Water Resources Management (2005) 19: 63–75

Eelgrass as a Bioindicator Under the European Water Framework Directive

DORTE KRAUSE-JENSEN,[1,*] TINA MARIA GREVE,[1] and KURT NIELSEN[2]
[1]*Department of Marine Ecology, National Environmental Research Institute, Vejlsøvej 25, DK-8600 Silkeborg, Denmark;* [2]*Department of Freshwater Ecology, National Environmental Research Institute, Vejlsøvej 25, DK-8600 Silkeborg, Denmark*
*(*author for correspondence, e-mail: dkj@dmu.dk; Fax: +45-8920-1414)*

(Received: 7 November 2002; in final form: 3 May 2004)

Abstract. Eelgrass is the most widespread plant in temperate coastal waters. It is regarded as a useful indicator of water quality because water clarity regulates its extension towards deeper waters, i.e. the depth limit. This study analyses the use of eelgrass depth limits as a bioindicator under the Water Framework Directive (WFD). The WFD demands that ecological status is classified by relating the actual level of bioindicators to a so-called 'reference level', reflecting a situation of limited anthropogenic influence. The directive further demands that reference levels are defined for 'water body types' with similar hydromorphological characteristics, and that the classification thereby becomes 'type-specific'.

A large historic data set on depth limits of eelgrass around 1900 was used to characterise reference levels, and a large data set from the Danish National Monitoring and Assessment Programme to characterise actual depth limits. Data represented a wide range of Danish coastal water bodies that were grouped into 10 water body types based on differences in salinity and water depth.

The analyses clearly illustrate that the definition of ecological status classes markedly influence the assessment of ecological status according to the WFD. Moreover, the study demonstrates that the use of type-specific classification implies a risk of misinterpreting ecological status. Classification problems were pronounced in spite of a unique data material on reference conditions, and the problems are likely to be even greater in cases where reference conditions are less well defined. A more robust classification was obtained by using reference levels for individual sites in a site-specific classification.

In conclusion, when classifying water quality on the basis of eelgrass depth limits, site-specific reference levels are recommended if such data are available. If more general information on reference levels is used, local conditions known to affect depth limits must be taken into account.

Key words: bioindicator, classification, depth limit, eelgrass, monitoring, reference conditions, Water Framework Directive, water quality, *Zostera marina*

1. Introduction

Eelgrass (*Zostera marina*) is the most widely distributed marine angiosperm in the Northern Hemisphere (den Hartog, 1970). The depth limit of eelgrass, defined as the deepest water depth where eelgrass grows, is generally regarded as a useful bioindicator, mainly because depth limits respond predictably to eutrophication, being largely regulated by light availability. The clearer the waters, the deeper eelgrass and other seagrasses grow (Duarte, 1991; Nielsen *et al.*, 2002a). Increased concentrations of total nitrogen stimulate phytoplankton growth, thereby reducing water clarity, and eventually reduce depth limits (Nielsen *et al.*, 2002a, 2002b). Eelgrass meadows are highly productive (Duarte and Chiscano, 1999), they provide important habitats for benthic invertebrates and fish fry (e.g. Boström and Bonsdorff, 2000) and tend to reduce coastal erosion through stabilisation of the sediment (Rasmussen, 1973; Ward *et al.*, 1984). These qualities and the widespread occurrence of the plant add to the usefulness of eelgrass as a bioindicator. Moreover, eelgrass depth limits are easy to measure and a large historic data material allows assessment of reference levels of eelgrass depth limits in Danish coastal waters in situations of high water quality (Ostenfeld, 1908).

The depth limit of eelgrass is therefore likely to be a useful indicator under the European Water Framework Directive (WFD, European Union, 2000) as well. The directive aims to achieve 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 directive thereby challenges us to identify useful biological indicators that respond predictably to human impact and can be quantified with sufficient precision.

The WFD demands that ecological status is quantified and expressed as a so-called 'Ecological Quality Ratio' (EQR), defined as the ratio between the actual level of a biological indicator and the reference level of the indicator (Table I). The reference level or reference condition is defined as the level of the indicator in an 'undisturbed' ecosystem with 'no or only very minor' anthropogenic influence. Ideally, reference levels should be defined based on information on existing, undisturbed water bodies, but widespread eutrophication is typically a hindrance to this approach and makes it necessary to define reference levels based on historical data, modelling or expert judgement instead. According to the WFD reference levels must be defined for so-called water body types, and the classification thereby becomes 'type-specific.'

Table I. Classification according to the Water Framework Directive. Ecological quality ratio (EQR) = Observed value of indicator/Reference level of indicator

Value of EQR	Deviation from reference	Ecological status
$\rightarrow 1$	None/minimal	High
	Slight	Good
	Moderate	Moderate
	Major	Poor
$\rightarrow 0$	Severe	Bad

Table II. Definitions of high, good and moderate ecological status based on macroalgae and angiosperms in coastal waters (European Union, 2000)

Status	Definition
High status	All disturbance-sensitive macroalgal and angiosperm taxa associated with undisturbed conditions are present. The levels of macroalgal cover and angiosperm abundance are consistent with undisturbed conditions.
Good status	Most disturbance-sensitive macroalgal and angiosperm taxa associated with undisturbed conditions are present. The levels of macroalgal cover and angiosperm abundance show slight signs of disturbance.
Moderate status	A moderate number of disturbance-sensitive macroalgal and angiosperm taxa associated with undisturbed conditions are present. Macroalgal cover and angiosperm abundance is moderately disturbed and may be such as to result in an undesirable disturbance to the balance of organisms present in the water body.

Depending on the degree of deviation from reference levels, the WFD defines five 'ecological status classes': 'high status,' 'good status,' 'moderate status,' 'poor status' and 'bad status' (Table I). 'High status' is obtained when the biological indicators meet reference levels and have EQR values close to 1. 'Good status' is achieved when the biological indicators differ only slightly from their reference levels. At moderate, poor and bad status the biological indicators show moderate, major and severe deviation from reference levels, respectively. As the WFD requires that all European surface waters must reach at least 'good status,' definition of the boundary value between good and moderate status is of utmost importance. Regarding coastal benthic flora, the WFD defines ecological status based on species composition, cover and abundance of seagrasses and macroalgae (Table II).

This study aims to analyse how the depth limit of eelgrass in Danish coastal waters can be used as a bioindicator of water quality under the WFD. More specifically, the study aims to (1) assess reference levels for eelgrass depth limits based on historical data, (2) define ecological status classes for Danish coastal waters based on eelgrass depth limits and (3) evaluate possibilities and limitations of using this indicator to assess water quality as defined by the WFD.

2. Materials and Methods

2.1. WATER BODIES

In order to implement the WFD, 'bodies of surface water' which can be e.g. specific estuaries or coastal areas, must be divided into 'water body types' based on similarities in physico-chemical and hydromorphological characteristic. The WFD requires that water body types are defined based on salinity and mean depth as a

Table III. Classification of Danish coastal waters into water body types based on mean water depth and salinity. The last column states the total number of coastal waters of each type that was included in the present analysis

Type	Type characteristics	Mean depth (m)	Salinity (psu)	No. of observations (1901)	No. of observations (1989–2000)
A1	Estuary	0–3	7–18	27	194
A2	Estuary	0–3	>18	2	71
B1	Estuary	3–8	7–18	2	128
B2	Estuary	3–8	>18	17	301
C1	Estuary	>8	7–18	26	462
C2	Estuary	>8	>18	15	314
D1	Threshold-estuary		7–18	5	166
D2	Threshold-estuary		>18		19
E	Sluice-regulated estuary				7
F	Inner estuary				263
Total observations				95	1925

minimum (EU 2000, Annex II). Earlier studies have already suggested a division of Danish water bodies into 10 estuarine water body types based on differences in salinity and mean water depth and the present investigation relies on this typology (Table III, Nielsen *et al.*, 2001). The water body types include three depth categories: shallow (0–3 m), intermediate water depth (3–8 m) and deep (>8 m). For each water depth category, there are two salinity groups: low salinity (7–18 psu) or high salinity (>18 psu). In addition, the water body types include ‘threshold estuaries’ of low and high salinity, ‘sluice-regulated estuaries’ and ‘inner estuaries’.

2.2. EELGRASS DATA

The analysis encompasses historical and actual data on eelgrass depth limits from a wide range of coastal Danish water bodies. Historical data used to assess depth limits under reference levels were based on the investigation by Ostenfeld and Petersen in 1901 (Ostenfeld, 1908). This investigation is the oldest nationwide eelgrass survey in Denmark and constitutes an excellent basis for defining eelgrass depth limits under ‘undisturbed conditions’. Nutrient levels were generally low in 1900 compared to recent years (Conley, 2000), though some inner estuaries may have been affected by nutrients even at that time. As the historical survey assessed eelgrass depth limits on the basis of samplings by grab, only relatively dense eelgrass cover was identified and data thus represent a conservative estimate of the absolute depth limit. Small eelgrass patches and individual seedlings may have occurred in deeper waters. The investigation included observations of eelgrass depth limits in 95 sites and these data represented seven of the coastal water bodies (Table III).

Data on actual depth limits of eelgrass were obtained through the Danish National Monitoring and Assessment Programme and included a total of 1925 observations representing 10 coastal water bodies over the period 1989–2000 (Table III). The Danish counties performed the investigations according to common guidelines (Krause-Jensen *et al.*, 2001). Divers identified actual depth limits as the depth of the deepest occurring eelgrass shoots and the actual data thereby represent maximum estimates of depth limits. Actual data from sites where eelgrass was found down to the maximum depth of the area were omitted from the analysis as these meadows were unable to improve their distribution range in response to improved water clarity.

Simple descriptive statistics, i.e. means and quantiles, were calculated for each water body type and used to characterise historic and actual depth limits of eelgrass. Comparisons between actual and reference depth limits were made for the seven water body types represented in both investigations.

3. Results and Discussion

3.1. HISTORICAL AND ACTUAL DEPTH LIMITS OF EELGRASS

Historical depth limits of eelgrass showed an average of 4.3–8.5 m in the various water body types, while actual eelgrass depth limits showed an average of 1–5.4 m (Figure 1). Eelgrass thus generally grew deeper 100 years ago than today. This pattern appeared in spite of the fact that the historical depth limits represented conservative estimates while actual depth limits were maximum estimates. The data therefore leave no doubt that depth limits of eelgrass have declined markedly since 1900. This trend in the Danish eelgrass meadows has also been documented in other studies (Boström *et al.*, 2003; Nielsen *et al.*, 2003). Both historic and present-day data showed a tendency towards eelgrass growing deepest in deep water body types (C1, C2 and D1). This picture probably reflects that deep areas are often situated along open coasts where water renewal is frequent and the water quality therefore inherently good as compared to more shallow and enclosed water bodies (Greve and Krause-Jensen, in press).

It is important to note that each water body type represented a wide range in eelgrass depth limits even under reference conditions (Figure 1). This characteristic was most pronounced in water bodies of type A1, but also appeared in water bodies of type B2, C1 and C2. The wide range in reference conditions is a problem because the WFD operates with just one reference value for each water body type and this value should therefore be well-defined and relevant for all water bodies belonging to a given water body type.

If water quality were the only factor regulating depth limits, a small range in reference depth limits would have been expected, as nutrient concentrations were generally low a century ago. The wide range in reference conditions indicates that variables other than water quality contribute to the regulation of depth limits. Though each water body type represents a limited spectrum of water depths and

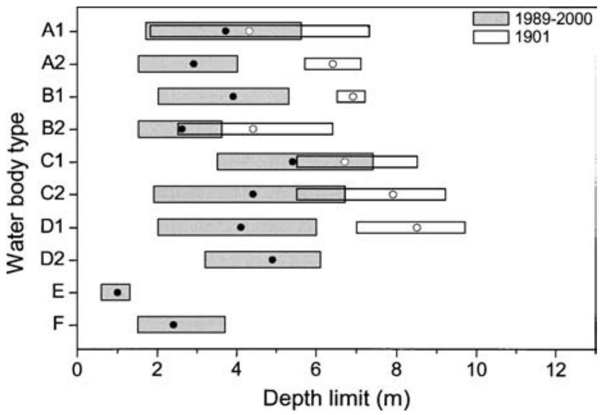

Figure 1. Depth limits of eelgrass in 10 different water body types. Open circles represent means and open bars the range (10–90 percentiles) of reference depth limits. Reference data represent conservative estimates of depth limits in 1901 (Ostenfeld, 1908) and include a total of 95 observations in water bodies distributed with 2–27 observations within each water body type. Solid circles represent means, and solid bars the range (10–90 percentiles) of maximum actual depth limits based on investigations under the National Danish Monitoring Programme in 1989–2000. The actual data include a total of 1925 estimates of depth limits distributed with 7–462 observations within each water body type.

salinities, they may also differ in e.g. exposure levels, residence time and sediment composition which may also affect depth limits. A more sophisticated definition of water body types that includes these variables in addition to depth and salinity would define the water body types more specifically and thereby reduce the range in reference conditions by increasing the number of types. But as the directive intends to use relatively few water body types, this may not be a feasible approach. The inaccuracy connected with assessment of depth limits based on sampling with grab may also have contributed to increasing the range in historic depth limits.

3.2. DEFINITION OF ECOLOGICAL STATUS CLASSES

The average reference depth limit within each water body type was assumed to correspond to the maximum EQR level (1). The boundaries of each status class were subsequently defined according to two sets of definitions—one non-restrictive and one restrictive—to illustrate how different boundaries affect the classification. The non-restrictive definition used an EQR of 0.90 to distinguish between high and good ecological status, and an EQR of 0.75 to distinguish between good and moderate ecological status (Example 1, Figure 2). In the restrictive definition, an EQR of 0.95 defined the boundary between high and good status and an EQR of 0.85 defined the boundary between good and moderate status (Example 2, Figure 2).

The depth limits defining the various ecological status classes obviously varied markedly depending on the applied criteria, and the definition of ecological status classes thus greatly influenced the assessment of ecological status according to the

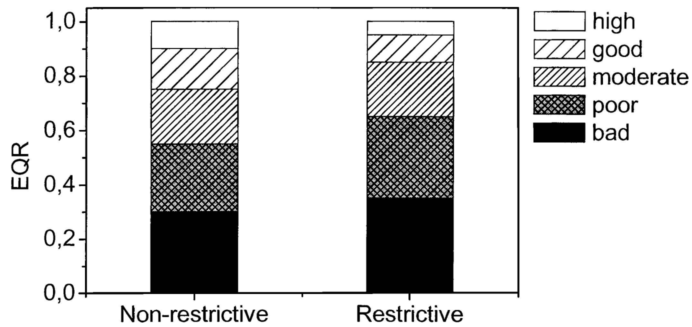

Figure 2. Examples of how ecological status classes can be defined according to the European Water Framework Directive. The ecological status classes are defined based on ecological quality ratios (EQR) representing the deviation of actual levels of a quality element from reference conditions. The left column represents non-restrictive criteria while the right column represents more restrictive criteria.

WFD (Table IV). When the non-restrictive definition of status classes was used, a depth limit of 5.5 m in a coastal water body of type B1 resulted in good ecological status while a similar depth limit in a coastal water body of type C2 resulted in only moderate status (Table IV). When the restrictive definition of status classes was used, a depth limit of 5.5 m resulted in only moderate status in areas of both type B1 and C2 (Table IV).

The depth limits required to fulfil the demands of good ecological status varied from 3.2 m in water body type A1 to 6.4 m in water body type D1 when the non-restrictive criteria were used. The restrictive criteria caused the depth limits required for good status to vary from 3.6 m in type A1 to 7.3 m in type D1.

3.3. ASSESSING ECOLOGICAL STATUS

The ecological status of each water body was assessed by comparing actual depth limits with those defining the status classes, and in this context attention must be drawn to two facts.

Firstly, the use of type-specific reference conditions caused classification errors. In the water body types A1 and A2, high and good status thus required depth limits deeper than the average water depth defined for these water body types (e.g. type A1: 0–3 m). Consequently, some areas could not obtain the environmental status required by the WFD because of their shallow depth alone. These areas and other areas exhibiting low reference depth limits achieved a false-positive classification. The opposite classification error, i.e. a false-negative classification, occurred in areas with high reference depth limits. These problems arose because the water body types were broadly defined and the type-specific reference conditions were therefore not relevant for all water bodies. It is alarming that these classification problems should arise in an example such as the present one, which represents a unique data material for assessing both reference levels and actual values of a

Table IV. Type-specific classes of ecological status based on eelgrass depth limits (m). The ecological status classes are defined for various water body types based on reference depth limits of eelgrass. The average reference depth limit of each water body is assumed to correspond to an Ecological Quality Ratio (EQR) of 1. Status classes are defined based on non-restrictive criteria (upper panel) and more restrictive criteria (lower panel) as defined in Figure 2

Environmental status: EQR:	High >0.90	Good 0.90–0.75	Moderate 0.75–0.55	Poor 0.55–0.30	Bad 0.30–0
Non-restrictive criteria					
A1	>3.8	3.8–3.2	3.2–2.3	2.3–1.3	1.3–0
A2	>5.8	5.8–4.8	4.8–3.5	3.5–1.9	1.9–0
B1	>6.2	6.2–5.1	5.1–3.8	3.8–2.1	2.1–0
B2	>3.9	3.9–3.3	3.3–2.4	2.4–1.3	1.3–0
C1	>6.0	6.0–5.0	5.0–3.7	3.7–2.0	2–0
C2	>7.1	7.1–6.0	6.0–4.4	4.4–2.4	2.4–0
D1	>7.7	7.7–6.4	6.4–4.7	4.7–2.6	2.6–0
Restrictive criteria					
	>0.95	0.95–0.85	0.85–0.65	0.65–0.35	0.35-0
A1	>4.0	4.0–3.6	3.6–2.8	2.8–1.5	1.5–0
A2	>6.1	6.1–5.4	5.4–4.2	4.2–2.2	2.2–0
B1	>6.5	6.5–5.8	5.8–4.5	4.5–2.4	2.4–0
B2	>4.1	4.1–3.7	3.7–2.8	2.8–1.5	1.5–0
C1	>6.3	6.3–5.7	5.7–4.3	4.3–2.3	2.3–0
C2	>7.5	7.5–6.8	6.8–5.2	5.2–2.8	2.8–0
D1	>8.1	8.1–7.3	7.3–5.6	5.6–3.0	3.0–0

bioindicator. Eelgrass depth limits may not be unique in exhibiting large ranges in reference conditions, and even larger problems are likely to arise in cases where less precise information on reference levels is available.

Secondly, the data clearly illustrated that the definition of status class boundaries has marked ecological and economical consequences. When the non-restrictive criteria were applied, more areas obviously fulfilled the requirements of high or good environmental quality, as opposed to the restrictive criteria (Figure 3). This effect was pronounced for the water body type C1 of which only half as many areas reached the requirements of high or good status when the restrictive criteria were used.

Due to the conservative estimate of reference conditions, our data tended to overestimate the actual ecological status. The finding that some areas reach high ecological status (Figure 3) is thus unrealistic due to the high present levels of eutrophication (Conley *et al*., 2000; Richardson and Heilmann, 1995). Our example therefore serves to illustrate aspects of the use of the WFD in practice rather than to assess the actual ecological status of Danish coastal waters.

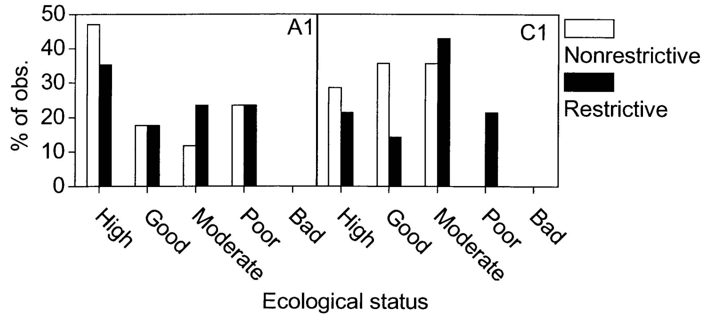

Figure 3. The bars represent the fraction of coastal water bodies of water body types A1 and C1 displaying a high, good, moderate, poor and bad environmental quality. Empty bars represent assessments based on non-restrictive criteria while filled bars represent assessments based on restrictive criteria.

3.4. TYPE-SPECIFIC VERSUS SITE-SPECIFIC CLASSIFICATION

Using reference levels for individual sites in a site-specific classification was found to reduce misinterpretations of ecological status. Examples from six selected coastal water bodies distributed evenly between water body types A1 and C1 illustrate that site-specific status classes can differ markedly from type-specific ones (Table V). For the three coastal water bodies belonging to type A1, the depth limit separating moderate and good environmental quality ranged from 1.9 to 6.2 m when site-specific classification was used (Figure 4), while a common value of 3.6 m was found when type-specific classification was used (Table IV, restrictive criteria). The actual depth limits of eelgrass in these areas ranged from 2.1 m to 6.6 m, and site-specific classification defined the environmental quality of the water bodies Bredningen

Table V. Assessment of ecological status based on type-specific (Table IV) and site-specific reference levels (Figure 4) using the restrictive definition of status classes. Examples from 6 coastal water bodies distributed evenly between water body types A1 and C1. Data illustrate general differences between the use of type- and site-specific reference levels but tend to overestimate the actual ecological status, because estimates of reference levels are based on conservative estimates of depth limits while present depth limits represent maximum estimates

Type	Water body	Depth limit 1900	Depth limit 1990s	Ecological status type-specific	Ecological status site-specific
A1	1. Bredningen	2.1	2.1	Poor	High
	2. Odense Fjord	5.5	4.1	Good	Moderate
	3. Fakse Bugt	7.3	6.6	High	Good
C1	1. Sejerø Bugt	6.4	4.6	Moderate	Moderate
	2. Nivå Bugt	7.3	6.0	Good	Moderate
	3. Amager Strand	8.2	7.3	High	Good

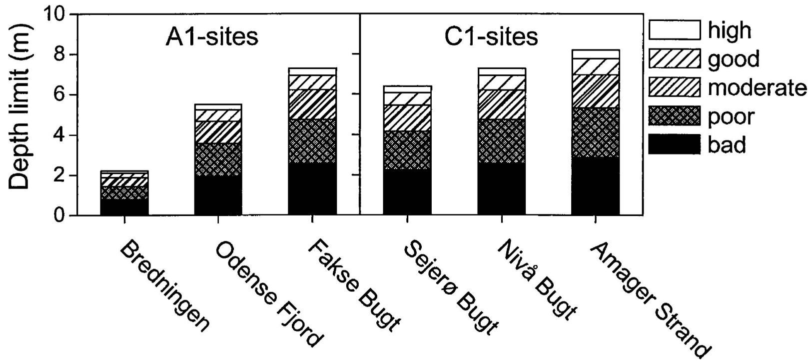

Figure 4. Examples of site-specific classes of ecological status defined for six coastal water bodies distributed evenly between water body types A1 and C1. The ecological status classes are defined based on reference depth limits of eelgrass using the restrictive criteria illustrated in Figure 3.

and Fakse Bugt as acceptable, while the quality in Odense Fjord was defined as unacceptable. By contrast, type-specific classification caused the quality of Odense Fjord and Fakse Bugt to be defined as acceptable, while that of Bredningen was unacceptable. Bredningen showed the largest divergence between type- and site-specific classification, the conclusion being poor status according to type-specific classification as opposed to high status according to site-specific classification. None of these findings may be indicative of the actual state of this water body because the very low reference depth limit of 2.2 m may be attributed to other limitations than light, e.g. local morphometry or sediment conditions that do not allow deep eelgrass populations.

In general, the depth limit of eelgrass is not a useful bioindicator in very shallow areas where factors other than light play the major regulating role, and the assessment of ecological status of such areas should instead be based on other bioindicators such as the abundance or species composition of shallow-water flora or fauna. The inclusion of more bioindicators in the assessment of ecological status would also generally reduce the risk of classification errors. The use of site-specific reference conditions facilitates the adjustment of monitoring activities according to local conditions and specification of the most useful bioindicators for a given area. Site-specific classification would therefore provide more confidence in the assessment of status classes while at the same time respecting natural variability.

In cases where site-specific reference conditions are not available, it is necessary to use more general information on reference levels in order to implement the WFD. One solution would be to use more integrated historic information, if available, on light-regulated depth limits. For example, Ostenfeld (1908) stated that eelgrass depth limits in western Kattegat, Denmark, showed a maximum of 11 m while those of the Danish estuaries were generally around 5 m in 1900, and this type of general information is useful when site-specific information is lacking.

Another solution would be to use empirical modelling e.g. of relations between nutrient concentrations and depth limits or between water clarity and depth limits to derive average reference depth limits from information on reference nutrient concentrations or water clarity. This approach is possible for Danish coastal waters using the models of Nielsen *et al.* (2002). Reference nutrient concentrations in the range 650–800 μg N L^{-1} have been reported for inner estuaries while levels of 200–300 μg N L^{-1} have been reported for outer parts of the estuaries (Conley *et al.*, 2000; Nielsen *et al.*, 2003). The model predicts corresponding reference depth limits of 2.8–3.2 m in inner estuaries and 5.7–7.7 m in outer estuaries. Such general information on reference depth limits could then be graduated according to location. For example, reference depth limits are relevant only for areas of sandy or silty bottom where eelgrass can grow, reference depth limits of shallow areas should match the maximum water depth of the area and reference depth limits should depend on the location of sites along the estuarine gradients.

3.5. MORE QUANTITATIVE INFORMATION ON BIOINDICATORS IS NEEDED

The WFD has recently inspired much research into the development of good biological indicators, but until now only few quantitative studies on the use of benthic vegetation as a bioindicator under the WFD have been published. An 'ecological evaluation index' relating the abundance of opportunistic species to the abundance of late-successional species (Orfanidis *et al.*, 2001, 2003; Panayotidis *et al.*, 2004) and the depth limits of individual macrophyte species (Domin *et al.*, 2004; Kautsky *et al.*, 2004) have recently been suggested as indicators of water quality under the WFD. It has also recently been demonstrated how modelling and historical data can be used to assess reference levels of benthic vegetation and other biological indicators in specific water bodies (Domin *et al.*, 2003; Nielsen *et al.*, 2003). Earlier quantitative studies relating benthic vegetation indicators to water quality are also relevant in connection with the WFD, but proper monitoring according to the WFD demands more information in this field.

Moreover, while most of the existing studies have documented how seagrass meadows and perennial algal vegetation deteriorate along with increased eutrophication, there are only few examples demonstrating how and at what rate the vegetation responds to improved water quality. The eutrophication process may not be directly reversible, as negative feed-back effects of eutrophication may hinder or delay the return to the former state even after nutrient concentrations have been reduced (e.g. Duarte, 1995; Scheffer *et al.*, 2001). Negative feed-back effects of eelgrass loss may involve alteration in sediment conditions, which may render some areas unsuitable for seagrass growth, increased resuspension of sediments that keep the waters turbid, and the fact that recolonisation can be a lengthy process, especially if mother populations are distant. For example, a general reduction in nutrient concentrations in coastal Danish waters over the last decade has not yet led to increased depth penetration of eelgrass. On the contrary, depth limits have continued to decrease over this period (Ærtebjerg *et al.*, 2003). Return to high or good environmental status may therefore not always be achieved immediately upon reductions in nutrient load.

4. Conclusion

The analyses of historic and actual eelgrass depth limits identified some general conditions that affect the assessment of environmental quality using the WFD. The study clearly demonstrated that the boundaries between quality classes markedly affect the assessment of environmental quality and therefore need to be carefully defined. Moreover, the use of broadly defined water body types and type-specific reference conditions implied a risk of misinterpreting the ecological status. Classification problems were pronounced in spite of a unique data material on reference conditions being available, and the problem is likely to be even greater in cases where reference conditions are less well defined. Site-specific classification seems to be a robust alternative to type-specific classification, which may be considered for the implementation of the WFD. When information on site-specific reference conditions is lacking, more general information on reference conditions can be used but must be adjusted to local conditions. The study emphasises that additional quantitative studies of reference conditions and of the response of bioindicators to water quality are needed in order to optimise future monitoring strategies.

Acknowledgements

Erik Bonsdorff and Jesper Andersen are thanked for constructive criticism to earlier versions of this manuscript and Anna Haxen for linguistic corrections. Dorte Krause-Jensen gained financial support from EU (#EVK3-CT-2001-00065 'CHARM'), Tina Maria Greve from the Danish Research Academy, and Kurt Nielsen from the Danish EPA.

References

Ærtebjerg, G., Andersen, J. and Hansen, O. S., 2003, *Nutrients and Eutrophication in Danish Marine Waters—A Challenge for science and Management*, Ministry of the Environment, Copenhagen, pp. 68–75.

Boström, C. and Bonsdorff, E., 2000, 'Zoobenthic community establishment and habitat complexity—the importance of seagrass shoot density, morphology and physical disturbance for faunal recruitment', *Mar. Ecol. Prog. Ser.* **205**, 123–138.

Boström, C., Baden, S. P. and Krause-Jensen, D., 2003, 'The seagrasses of Scandinavia and the Baltic Sea', in E. P. Green and F. T. Short (eds), *World Atlas of Seagrasses*, California University Press, California, p. 310.

Conley, D. J., 2000, 'Biogeochemical nutrient cycles and nutrient management strategies', *Hydrobiologia* **410**, 87–96.

Conley, D. J., Kaas, H., Møhlenberg, F., Rasmussen, B. and Windolf, J., 2000, 'Characteristics of Danish estuaries', *Estuaries* **23**, 820–837.

den Hartog, C., 1970, *The Seagrasses of the World*, North Holland Publishing Company, Amsterdam.
Domin, A., Schubert, H., Krause, J. C. and Schiewer, U., 2004, 'Modelling of pristine depth limits for macrophyte growth in the southern Baltic Sea', *Hydrobiologia* **514**, 29–39.
Duarte, C. M., 1991, 'Seagrass depth limits', *Aquat. Bot.* **40,** 363–377.
Duarte, C. M., 1995, 'Submerged aquatic vegetation in relation to different nutrient regimes', *Ophelia* **41**, 87–112.
Duarte, C. M. and Chiscano, C. L., 1999, 'Seagrass biomass and production: A reassessment', *Aquat. Bot.* **65**, 159–174.
European Union, 2000, *Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water polic, Legislative Acts and other instruments*, ENV221 CODEC 513, European Union.
Greve, T. M. and Krause-Jensen, D., in press, 'Predictive modelling of eelgrass (*Zostera marina* L.) depth limits', *Mar. Biol.*
Kautsky, L., Andersson, C. and Dahlgren, S., 2004, 'Framtagning av nya bedömningsgrundar för kust och hav enlight ramdirektivets krav— Förslag til ekologisk kvalitetskvot (EQR) för den biologiske parametern makrovegetation', Botanical institute, University of Stockholm, *Report 2004-01-12* (in Swedish).
Krause-Jensen, D., Laursen, J. S., Middelboe, A. L., Stjernholm, M. and Manscher, O., 2001, 'Bundvegetation', chap 12 in *NOVA—Teknisk anvisning for marin overvågning*, NERI, Denmark (in Danish), http://www.dmu.dk/1_om_dmu/2_tvaer-funk/3_fdc_mar/programgrundlag/tekanv/Kap12_Bundveg18_04-01_rev2000_6.doc.
Nielsen, K. Sømod, B. and Christiansen, T., 2001, 'Typology and quality elements—Danish marine areas—Water Framework Directive—Phase I,' NERI, Denmark, *Technical Report no. 369* (in Danish).
Nielsen, K., Sømod, B., Ellegaard, C. and Krause-Jensen, D., 2003, 'Assessing reference conditions according to the European Water Framework Directive using modelling and analysis of historical data: An example from Randers Fjord, Denmark,' *Ambio* **32**, 287–294.
Nielsen, S. L., Sand-Jensen, K., Borum, J. and Geertz-Hansen, O., 2002a, 'Depth colonisation of eelgrass (*Zostera marina*) and macroalgae as determined by water transparency in Danish coastal waters', *Estuaries* **5**, 1025–1032.
Nielsen, S. L., Sand-Jensen, K., Borum, J. and Geertz-Hansen, O., 2002b, 'Phytoplankton, nutrients and transparency in Danish coastal waters', *Estuaries* **5**, 930–937.
Orfanidis, S., Panayotidis, P. and Stamatis, N., 2001, 'Ecological evaluation of transitional and coastal waters: A marine benthic macrophytes-based model,' *Mediterranean Mar. Sci.* **2,** 45–65.
Orfanidis, S., Panayotidis, P. and Stamatis, N., 2003, 'An insight into the ecological evaluation index (EEI),' *Ecol. indicators* **3**, 27–33.
Ostenfeld, C. H., 1908, 'Ålegræssets (*Zostera marina's*) udbredelse i vore farvande', in C. G. J. Petersen (ed), *Beretning til Landbrugsministeriet fra den danske biologiske station XVI*, Copenhagen, Centraltrykkeriet.
Panayotidis, P., Montesanto, B. and Orfanidis, S., 2004, 'Use of low-budget monitoring of macroalgae to implement the European Water Framework Directive', *J. Appl. Phycol.* **16**, 49–59.
Richardson, K. and Heilmann, J. P., 1995, 'Primary production in the Kattegat—past and present', *Ophelia* **41**, 317–328.
Scheffer, M., Carpenter, S. R., Foley, J.A., Folke, C. and Walker, B., 2001, 'Catastrophic shifts in ecosystems', *Nature* **413,** 591–596.
Ward, L. G., Kemp, M. and Boynton, W. R., 1984, 'The influence of waves and seagrass communities on suspended particulates in an estuarine embayment', *Mar. Geol.* **59**, 85–103.