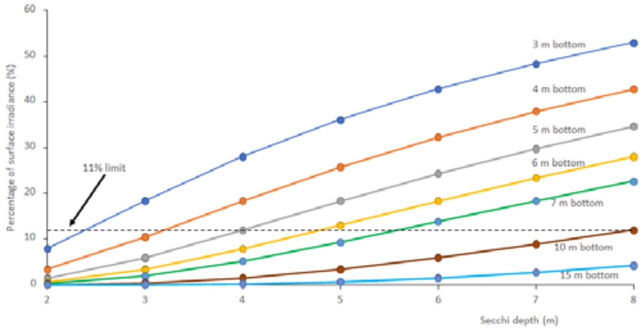

Fig. 2. Light limit for eelgrass growth for different Secchi depths.

Although the historical records for eelgrass provide information on distribution, they do not provide data on abundance. This is a potential issue, since in the WFD Annex V definition, *High* status for *composition and abundance of other aquatic flora* is defined as (a) 'all disturbance-sensitive macroalgal and angiosperm taxa associated with undisturbed conditions are present'; and (b) 'the levels of macroalgal cover and *angiosperm abundance* are consistent with undisturbed conditions'. *Good* status is defined as (a) 'most disturbance-sensitive macroalgal and angiosperm taxa associated with undisturbed conditions are present'; and (b) 'the level of macroalgal cover and *angiosperm abundance* show slight signs of disturbance'.

Van Calster et al (2021) note in their legal opinion on the application of the WFD that the use of 'temporally based reference conditions', commonly termed the historical method, is potentially inferior to other approaches for defining reference conditions. The lack of eelgrass abundance data, comparable data on macroalgae for the same time period, together with other aspects discussed in the next section, are examples of the potential limitations in using historical data.

A second question related to the selection of eelgrass as representative of the BQE is that the WFD specifically refers *taxa*, suggesting that this should be a community-based approach, rather than a monospecific one, and additionally mandates that both macroalgae and angiosperms be considered.

In their review of the Danish 2nd cycle RBMP, Herman et al (2017) state that '*the panel stresses the generality of the required "angiosperm vegetation" indicator, so that it may occasionally differ from the single "Zostera maximum depth" indicator, at least in principle*.'

As a final note, the assumption that the situation in 1900 corresponds to an undisturbed condition would automatically define the eelgrass depths recorded as *High* status. Since *Good* status will correspond to lower depths, management measures designed to meet *Good* status would be sufficient for WFD compliance, although from a restoration perspective, *High* status is desirable.

Factors influencing eelgrass distribution

Coastal systems are subject to both anthropogenic and natural pressures—abundance and distribution of different species and communities is conditioned by multiple stressors. Eelgrass covered an estimated 6700 km^2 in 1900 (Ostenfeld, 1908; Petersen, 1914), and although no data currently exist for spatial cover, the potential area is calculated to be 2200 km^2 (Staehr et al, 2019).

The distribution of eelgrass in Danish fjords and coastal waters is constrained by two main factors: light availability and substrate conditions; in addition, pathogen outbreaks have significantly affected *Zostera* in the past, climate change affects it presently and in the future (Krause-Jensen et al, 2021), and stressors such as herbicide application (e.g. Wilkinson et al, 2017) may also play a part.

These various factors are briefly reviewed below.

Light availability

The underwater light climate determines the availability of sufficient light at the sea bottom for eelgrass growth. This is a necessary factor for eelgrass development, but not sufficient by itself.

Nielsen et al (2002a) analyse a substantial data set on chlorophyll, total nitrogen (TN), and Secchi depth, as a proxy for light availability. These authors state that '*Secchi depth is strongly dependent on nutrient concentrations*', but the references cited to support the statement are from freshwater lakes, where dependencies can be very different from marine systems.

Light attenuation in the water column is a function of the various particulate and dissolved constituents of seawater, and of the water itself, and is due to both absorption and scattering of photons (Eq. 1).

$$K_d = K_{chl} + K_{POM} + K_{PIM} + K_s + K_w \qquad \text{(Eq. 1)}$$

Where:

K_d: light extinction coefficient (m^{-1});

K_{chl}: light extinction due to phytoplankton algae (m^{-1});

K_{POM}: light extinction due to detrital (non-living) particulate organic matter, or POM (m^{-1});

K_{PIM}: light extinction due to particulate inorganic matter (PIM), i.e. suspended silt and clay (m^{-1});

K_s: light extinction due to coloured dissolved organic matter, or CDOM (m^{-1});

K_w: light extinction due to water molecules (m^{-1}).

Phytoplankton and CDOM are considered to be the main factors responsible for absorption of light, whereas PIM and POM are mainly responsible for scattering (Kirk, 1994). The contribution of water molecules (K_w) to the value of K_d is typically negligible.

Pedersen et al (2014) studied the components responsible for K_d in Roskilde Fjord, a shallow Danish estuary, and provide a comprehensive analysis in time and space (Table 1).

The dataset covers two distinct periods, 1985 and 2008-2009, but no major differences seem to exist in the relative contributions of phytoplankton and CDOM.

Table 1. Partitioning of light attenuation components in Roskilde Fjord. Table adapted from Pedersen et al, 2014.

Station[4]	Period	Average Z_d (m)	Average K_d (m^{-1})	Phytoplankton attenuation (%)	POM attenuation (%)	CDOM + PIM attenuation (%)
2	1985	2.4	0.8	18.0	16.9	65.1
3	1985	N/A	0.6	17.4	22.1	60.5
4	1985	3.5	0.5	21.4	21.3	57.2
1	2008–2009	3.8	0.7	12.0	4.0	84.1
2	2008–2009	4.2	0.5	12.9	3.9	83.2
3	2008–2009	4.0	0.5	17.3	5.5	77.2
4	2008–2009	4.6	0.5	17.2	6.2	76.7
Mean		3.7	0.6	16.6	11.4	72.0

The most striking aspects of these data are (i) the relatively low contribution (μ = 16.6%) of phytoplankton; and (ii) the high contribution (μ = 72.0%) of CDOM + PIM, of which CDOM is the main constituent.

These results contrast sharply with the statement by Nielsen et al (2002a, 2002b) that emphasises '*the strong dependence of Secchi depth on phytoplankton biomass...*'

However, the work by Pedersen et al (2014) focused on Roskilde fjord, so an analysis of its wider application to Danish waters is warranted—this is addressed in the conclusions of this report. Nevertheless, it is accepted that CDOM plays an important role in light attenuation in the Baltic proper (Kowalczuk et al, 2005; Kowalczuk et al, 2006).

Source control of land-based nutrient emissions is a common remedial action aimed at reducing pelagic algal biomass and increasing water transparency, thereby promoting the restoration of submerged aquatic vegetation (SAV). However, it is unclear whether major changes in underwater light climate will be obtained in Danish coastal waters through a reduction in phytoplankton concentration (chlorophyll is normally used as a proxy), given that it accounts on average for less than 17% of light attenuation.

If no phytoplankton were present in the coastal water, Secchi depth would only increase by about 20%, which would certainly not achieve the objective of changing the estimated potential cover of 2200 km^2 (Staehr et al, 2019) to the 6700 km^2 estimated by Ostenfeld (1908) and Petersen (1914).

The question then is whether land-based control of nutrient loading will have a significant effect on coloured dissolved organic matter, since it accounts for over 70% of light extinction—it appears that CDOM emissions from diffuse sources are rather low, when compared to the discharge of dissolved inorganic nitrogen (DIN)—it is therefore uncertain to what extent a reduction in nitrogen loads will significantly improve water clarity and therefore the potential for eelgrass restoration.

[4] Station 1 (1985) in the original table was omitted since the values are clearly different from all the others (phytoplankton attenuation = 45.6% compared to a mean of 16.6% for all other sites). In 2008-2009, Station 1 (data shown) has the lowest percentage contribution from phytoplankton (12%).

Exudation of dissolved organic matter during algal photosynthesis is well known (e.g. Thornton, 2014) and therefore the possibility that loading of inorganic nitrogen *indirectly* contributes to CDOM should be considered (Carstensen, pers. com.). Kinsey et al (2018) performed a series of experiments with phytoplankton and bacteria, and conclude '*Our experimental results suggest that at least a portion of open-ocean CDOM is produced by autochthonous processes and aggregation likely facilitates microbial reprocessing of organic matter into refractory DOM*.' This suggests that a clear link between phytoplankton and CDOM is at present more an area for scientific research than a set of well-established quantitative relationships appropriate for management of coastal systems.

External nutrient loading

Enrichment of seawater with nitrogen (N) and phosphorus (P) can lead to eutrophication, one of the symptoms (*sensu* Bricker et al, 2003) of which is an undesirable growth of phytoplankton. In the case of Danish coastal waters, nitrogen is commonly identified as the limiting nutrient for primary production, based on 30-year time series presented in the Danish Marine Areas 2018 (Hansen & Høgslund, 2019) and 2019 (Hansen & Høgslund, 2021) reports, although 1993-1995 and 1998-2000 showed N/P ratios higher than the Redfield ratio of 16 (in atoms).

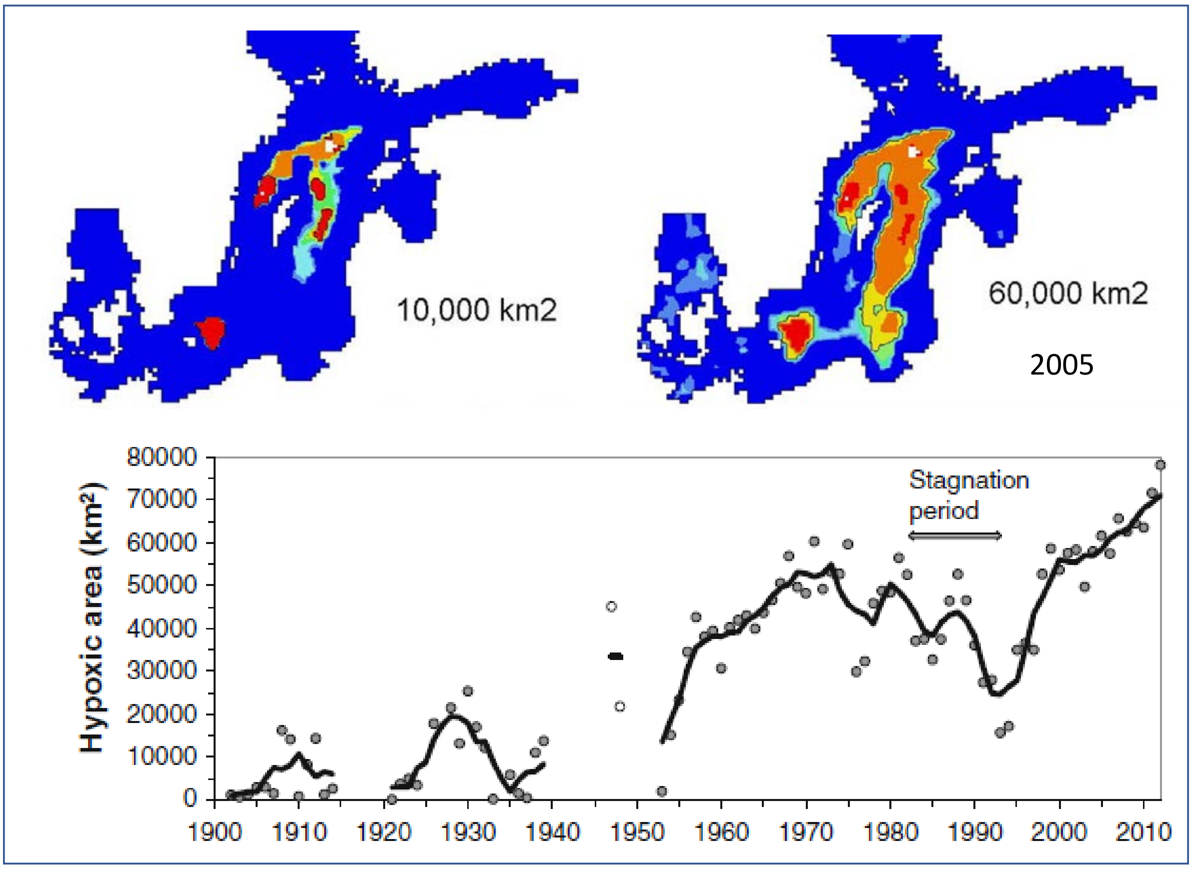

Fig. 3. Upper pane: area of hypoxia (low dissolved oxygen) in the bottom waters of the Baltic Sea in 1905-1906 and a century later (Savchuk et al, 2008). Lower pane: timeline of hypoxia in the Baltic from 1900 to 2010 (Carstensen et al, 2014).

Since the issues discussed herein are related to the control of nitrogen emissions from land-based sources, as per the Danish proposals for the third cycle of the WFD (see review by Oenema, 2021) we will centre our discussion on nitrogen loading as a pressure on the coastal system.

Although this document is focused on the marine ecosystem, source apportionment of nitrogen loads is key to the discussion. Nitrogen discharges from the territory of Denmark are principally due to diffuse sources, given that point-sources are largely controlled.

Conley et al (2007) estimate that in 1900 about 64% of the annual nitrogen loading from Denmark was due to point sources, whereas by 2003 than number had fallen to 10%.[5]

However, Denmark is at the boundary of the Baltic and the North Sea, and nitrogen loading to the Baltic has increased very substantially in the past century, as evidenced by the reduction of dissolved oxygen in the water (Fig. 3). Furthermore, Carstensen et al (2014) show that hypoxic conditions in the Baltic show a quasi-linear upward trend in the last two decades, and now affect 80,000 km^2 (Fig. 3).

A mathematical modelling analysis of chlorophyll distribution in Danish marine waters (DHI, 2015) was carried out as part of the WFD Cycle 2 work programme. Fig. 4 shows the percentage of chlorophyll concentration that can be explained by nitrogen loading from Danish land-based sources.

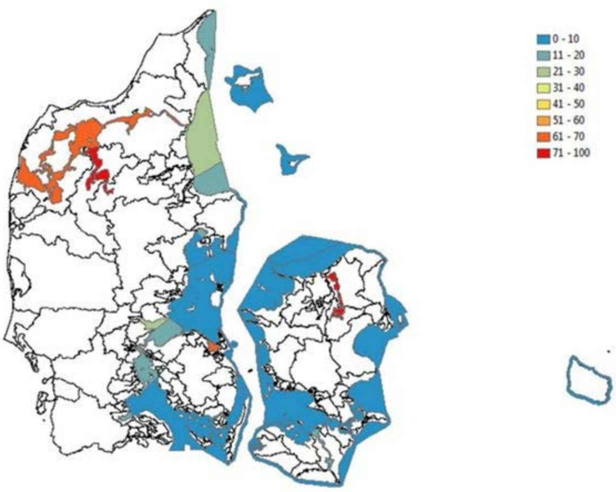

Fig. 4. Percentage of Chl indicator that can be explained by Danish N loading from land.

Mechanistic models of this kind simulate primary production under different scenarios of nutrient supply and compare the resulting phytoplankton (chlorophyll) concentrations—this is the only way to analyse the relative contribution of different nutrient sources, in this case the Danish loading and the Baltic proper. The model results are striking: in the waters around Zeeland and eastern Jutland, the Danish contribution generally accounts for less than 20% of the chl, rising to 20-30% in NE Jutland. The only areas where the Danish land sources of N account for a high (>60%) proportion of chl are the rather enclosed parts of NW Jutland, i.e. the three water areas defined in the model for the Limfjord (DHI, 2015), which include e.g. Bjørnholms Bugt, Riisgårde Bredning, Skive Fjord, and Lovns Bredning.

Substrate conditions

Eelgrass requires an appropriate substrate for growth, and therefore this is another necessary but not sufficient factor for restoration. Coastal systems in Denmark, just as elsewhere, have a broad range of human uses, one of the oldest of which is fisheries.

In Denmark, as in the Netherlands and Ireland, bottom trawlers engage in a sub-tidal blue mussel (*Mytilus edulis*) fishery. Recent data on the Danish mussel fishery are relatively sparse, but Dolmer & Frandsen (2002) refer an area of 893 km^2 licensed for bottom trawling in 2002. With respect to habitat protection, these authors state that '*fishery for mussels is rather restrictive in Limfjorden. The vessels must be licensed, be below a maximum size, and fishing is banned at water depth more shallow*

[5] These are annual estimates, so it is possible that there may be significant seasonal variation.

than two or three metres in order to reduce fishery impact on benthic flora and fauna and in particular to protect the seagrass beds'.

In practice, this means that in areas of depth greater than 3 m, the potential for restoration of eelgrass as a consequence of increased water transparency is easily compromised by bottom trawling for mussels.

Krause-Jensen et al (2021) discuss the interaction between mussel harvest and eelgrass recovery, since the trawling activity is responsible for the destruction of seagrass beds.

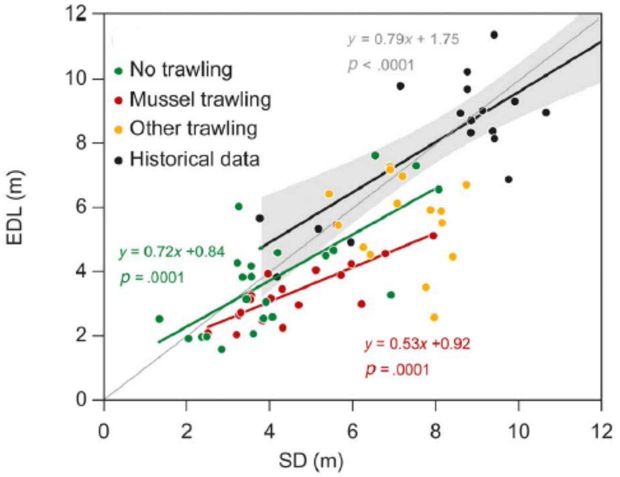

Fig. 5. Relationship between eelgrass depth and mussel trawling (Krause-Jensen et al, 2021).

According to those authors, mussel harvest in Danish waters peaked in the 1980s, with an annual production of 120,000 t live weight, but due to a decline in stocks the current harvest is around 40,000 t y^{-1}.

Krause-Jensen et al (2021) identify mechanical removal (i.e. dredging), increased water column turbidity due to resuspension, and particle settling on leaves as possible factors suppressing eelgrass recovery. Fig. 5 shows quantitative data for areas that are trawled or undisturbed: the red data points, associated with mussel trawling, have much less scatter than the green data points, which in some cases show values comparable with the historical data (black points).

Erfetemeijer & Lewis (2006) reviewed the impacts of various types of dredging on seagrasses, using 45 case studies worldwide, and concluded that through direct and indirect effects, the consequences of dredging on the distribution of marine angiosperms are rather severe.

The WFD does not consider the effect of fishing in general and bottom trawling in particular as a factor in the assessment of ecological status, a gap that has often been criticised. As a consequence, any measures to be put in place disregard the effect of fishing on biological quality elements. The practical consequence of this for eelgrass restoration in Danish waters is that measures designed to improve water clarity must necessarily be accompanied by (non-WFD) measures for the preservation or improvement of suitable habitat in order to be successful.

Measures such as ecosystem-based management (EBM) of mussel leases, including criteria based on seabed mapping, stocking density, and no-take periods are not mandated by the WFD, but because successful eelgrass restoration requires a multi-stressor management approach, these and other considerations must be taken into account.

Pathogen events

The wasting disease of *Zostera* in the 1930s led to an estimated reduction in cover of about 90% in North America and Europe (Muehlstein, 1989), such that in Denmark, the estimated 6700 km^2 in 1900 (Ostenfeld, 1908; Petersen, 1914), would have been reduced to 650-700 km^2.

The causes of the disease are still unclear: candidates include alterations in seawater temperature and/or other environmental factors, or pathogenic microorganisms. An extensive literature review by

Muehlstein (1989) identified the slime mould *Labyrinthula sp*. as the primary etiological agent. In 1984, the *Zostera* wasting disease recurred in the U.S. (Maine, New Hampshire, and Massachusetts) and was also detected in France (Short et al, 1987); the pathogen was identified as the slime mould *Labyrinthula zosterae*.

Marine ecosystems rarely respond in a linear manner to stressors, but instead non-linear changes are observed at tipping points. Equally, after a shift has occurred, the return pathway following removal of the stressors can be complex (Fig. 6).

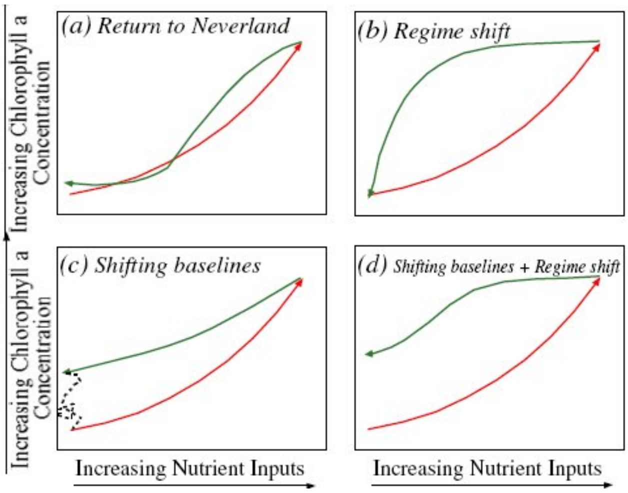

Fig. 6. Regime shift and shifting baselines (Duarte et al., 2008).

In the case of the eelgrass wasting disease of the 1930s, Muehlstein (1989) reports that by the early 1950s (i.e. twenty years later), eelgrass on the Atlantic coast of the U.S. and Canada was almost fully recovered, although it remained absent in '*Southern New Jersey, isolated bays in Connecticut, Delaware, Chincoteague Bay in Maryland, and Virginia*'. We were unable to find comparable information for Denmark with respect to the recovery after the 1930s wasting disease, but the overarching message is that regime shift can significantly change the pathway of recovery, and baseline shifts such as climate change mean that it is unlikely that an ecosystem will easily or quickly return to the *status quo ante*.

Other stressors

Krause-Jensen et al (2021) highlight the sensitivity of eelgrass to climate change: the Baltic Sea has been warming rapidly (0.4 °C per decade since 1990), and the rise in temperature increases light requirements and mortality rates. For Danish eelgrass populations, experimental work shows that temperatures above 25 °C result in a negative carbon balance and a twelve-fold increase in mortality rates (Nejrup & Pedersen, 2008).

Synthesis

In summary, a range of factors influence the distribution and abundance of eelgrass in Danish waters, and management measures to ensure successful restoration must be designed with an understanding that this will only be achieved by addressing multiple stressors in a holistic manner.

A focus on only one part of the problem, such as the issue of water transparency, may well fail to provide an acceptable outcome with respect to eelgrass restoration. Such sectorial measures will most likely (a) not result in successful compliance with the WFD for the eelgrass BQE, leading to further investigation by the European Commission; (b) result in social costs for the Danish citizens with respect to the expense of land-based emission controls and increased unemployment in the agricultural sector, and as discussed above may not *per se* significantly improve water transparency; and (c) deny the expectations of improved ecosystem services from eelgrass created by such measures.

Indicators for assessing eelgrass reference conditions

The ‘transparency’ indicator

For the BQE *composition and abundance of other aquatic flora*, represented by *Zostera marina* (eelgrass), Denmark has selected the SQE *transparency* as a key indicator for quality assessment. This is based on the concept that suspended particulate matter (SPM) conditions light penetration and thus development of eelgrass, due to light limitation. As stated above, the role of CDOM cannot be ignored.

Since microscopic pelagic algae (i.e. phytoplankton) depend on light energy and inorganic nutrients such as N and P for growth, this SQE may also be seen as a proxy for eutrophication, on the premise that the loading of N and P from land-based sources will drive increased pelagic primary production, which will result in a higher light extinction coefficient K_d, less light availability at the benthic boundary layer, and loss of eelgrass in deeper areas.

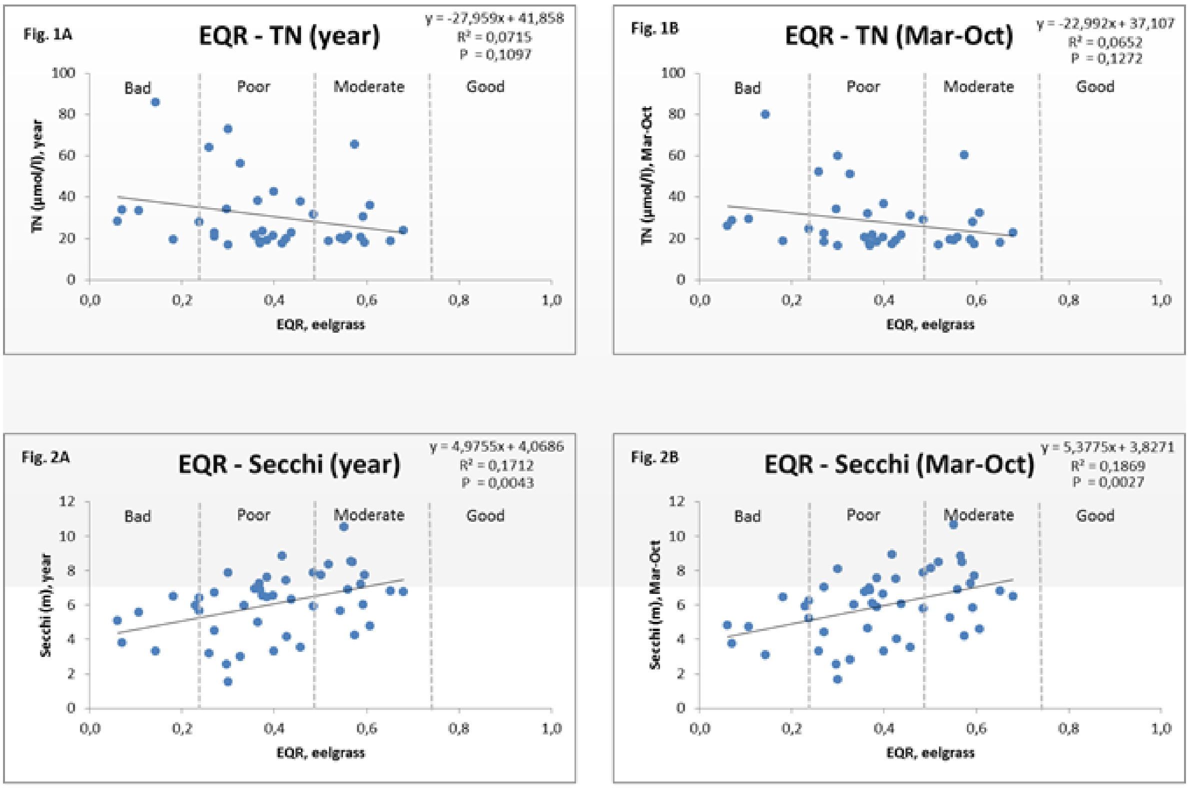

Fig. 7. Graphs of total nitrogen (TN) and Secchi depth as a function of the eelgrass EQR (Henriksen et al, 2014).

The assumption when using the underwater light climate, measured for instance by means of a Secchi disk, as a proxy for eelgrass distribution, is that this BQE will be at *Good* or *High* Ecological Quality Status (EQS) if the SPM in the water, part of which is phytoplankton, allows for sufficient light penetration.

As discussed above (see Table 1 and accompanying text), phytoplankton appears to contribute only a relatively small proportion of light attenuation. More broadly, appropriate water clarity is necessary but not sufficient to restore eelgrass populations.

Furthermore, the use of an SQE as the primary (the only?) indicator is contrary to the spirit of the WFD, which promotes an ecosystem-based approach and emphasises the use of BQE. Water clarity will not provide a robust assessment of the change in distribution and abundance of eelgrass, it can only establish the potential for such a recovery. Given the multi-stressor aspects discussed above, the only indicators that make sense for evaluating whether measures are successful are eelgrass distribution and abundance themselves, by means of an appropriate monitoring programme.

This is in agreement with Herman et al (2017): "*the Panel recommends reviewing the approach for this WFD indicator by starting from the basic observation that not K_d, but survival and restoration of aquatic angiosperm vegetation is the real criterion.*"

By definition, an indicator must correlate with what it aims to indicate, and in this case, since the ambition is to relate transparency to water column chlorophyll concentration, this SQE should be sensitive to the associated *pressure*, i.e. nitrogen loading.

The graphs shown in Fig. 7, produced as part of the Danish contribution to the WFD intercalibration (phase 2), are not particularly encouraging.

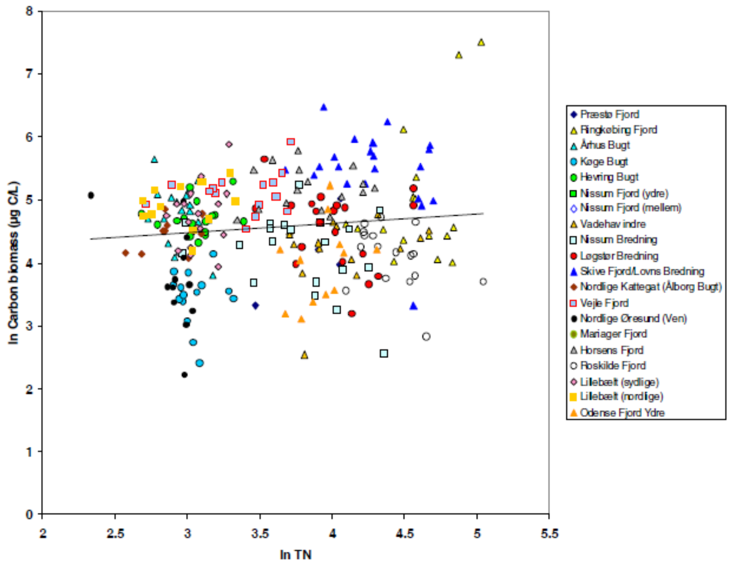

Fig. 8. Relationship between phytoplankton biomass and total nitrogen.

The coefficient of determination (r^2) is in all cases too low to justify a regression line, and the scatter of points across the various status classes, or EQR (*High* is not shown in the graphs) shows little or no trend both for total nitrogen and Secchi depth. The authors explain that '*Phytoplankton indicators were established for total phytoplankton biovolume and carbon biomass.*

The indicators were derived from relationships between concentrations of total nitrogen (TN) [6] *and biovolume or carbon biomass of the phytoplankton community*.' The data used to derive the relationship between TN and phytoplankton carbon (Fig. 8) shows a substantial scatter of measured values, which again suggests that the line drawn is illustrative, rather than a representation of a significant trend.

Nielsen et al (2002b) also analyse the relation between chlorophyll and total nitrogen, and, although statistically significant, TN explains only about 55% of the chlorophyll variation. In conclusion, as evidenced by Pedersen et al (2014), the use of Secchi depth as a proxy for phytoplankton concentration merits a more detailed analysis.

Nitrogen loading and the 'Transparency' indicator

Since the concentration of phytoplankton is related to N and P availability, a further assumption is that by reducing the land-based loading of N and P, the algal biomass in the coastal waters (*sensu* WFD)

---

[6] Carbon biomass is another way to quantify phytoplankton and assuming, as the authors appear to do, that all TN is phytoplankton, can be readily converted to chlorophyll. The maximum value in the upper pane (100 µmol L^{-1} TN) would thus correspond to 196 µg L^{-1} of chlorophyll: 100 µmol L^{-1} X 14 (atomic mass of N) X 7 (C:N ratio) / 50 (C:Chl ratio) = 196 µg L^{-1} chl. Values from the graph are centred around 40 µmol L^{-1}, or ≈80 µg L^{-1} chl.

will be lower, and therefore eelgrass will colonise deeper water, restoring the spatial coverage to levels considered appropriate (i.e. *Good* or *High* EQS).

A reduction in the flux of nitrogen to the coastal zone will undoubtedly reduce the concentration of nitrogen in seawater, and this will have an effect on primary production. The degree to which that effect will be evident depends on a number of factors, including water column turbidity, water residence time, and top-down control of phytoplankton, e.g. by bivalve filter-feeders.

The degree to which the control of land-based nitrogen emissions from Denmark will contribute to reduce N flux is also a matter for consideration, viz. the review above of loading from the Baltic Sea to Danish coastal waters, which has changed significantly over the last century.

Krause-Jensen et al (2021) show the change in Secchi depth and nitrogen loading from land over a period of one hundred and forty years (Fig. 9). A similar plot for chlorophyll is not available over such a long period, but although the nitrogen loading starts decreasing steadily from the early 1980s onwards, the response in terms of water transparency is not obvious.

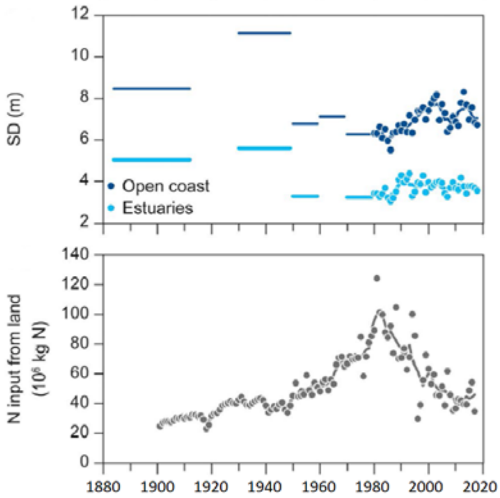

Fig. 9. Secchi depth and nitrogen input from land (Krause-Jensen et al, 2021).

In estuarine waters, Secchi depth in the period 1980-2020 ranges between 3 and 4 m, but no trend can be observed. In coastal waters, there seems to be a slight upward trend, although the fitted line reflects a rather noisy distribution in time, with Secchi depth decreasing in the early 2000s and then increasing again, only to show a downward trend in 2010-2020.

Overall, one would expect a clearer response from this indicator, if in fact transparency were the most appropriate metric to relate nitrogen loading to the eelgrass BQE.

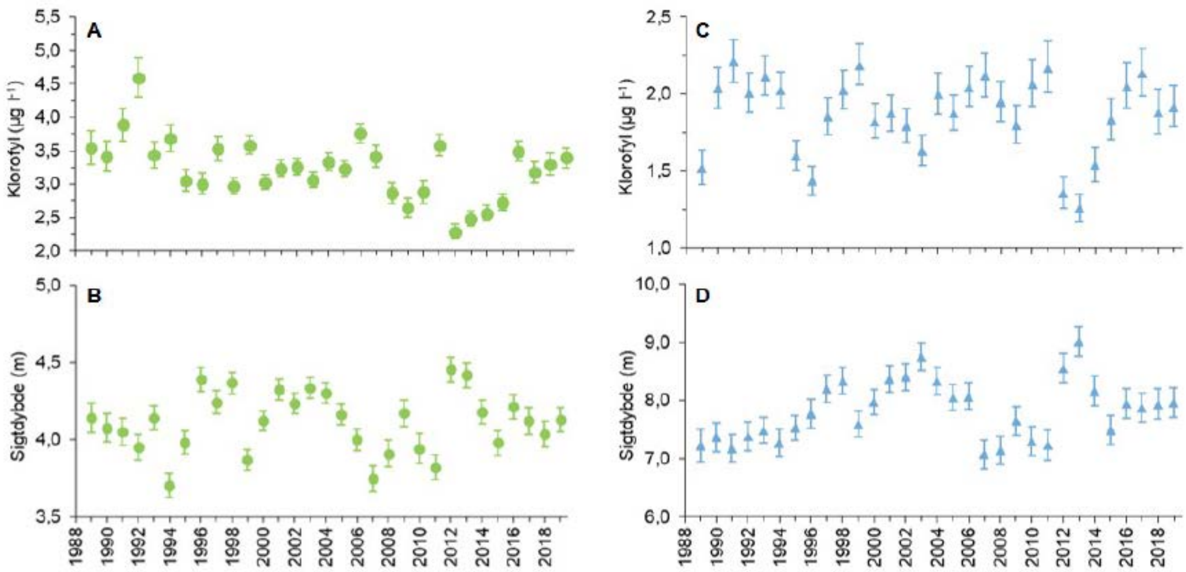

Fig. 10. Chlorophyll (upper pane) and Secchi depth (lower pane) for fjords and coastal waters (left) and inland waters (right) (Hansen & Høgslund, 2021).

Fig. 10 shows chlorophyll concentrations and Secchi depth for fjords and coastal waters (left panes) over the period 1988-2019. Without plotting one parameter against the other it is challenging to understand how they correlate, but there does not seem to be much change in the reported chlorophyll values during a period (1980-2010) when the N load from land was reduced from one hundred thousand tonnes per year to about 40% of that value.

The chlorophyll concentration observed is always below 5 μg L^{-1}, the 'Low' threshold defined in Bricker et al (2003), but an analysis of monthly Copernicus data obtained at a 1 km^2 resolution for the present report shows values in NE Denmark that reach 14 μg L^{-1} in July 2000 (Fig. 11).

Unfortunately, we did not find a long-term record of satellite measurements to verify whether seasonal peaks in earlier or later years were significantly different from those in 2000.

Note that chlorophyll directly calculated from TN (Fig. 7) had typical values of the order of 80 μg L^{-1}. A similar calculation for the line in Fig. 8 gives carbon values of ≈90 μg C L^{-1} ($e^{4.5}$), i.e. ≈2 μg Chl L^{-1}.

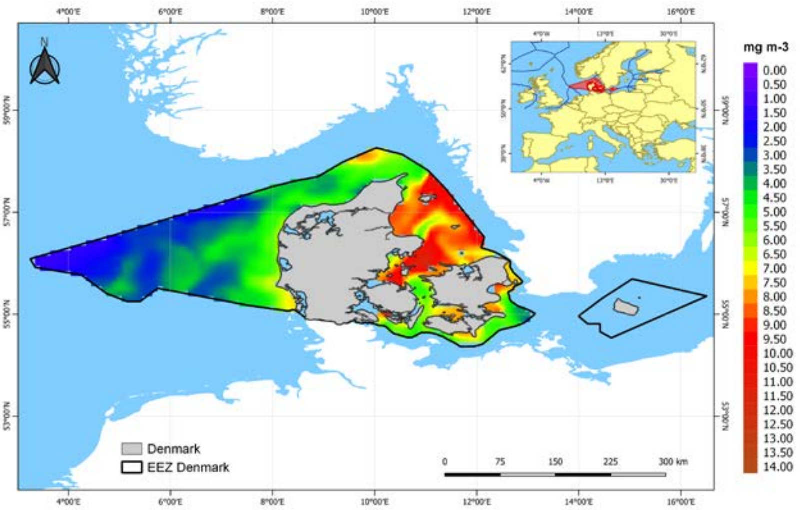

Fig. 11. Chlorophyll determined by remote sensing (Copernicus MEMS) in July 2000 for the Danish EEZ (source: this study).

In their review of the Danish 2nd cycle RBMPs, Herman et al (2017) write "*Even though a significant correlation of summer (June to August) averages of K_d with N load is reported for 16 out of 22 stations [p 94], the slopes of these relations are very low [p 94], and no material changes in yearly averages are observed over time despite changes in N loading. Similarly, in the mechanistic modelling, slopes for change of K_d as a function of N load are usually small, and the model is not able to reproduce the reference (observed around 1900) K_d values by modelling reference loads of 1900.*"

They further note in their concluding remarks, "*…the relationship between K_d in coastal waters and external nutrient loading is sometimes very weak. Further, K_d and the insufficient relationship have different consequences for and are differently treated in the mechanistic and the statistical modelling exercises. In the statistical modelling approach, for example, the use of K_d in some cases causes impossible N load reduction requirements of above 100%.*"

A final note on the use of appropriate indicators for the eelgrass BQE is that (i) both distribution and abundance, monitored *in loco* using e.g. underwater autonomous vehicles (UAV) or some form of remote sensing, should be measured; (ii) the SQE *Transparency* should be measured as a supporting parameter only, e.g. to identify areas where eelgrass recovery is possible with respect to light availability but has not occurred; and (iii) the HME *Structure and Substrate of the Coastal Bed* should be considered as a further supporting element, which could be used as a metric to incorporate the substrate conditions discussed above.

Analysis of proposed nutrient management measures

As a primary management measure, Denmark plans to set a target for land-based nitrogen emissions, leading to a reduction in present-day loads. The underlying assumption is that the improvement in water clarity due to this reduction would allow the reference condition for the eelgrass BQE to be met.

For the reasons discussed earlier, there is no guarantee that this would be achieved. The *adequacy* of the proposed measures appears questionable, since (i) only the water clarity issue is addressed, when a multi-stressor approach is clearly necessary; and (ii) the relationship between nitrogen loading and water clarity, and of both with chlorophyll, also requires further analysis.

With respect to the *consistency* question, Denmark initially considered reducing the nitrogen loading from land to values calculated for the year 1900, based on the rationale that these represent negligible anthropogenic input. Despite my serious reservations as to whether this approach would enable successful eelgrass restoration, at least there is consistency in applying load reduction measures based on emissions in 1900 when addressing ecological conditions determined for the same period.

However, Timmermann (2020) notes that '*... results... regarding the estimate of nutrient inputs to the sea in 1900, indicate that nutrient inputs were significantly greater than the levels seen in present-day watercourses with minor human impact. The reason is that the extent of agricultural activity, the loss of nutrients from these activities and inputs of urban wastewater were already substantial at that time.*'

That author further states, '*Strictly speaking, the input in 1900 cannot be regarded as a reference input for the Water Framework Directive and consequently, should not be used to calculate the reference condition for, for example, the chlorophyll indicator in coastal waters for use in Water Plan 3.*'

No justification is provided for this statement—on the contrary, it is difficult to understand, if a historical approach to reference conditions has been selected, how measures addressing nutrient emissions could be derived in any other way than by using historical loading for the same period.

Timmermann refers that '*The apparent discrepancy between the historical eelgrass observations and the preliminary results from the 1900 project, which indicate extensive human impact, may be due to the time delays in the manifestation of the effects. It takes time (several years) before increased nutrient inputs fully impact light conditions.*' This is certainly a possibility, but the nutrient loads (Fig. 9) remain roughly constant until 1920, and there is no decline reported in eelgrass distribution until the wasting disease of the 1930s.

As an alternative, the same author proposes a '*reference input is based on concentrations in smaller watercourses, which drain catchment areas with a very small level of cultivation multiplied by a present-day waterflow.*'

This methodology aims to arrive at a lower nitrogen loading than that determined for the year 1900, although no quantitative data are presented, but it is impossible to understand how the link between the ecosystem conditions in 1900 and the loading calculated from 'smaller watercourses' is established.

Conclusions

On the basis of the evidence reviewed in this work, and with reference to the stated objectives, we conclude the following:

1. The use of historical data for eelgrass distribution as a reference condition for the *composition and abundance of other aquatic flora* BQE is appropriate, although nothing can be stated concerning abundance. The BQE further requires an analysis of taxa, which is not addressed, and additionally refers macroalgae as part of the BQE. These aspects make it challenging for the historical data on distribution to be considered satisfactory to define the BQE;
2. The only indicator chosen is the supporting quality element '*Transparency*', rather than the BQE itself, which goes against the spirit of the WFD, since the directive emphasises the use of *biological* elements. An analysis of the power of this indicator to deal with eutrophication and its relation to nutrient loading shows several shortcomings. The addition of the *structure and substrate of the coastal bed* SQE would be helpful in dealing with the additional pressure of mussel dredging;
3. The *adequacy* of the proposed measures (reduction in nitrogen loading from land) is rather uncertain, primarily because these fail to address the restoration issue in a holistic manner. In addition, there are a number of uncertainties at a sectorial level with respect to the relationship between nitrogen loading and water clarity, and of both with chlorophyll;
4. The *consistency* of the proposed measures, if based on N loading determined for small catchments at the present time, the consequences of which are then presumed to be the restoration of eelgrass to the conditions observed in 1900, lacks a clear justification.

My overarching concern is that this whole question is seen as a conflict between environment and agriculture, having been reduced to the specific issue of setting a target for nutrient emissions, when in fact it is a much broader problem, and the tools to resolve it do not appear to be in place.

Restoration of eelgrass is a fundamental objective for the Danish marine ecosystem and can only be achieved by addressing the problem with a multi-stressor management approach, and in addition by taking into account what appear to be critical transboundary issues—the results obtained through mathematical modelling (DHI, 2015) suggest that, apart from more enclosed areas such as the Limfjord and other embayments, land-based nitrogen reductions will not have a major effect on water column chlorophyll. A corollary of this is that the water transparency related to chl light attenuation will not be significantly improved.

While it is not within the remit of this report to outline the characteristics of the type of holistic approach required, and in the full knowledge that the Danish scientific and management community are in an excellent position to develop this, the following suggestions might be considered:

(i) The use of eelgrass biomass and cover as the indicators of choice, following the comments of Herman et al (2017) and this document—supporting elements are of course desirable;

(ii) Research into the partitioning of different contributing factors to light attenuation, particularly the role of CDOM, and understanding if/how it can be related to both nutrient loading (direct) and phytoplankton exudates (indirect);

(iii) Development of an integrated multi-model framework combining (a) the land component using hydrological and nutrient loading models such as SWAT or E-HYPE; (b) hydrodynamic models from DHI, which are already in use, and water quality/ecological models able to represent eelgrass growth, taking into account the issue of CDOM discussed above; (c) models considering other aspects such as mussel dredging effort and substrate changes. Nobre et al (2010) provide an example of this kind of integrated framework;

(iv) Consideration of potential complementary approaches for limiting phytoplankton, for instance by means of top-down control by filter-feeders, such as proposed by Petersen et al

(2016) for different Danish fjords. This could and probably should be done selectively for marine areas and fjords where it would be most effective. In the eastern US, top-down control from bivalve aquaculture has been proposed as part of a nutrient credit trading system within an integrated catchment management framework (Ferreira & Bricker, 2019);

(v) Stakeholder review and agreement on a comprehensive set of aims, measures, and expected achievements, including compliance with relevant legislation but extending well beyond that to meet agreed sustainability targets.

From a compliance perspective, it is important to satisfy the requirements of the WFD, but more importantly, from the perspective of Danish society, it is fundamental that a credible long-term policy be put in place to ensure successful restoration of eelgrass communities and the key ecosystem services they provide.

Acknowledgements

The author is indebted to D. Krause-Jensen and J. Carstensen, both from Aarhus University, and to A. Newton, for enlightening discussions during the course of this work. I wish to thank R. Marteleira for an analysis of historical nitrogen loads and D. Gomes for the satellite images of chlorophyll in the Danish EEZ, and I am grateful to the colleagues who were part of this transdisciplinary review team.

References

Bricker, S.B., J.G. Ferreira, T. Simas, 2003. An Integrated Methodology for Assessment of Estuarine Trophic Status. Ecological Modelling, 169(1), 39-60.

Carstensen, J., Conley, D.J., Bonsdorff, E., Gustafsson, B.G., Hietanen, S., Janas, U., Jilbert, T., Maximov, A., Norkko, A., Norkko, J., Reed, D.C., Slomp, C.P., Timmermann, K., Voss, M., 2014. Hypoxia in the Baltic Sea: Biogeochemical Cycles, Benthic Fauna, and Management. Ambio 43:26–36. DOI 10.1007/s13280-013-0474-7

Conley, D. J., Carstensen, J., Ærtebjerg, G., Christensen, P. B., Dalsgaard, T., Hansen, J. L. S., & Josefson, A. B., 2007. Long-term changes and impacts of hypoxia in Danish coastal waters. Ecological Applications, 17, https://doi.org/10.1890/05-0766.1

Dennison, W. C., 1987. Effects of light on seagrass photosynthesis, growth and depth distribution. Aquatic Botany, 27(1), 15–26. https:// doi.org/10.1016/0304-3770(87)90083-0

DHI, 2015. Modeller for Danske Fjorde og Kystnære Havområder – del 2. Mekanistiske modeller og metode til bestemmelse af indsatsbehov. DHI, 56 pp.

Dolmer, P., Frandsen, R.P., 2002. Evaluation of the Danish mussel fishery: Suggestions for an ecosystem management approach. Helgol. Mar. Res. 56:13–20 DOI 10.1007/s10152-001-0095-6

Duarte, C.M., Conley, D.J., Carstensen, J., Sánchez-Camacho, M., 2008. Return to Neverland: Shifting Baselines Affect Eutrophication Restoration Targets. Estuaries and Coasts. DOI 10.1007/s12237-008-9111-2

Duarte, C. M., 1991. Seagrass depth limits. Aquatic Botany, 40(4), 363–377. https://doi.org/10.1016/0304-3770(91)90081-F

EMODnet, 2018. EMODNET High Resolution Seabed Mapping (HRSM). National Coastlines and Baselines – data set collection for European Countries. 32 pp.

Erftemeijer, P.L.A., Robin Lewis III, R.R., 2006. Environmental impacts of dredging on seagrasses: A review. Mar. Pol. Bull. 52 (12), 1553-1572.

Ferreira, J.G., Bricker, S.B., 2019. Assessment of Nutrient Trading Services from Bivalve Farming. In: Goods and Services of Marine Bivalves. Aad C. Smaal, Joao G. Ferreira, Jon Grant, Jens K. Petersen, Øivind Strand, Editors. p. 551-584.

Hansen J.W. & Høgslund S. (red.) 2019. Marine områder 2018. NOVANA. Aarhus Universitet, DCE – Nationalt Center for Miljø og Energi, 156 s. - Videnskabelig rapport fra DCE nr. 355 http://dce2.au.dk/pub/SR355.pdf

Hansen J.W. & Høgslund S. (red.) 2021. Marine områder 2019. NOVANA. Aarhus Universitet, DCE – Nationalt Center for Miljø og Energi, *In Prep*.

Henriksen, P., Josefson, A., Würgler Hansen, J., Krause-Jensen, D., Dahl, K., Dromph, K., 2014. Danish contribution to the EU Water Framework Directive intercalibration phase 2. Technical Report from DCE – Danish Centre for Environment and Energy, No. 37.

Herman, P., Newton, A., Schernewski, G., Gustafsson, B., Malve, O., 2017. International evaluation of the Danish marine models. Implement Consulting Group, 49 pp.

Kinsey, J.D., Gabrielle Corradino, G., Ziervogel, K., Schnetzer, A., Osburn, C.L., 2018. Formation of Chromophoric Dissolved Organic Matter by Bacterial Degradation of Phytoplankton-Derived Aggregates. Frontiers in Marine Science. doi: 10.3389/fmars.2017.00430

Kirk, J.T., 1994. Light and photosynthesis in aquatic ecosystems , 2nd Ed. Cambridge: Cambridge University Press, 509 pp.

Kowalczuk, P., Olszewski, J., Darecki, M., Kaczmarek, S., 2005. Empirical relationship between coloured dissolved organic matter (CDOM) absorption and apparent optical properties in Baltic Sea waters. International Joumal of Remote Sensing 26(2),345-370.

Kowalczuk, P., Stedmon, C.A., Markager, S., 2006. Modeling absorption by CDOM in the Baltic Sea from season, salinity and chlorophyll. Marine Chemistry 101, 1–11.

Krause-Jensen, D., Duarte. C.M., Sand-Jensen, K., Carstensen, J., 2021. Century-long records reveal shifting challenges to seagrass recovery. Global change Biology 27(3). DOI: 10.1111/gcb.15440

Muehlstein, L.K., 1989. Perspectives on the wasting disease of eelgrass *Zostera marina*. Dis. Aquat. Org. 7: 211-221.

Nejrup, L. B., Pedersen, M. F., 2008. Effects of salinity and water temperature on the ecological performance of *Zostera marina*. Aquatic Botany, 88(3), 239–246.

Nielsen, S.L., Sand-Jensen, K., Borum, J., Geertz-Hansen, O., 2002a. Depth Colonization of Eelgrass (Zostera marina) and Macroalgae as Determined by Water Transparency in Danish Coastal Waters. Estuaries, 25(5), 1025-1032.

Nielsen, S.L., Sand-Jensen, K., Borum, J., Geertz-Hansen, O., 2002b. Phytoplankton, Nutrients, and Transparency in Danish Coastal Waters. Estuaries, 25(5), 930-937.

Nobre, A. M., Ferreira, J. G., Nunes, J. P., Yan, X., Bricker, S., Corner, R., Groom, S., Gu, H., Hawkins, A., Hutson, R., Lan, D., Lencart e Silva, J. D., Pascoe, P., Telfer, T., Zhang, X., Zhu, M., 2010. Assessment of coastal management options by means of multilayered ecosystem models. Estuarine, Coastal and Shelf Science, 87, 43-62.

Oenema, O., 2021. Reference conditions in Danish coastal waters under the European Union Water Framework Directive. An integrated legal and scientific appraisal. Annex 2 - Estimations of background N loads to rivers and coastal waters in Denmark for defining reference conditions – a review. 18 pp.

Ostenfeld, C., 1908. Aalegræsset (*Zostera marina's*) Vækstforhold og Udbredelse i vore Farvande. In C. G. J. Petersen (Ed.), Beretning til Landbrugsministeriet fra den danske biologiske station (pp. XVI: 1–61). Centraltrykkeriet.

Pedersen, T.M., Sand-Jensen, K., Markager, S., SNielsen, S.L., 2014. Optical changes in a eutrophic estuary during reduced nutrient loadings. Estuaries and Coasts 37: 880–892. doi:10.1007/s12237-013-9732-y.

Petersen, J. C. G., 1914. Om bændeltangens (*Zostera marina*) aars-produktion i de danske farvande. In H. Jungersen, & E. Warming (Eds.), Mindeskrift i anledning af hundredåret for Japetus Steenstrups fødsel (pp. 1–20). Bianco Lunos Bogtrykkeri.

Petersen, J.K., Saurel, C., Nielsen, P., Timmermans, K., 2016. The use of shellfish for eutrophication control. Aquacult Int 24, 857–878. doi.org:10.1007/s10499-015-9953-0

Savchuk, O.P., F. Wulff, S. Hille, C. Humborg, and F. Pollehne. 2008. The Baltic Sea a century ago—a reconstruction from model simulations, verified by observations. J. Mar Sys. 74: 485–494.

Short, F.T., Muehlstein, L.K., Porter, D., 1987. Eelgrass wasting disease: cause and recurrence of a marine epidemic. Biol. Bull. 173: 557-562.

Staehr, P. A., Göke, C., Holbach, A. M., Krause-Jensen, D., Timmermann, K., Upadhyay, S., & Ørberg, S. B., 2019. Habitat model of eelgrass in Danish coastal waters: Development, validation and management perspectives. Frontiers in Marine Science, 6(APR), 1–18. https://doi. org/10.3389/fmars.2019.00175

Timmermann, K., 2020. Reference inputs of nitrogen for Water Plan 3. Note from DCE - National Centre for the Environment and Energy. 6th January, 2020.

Thornton, D.C.O., 2014. Dissolved organic matter (DOM) release by phytoplankton in the contemporary and future ocean, European Journal of Phycology, 49:1, 20-46, DOI: 10.1080/09670262.2013.875596

Van Calster, G.A., Garnett, K., 2001. Reference conditions in Danish coastal waters under the European Union Water Framework Directive. An integrated legal and scientific appraisal. Annex 1 – Legal Opinion. 11 pp.

Wilkinson, A.D., Collier, C.J., Flores, F., Langlois, L., Ralph, P.J., Negri, A.P., 2017. Combined effects of temperature and the herbicide diuron on Photosystem II activity of the tropical seagrass *Halophila ovalis*. Nature Scientific Reports, 7:45404. DOI: 10.1038/srep45404