Journal of Experimental Marine Biology and Ecology 350 (2007) 46 – 72

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

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Seagrasses and eutrophication

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Received 19 February 2007; received in revised form 1 June 2007; accepted 4 June 2007

Abstract

This review summarizes the historic, correlative field evidence and experimental research that implicate cultural eutrophication as a major cause of seagrass disappearance. We summarize the underlying physiological responses of seagrass species, the potential utility of various parameters as indicators of nutrient enrichment in seagrasses, the relatively sparse available information about environmental conditions that exacerbate eutrophication effects, and the better known array of indirect stressors imposed by nutrient over-enrichment that influence seagrass growth and survival. Seagrass recovery following nutrient reductions is examined, as well as the status of modeling efforts to predict seagrass response to changing nutrient regimes.

The most common mechanism invoked or demonstrated for seagrass decline under nutrient over-enrichment is light reduction through stimulation of high-biomass algal overgrowth as epiphytes and macroalgae in shallow coastal areas, and as phytoplankton in deeper coastal waters. Direct physiological responses such as ammonium toxicity and water-column nitrate inhibition through internal carbon limitation may also contribute. Seagrass decline under nutrient enrichment appears to involve indirect and feedback mechanisms, and is manifested as sudden shifts in seagrass abundance rather than continuous, gradual changes in parallel with rates of increased nutrient additions. Depending on the species, interactions of high salinity, high temperature, and low light have been shown to exacerbate the adverse effects of nutrient over-enrichment. An array of indirect effects of nutrient enrichment can accelerate seagrass disappearance, including sediment re-suspension from seagrass loss, increased system respiration and resulting oxygen stress, depressed advective water exchange from thick macroalgal growth, biogeochemical alterations such as sediment anoxia with increased hydrogen sulfide concentrations, and internal nutrient loading via enhanced nutrient fluxes from sediments to the overlying water. Indirect effects on trophic structure can also be critically important, for example, the loss of herbivores, through increased hypoxia/anoxia and other habitat shifts, that would have acted as “ecological engineers” in promoting seagrass survival by controlling algal overgrowth; and shifts favoring exotic grazers that out-compete seagrasses for space. Evidence suggests that natural seagrass population shifts are disrupted, slowed or indefinitely blocked by cultural eutrophication, and there are relatively few known examples of seagrass meadow recovery following nutrient reductions.

Reliable biomarkers as early indicators of nutrient over-enriched seagrass meadows would benefit coastal resource managers in improving protective measures. Seagrasses can be considered as “long-term” integrators (days to weeks) of nutrient availability, especially through analyses of their tissue content, and of activities of enzymes such as nitrate reductase and alkaline phosphatase. The ratio of leaf nitrogen content to leaf mass has also shown promise as a “nutrient pollution indicator” for the seagrass *Zostera marina*, with potential application to other species. In modeling efforts, seagrass response to nutrient loading has proven difficult to quantify beyond localized areas because long-term data consistent in quality are generally lacking, and high inter-annual variability in abundance and productivity depending upon stochastic meteorological and hydrographic conditions.

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doi:10.1016/j.jembe.2007.06.024

Efforts to protect remaining seagrass meadows from damage and loss under eutrophication, within countries and across regions, are generally lacking or weak and ineffective. Research needs to further understand about seagrasses and eutrophication should emphasize experimental studies to assess the response of a wider range of species to chronic, low-level as well as acute, pulsed nutrient enrichment. These experiments should be conducted in the field or in large-scale mesocosms following appropriate acclimation, and should emphasize factor interactions (N, P, C; turbidity; temperature; herbivory) to more closely simulate reality in seagrass ecosystems. They should scale up to address processes that occur over larger scales, including food-web dynamics that involve highly mobile predators and herbivores. Without any further research, however, one point is presently very clear: Concerted local and national actions, thus far mostly lacking, are needed worldwide to protect remaining seagrass meadows from accelerating cultural eutrophication in rapidly urbanizing coastal zones.

Keywords: Biomarkers; Eutrophication; Food webs; Grazers; Nitrogen; Phosphorus; Seagrass; Watershed

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Contents

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1. Introduction

During the past several decades, catastrophic losses in seagrass meadows have been documented worldwide, especially in quiet, poorly flushed estuaries and coastal embayments and lagoons with reduced tidal flushing where nutrient loads are both concentrated and frequent (see reviews in Ralph et al., 2006; Hauxwell and Valiela, 2004; Orth et al., 2006; Short et al., 2006). Along with inland watershed inputs of nutrients transported to marine coasts by rivers and estuaries (Caraco 1995, Vitousek et al., 1997), rapidly increasing human population density on coastlands is more than double that in inland areas (Nichols and Small, 2002; McGranahan et al., 2007), contributing high nutrient loads to coastal waters through sewage and other secondary impacts of development (Vitousek et al., 1997; National Research Council [NRC], 2000). Seagrass decline in favor of macroalgae or phytoplankton is a typical response.

Cultural eutrophication or nutrient over-enrichment, especially of nitrogen and phosphorus, has degraded many coastal waters and has been invoked as a major cause of seagrass disappearance worldwide (e.g. Cambridge and McComb, 1984; Short and Wyllie-Echeverria, 1996; Bricker et al., 1999; Green and Short, 2003). As Ralph et al. (2006, p. 568) point out, “causality is rarely established for the loss of a particular meadow, and factors contributing to a decline are more often established through correlation and corroborating results from experimental studies”. Nevertheless, compelling evidence from a wealth of field observations and experiments, and a limited number of cases of seagrass recovery following cases of imposed oligotrophication (e.g. Walker et al., 2006), collectively support eutrophication as an underlying factor in seagrass disappearance. This review summarizes that information and also examines the physiological responses of seagrass species to nutrient enrichment, and the potential importance of factors that interact with nutrient enrichment to control seagrass survival in increasingly urbanized coastal zones.

2. Acute and chronic effects of nutrient over-enrichment on seagrasses

Historically the obvious, acute effects of cultural eutrophication have been a major research emphasis across aquatic ecology — high-biomass algal blooms, oxygen deficits, and sudden kills of fish and other fauna (Bricker et al., 1999; Burkholder 2001). The most common mechanism invoked for seagrass decline under nutrient over-enrichment is stimulation of high-biomass algal overgrowth (Shepherd et al., 1989), including phytoplankton but more commonly, epiphytes as well as macroalgae (e.g. Orth and Moore, 1983; Borum, 1985; Twilley et al., 1985; Dennison et al., 1993; Harlin, 1993; Dunton, 1994; Lapointe et al., 1994; Short et al., 1995; Wear et al., 1999; Hauxwell and Valiela, 2004; Ralph et al., 2006) (Figs. 1 and 2). Human activities such as coastal land disturbance in development, dredge-and-fill operations, some types of aquaculture, motorboating, and fishing practices such as trawling increase turbidity and act with algal overgrowth from nutrient enrichment to promote seagrass die-off. Other human-related changes such as increased temperatures from global warming, exotic species introductions, and trophic imbalances that lead to overgrazing may also interact with nutrient enrichment and other stressors to cause seagrass declines (Orth et al., 2006).

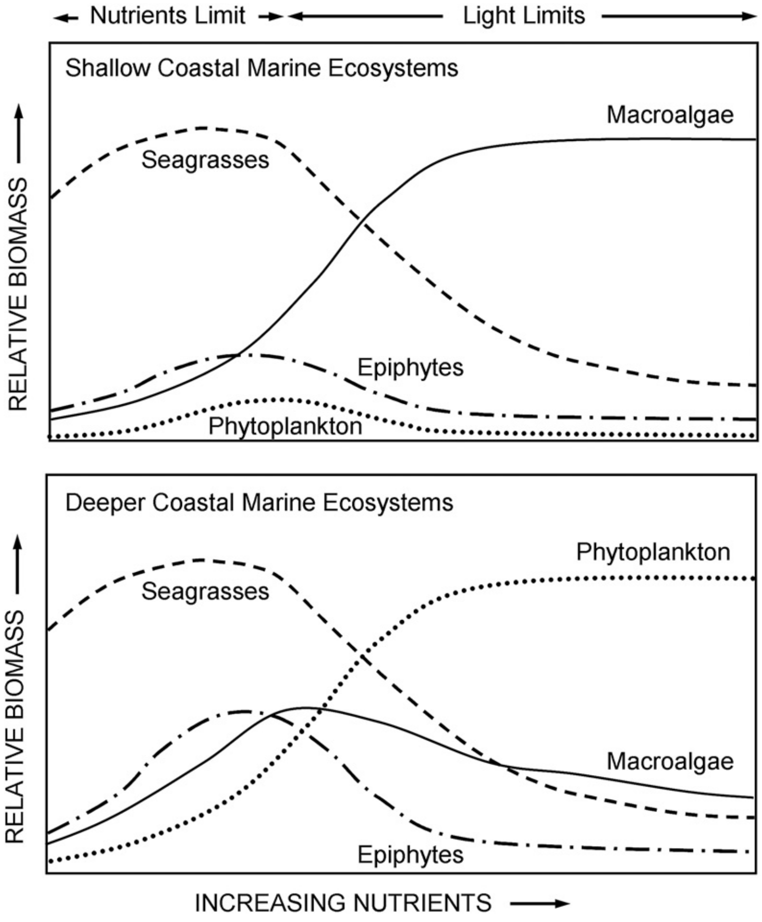

Fig. 1. Generalized shift in the biomass of major groups of primary producers with increasing nutrient enrichment to shallow and deeper coastal marine waters (upper and lower panels, respectively). In this generalized format, nutrients limit primary production under oligotrophic conditions. As eutrophication progresses with increasing nutrient enrichment, light becomes the primary limiting factor. Macroalgae (in shallow waters) or phytoplankton (in deeper waters) dramatically increase and become dominant, while seagrasses decline. Direct underlying mechanisms include competition for light and/or nitrogen and also, for some species, nitrate inhibition or ammonium toxicity. Upper panel reprinted from Harlin (1993), with permission from the publisher.

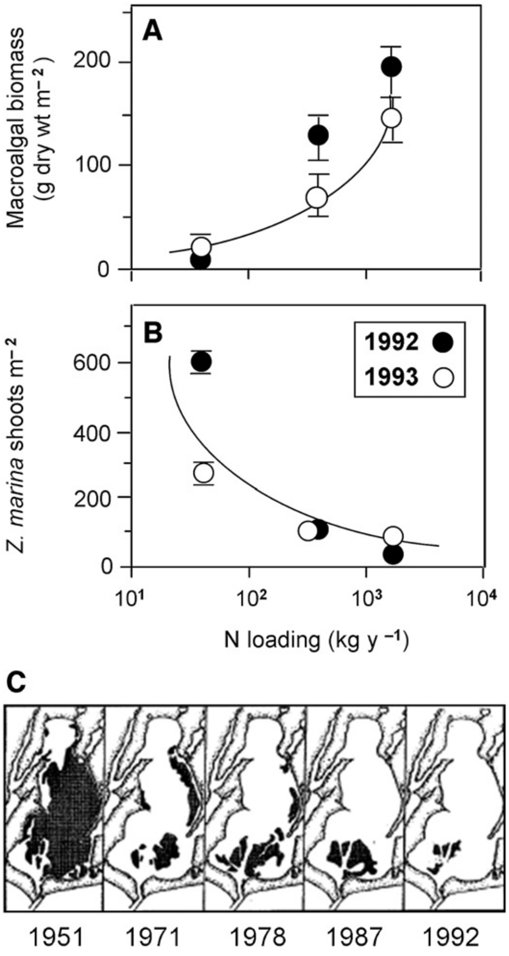

Fig. 2. Changes in plant abundance as (A) macroalgae (biomass) and (B) *Zostera marina* (shoot number) in response to N enrichment (modified from Deegan 2002; note that loading was estimated based on area of open water). (C) Change in spatial location and patch size of *Z. marina* distribution in Waquoit Bay in response to nutrient enrichment. From Valiela et al. (2000), with permission from the publisher.

Udy and Dennison (1997a) proposed three categories of seagrass response to nutrient enrichment and the associated environmental conditions, paraphrased as: (i) growth and physiology respond favorably to the additions in low-nutrient habitats where nutrients are the only environmental factor limiting growth; (ii) there is a positive physiological response but no increase in growth, in low-nutrient habitats where environmental factors other than the added nutrient limits growth; and (iii) there is neither a growth nor a physiological response, in high-nutrient environments where nutrient supplies are in excess. Touchette and Burkholder (2001) added a fourth category of seagrass response, wherein (iv) there is a negative physiological response and an inhibition of growth by the added nutrient (e.g., Burkholder et al., 1992; van Katwijk et al., 1997). Somewhat analogously, Hauxwell and Valiela (2004) suggested at least three mechanisms by which increased nutrient (N) supplies could promote seagrass decline — direct nitrate inhibition, light limitation, and unfavorable biogeochemical alterations in the habitat. All of these response categories have been documented in various seagrass species.

2.1. Nutrient sources

Seagrasses derive N and P, mostly as inorganic forms (N_i and P_i — also see Bird et al., 1998), from sediment pore water (especially ammonium, NH_4^+) and the water column (mostly nitrate, NO_3^- and phosphate, PO_4^{-3}) (see reviews in Short, 1987; Touchette and Burkholder, 2000; Romero et al., 2006). Groundwater inflows can contribute up to 800 μM NO_3^- from septic effluent leachate and other anthropogenic sources (Maier and Pregnall, 1990; Harman et al., 1996). Nutrient concentrations are often 10- to 100-fold higher in the sediments than in the water column (e.g. Sand-Jensen and Borum, 1991).

Seagrasses may be N-limited in nutrient-poor waters with sandy or (less so) organic sediments, and P-limited in carbonate sediments (but see Terrados et al., 1999). These plants receive most of their nutrients from the sediment when concentrations are low in the water column and sediments are enriched (e.g. Thursby and Harlin, 1982; Short and McRoy, 1984). Some authors have suggested that uptake of inorganic N by leaves and roots may be about the same under most circumstances (Romero e al., 2006), but highly enriched waters would favor leaf uptake. Leaf tissues apparently can have higher nutrient uptake affinities than root tissues (e.g. Pederson et al., 1997; Lee and Dunton, 1999a,b), and leaf tissues can substantially contribute to total nutrient acquisition (e.g. Iizumi and Hattori, 1982; Short and McRoy, 1984; Lee and Dunton, 1999a; Lepoint et al., 2002).

Phosphate levels in seagrass habitats typically range from ca. 0.1 to 1.7 μM in the water column, with higher concentrations in the sediment pore water (0.3 to 20 μM $PO_4^{-3}P$) (reviewed in Touchette and Burkholder, 2000). Ammonium ranges from 0 to 3.2 μM in the water column, and from ~1 to 180 μM NH_4^+N in sediment pore water depending on sediment characteristics (especially percent organic matter) and community composition (Touchette and Burkholder, 2000). Nitrate + nitrite has been reported at ~0.05 to 8 μM in the water column, and ~2 to 10 μM in the sediments (Touchette and Burkholder, 2000). Total water-column N_i typically is <3 μM during the day in habitats without land-based anthropogenic influence (Touchette and Burkholder, 2000). Nitrate supplies for most seagrasses are provided by leaf absorption from the water column (Terrados and Williams, 1997; Lee and Dunton, 1999a), except for groundwater inflows that can contribute as much as 800 μM NO_3^-N to seagrass beds if influenced by septic effluent leachate or other anthropogenic sources (Maier and Pregnall, 1990; Harman et al., 1996).

2.2. Seagrass physiological responses

Surprisingly little still is known about the basic nutritional physiology of many seagrass species, or the physiological mechanisms that control their responses to N and P gradients (Touchette and Burkholder, 2000). The limited available evidence to date has revealed an expected response of increased growth with low to moderate nutrient enrichment, especially from sediment sources (Table 1). Both N and P can be stored, as suggested by higher tissue N and P contents under nutrient enrichment; for example, nitrate generally is stored in vacuoles within the leaf cytosol (Touchette and Burkholder, 2000; and review in Ferdie and Forquerean, 2004).

There is also evidence for influences of nutrient enrichment on competitive interactions and shifts in seagrass dominance. For example, Fourqurean et al. (1995) experimentally fertilized natural seagrass meadows dominated by the normally late successional species, *Thalassia testudinum*, with bird guano for 8 yr. For the first 2 yr, *T. testudinum* production was positively affected by the nutrient enrichment, but gradually *Halodule wrightii*, normally an early-successional species in subtropical habitats, colonized the beds, and by the end of the study accounted for 97% of the aboveground seagrass biomass. Unfertilized control sites showed no change in *T. testudinum* biomass or shoot density, and were not colonized by *H. wrightii*. This shift in species dominance persisted at least 8 yr after nutrients were no longer added, suggesting that once an area has been fertilized, the system can be resistant to change. The authors hypothesized that replacement of *H. wrightii* by *T. testudinum* occurred because *T. testudinum* had not been able to decrease nutrient availability below the requirements of *H. wrightii*.

Certain seagrass species have shown somewhat unusual responses to nutrient enrichment in comparison to other vascular plants. For example, studies of N_i assimilation in several seagrass species have indicated limited or negligible product feedback inhibition, hypothesized to reflect an adaptation to oligotrophic habitats or an adaptive response to changing nutrient regimes (Burkholder et al., 1992). A biphasic rather than hyperbolic P_i uptake curve, with “surge” uptake, was described for *Zostera noltii* (Pérez-Lloréns and Niell, 1995). Stapel et al. (1996) found no evidence of a feedback inhibition mechanism in NH_4^+ uptake by *Thalassia hemprichii*, at least over the period tested. Roth and Pregnall (1988) compared nitrate uptake for *Zostera marina* from sandy (low N) versus organic (high N) sediments and low-N overlying waters. In short-term laboratory trials under N-enriched conditions, plants grown in sandy sediments showed a linear response in nitrate uptake, with no evidence of product feedback inhibition over time (6 days). A linear response in nitrate uptake was also shown by plants from N-rich sediments, but uptake was significantly lower. Thus, the low N-adapted plants apparently had conformed to maximize their response to water-column NO_3^- enrichment, relative to the plants that had been growing in sediment N-enriched conditions.

In outdoor mesocosms, Burkholder et al. (1992, 1994) experimentally documented water-column NO_3^- inhibition of *Z. marina* growing at relatively high temperatures in the southern limit of its range. Even at low levels of pulsed NO_3^- enrichment (~3.5 and ~7 μM initially added as one pulse per day, maintaining concentrations of <4 μM in the water — note, incorrectly described by Duarte, 1995 and Ralph et al., 2006), there was 75–95% shoot die-off relative to the un-enriched control plants. The effect was unrelated to light reduction from algal overgrowth. The authors hypothesized that lack of a product inhibition feedback mechanism had caused the plants to continue to take up nitrate, so that the sustained carbon demand for assimilation to amino acids had driven the plants into internal C limitation. Nitrate assimilation is a metabolically expensive process, requiring high cellular energy and carbon skeletons for amino acid synthesis (Turpin et al., 1991; Larsson, 1994). Most plants reduce nitrate during the day with energy from photosynthesis (Larsson, 1994).

In contrast, *Z. marina* reduces water-column nitrate day or night if it becomes available (Touchette and Burkholder, 2001) (Fig. 3). This species likely evolved in N_i-poor coastal waters. Sustained nitrate uptake and assimilation without product inhibition may have developed as a once-advantageous competitive strategy under temporary enrichment, but would be disadvantageous as coastal waters have become highly enriched from anthropogenic sources. In contrast, nitrate enrichment to the sediments, under control by a rich microbial

Table 1
Overview of seagrass responses to nutrient enrichment and/or eutrophication events

Species	Response	Mechanism	Nutrient(s)	Source
Temperate				
Posidonia oceanica L.	Die-off	Nutrient loading, turbidity	Water-column N_i+P_i	Cambridge and McComb (1984), Cambridge et al. (1986), Silberstein et al. (1986).
	Increased growth	N+P co-limitation	Sediment N_i+P_i	Alcoverro et al. (1997).
Posidonia sinuosa Cambridge et Kuo	Die-off	Shading by epiphytes	Water-column N_i	Pergent-Martini (1992)
	Die-off	Nutrient loading, turbidity, other human disturbance	Water-column $N+P_i$	Cambridge and McComb (1984), Cambridge et al. (1986), Silberstein et al. (1986)
Ruppia drepanensis Tineo ex Guss.	Die-off	NH_4^+ inhibition	Sediment N	Santamaría et al. (1994)
Ruppia maritima L.	Increased growth	N limitation	Water-column NO_3^-	Burkholder et al. (1994)
R. maritima	Die-off	Competition (*Zostera marina*); shading by epiphytes	Sediment N_i+P_i Water-column N_i+P_i	Orth (1977) Twilley et al. (1985)
R. maritima	Increased growth	P limitation	Water-column P_i	Harlin and Thorne-Miller (1981)
	No effect	–	Water-column NO_3^-	Harlin and Thorne-Miller (1981)
Zostera capricorni Ascherson[a]	Increased growth	N+P co-limitation (N_i alone — no effect)	Sediment N_i+P_i	Udy and Dennison (1997a)
Z. marina L.	Increased growth	N limitation	Sediment N_i+P_i	Orth (1977), Pedersen and Borum (1993), Peralta et al. (2003)
	Increased growth	N limitation	Sediment N_i	Kenworthy and Fonseca (1992), van Lent et al. (1995)
	Increased productivity	N limitation?	Sediment N_i+P_i	Wear et al. (1999)
	Decreased growth	NO_3^- inhibition	Sediment NO_3^- (>20 mM)	Peralta et al. (2003)
	Increased growth	N limitation	Water-column NH_4^+	Harlin and Thorne-Miller (1981)[b]
	Increased growth	N limitation (spring)	Sediment NH_4^+	Williams and Ruckelshaus (1993)
	No effect (epiphytes N-limited but controlled by grazers)	–	Water-column NH_4^+	Williams and Ruckelshaus (1993)
	Die-off or no effect	NO_3^- inhibition	Water-column NO_3^-	Harlin and Thorne-Miller (1981)
	Increased growth	P limitation	Sediment P_i	Murray et al. (1992)
	No effect	–	Sediment NH_4^+	Murray et al. (1992)
	No effect	–	Water-column P_i	Taylor et al. (1995)
	Decreased growth	Algal overgrowth, shading	Water-column NO_3^-, or $P_i+NO_3^-$ or NH_4^+	Taylor et al. (1999)
	No effect	–	Water-column P_i	Taylor et al. (1999)
	Increased growth	P limitation	Water-column P_i	Harlin and Thorne-Miller (1981)
	Die-off	NO_3^- inhibition	Water-column NO_3^-	Burkholder et al. (1992, 1994), Touchette and Burkholder (2001, 2002), Touchette et al. (2003), Touchette and Burkholder (2007-this volume)
	Die-off	NH_4^+ toxicity	Water-column $NH_4^++NO_3^-$	van Katwijk et al. (1997)
	Decreased growth	Algal overgrowth, shading	Water-column N_i+P_i	Neckles et al. (1993), Moore and Wetzel (2000)
	Decreased growth	Algal overgrowth, shading	Water-column $NH_4^++P_i$	Short et al. (1995)
	Die-off	Algal overgrowth, shading	Groundwater N_i	Short and Burdick (1996)
	Die-off	Algal overgrowth, shading	Groundwater N_i+P_i	Short et al. (2006)
	Die-off	Algal overgrowth, shading	N loading	Hauxwell et al. (2003)
	Increased growth	N limitation	Sediment N_i	van Lent et al. (1995)
	Increased growth	N limitation	Water-column Ni+Pi	De Casabianca et al. (1997)

(continued on next page)

Table 1 (*continued*)

Species	Response	Mechanism	Nutrient(s)	Source
Z. marina, *Zostera noltii* Hornemann	Die-off	Algal overgrowth	Water-column N+Pi	De Casabianca et al. (1997)
Zostera tasmanica Martens ex Ascherson (as *Heterozostera tasmanica*)[a]	Increased growth	N limitation	Sediment NH_4^++P_i	Bulthuis and Woelkerling (1981)
	Increased growth	N limitation	Sediment N_i (P_i — no effect)	Bulthuis et al. (1992)
Tropical/subtropical				
Cymodocea aequorea König (as *Cymodocea nodosa*)[c]	Increased growth	P limitation	P_i	Pérez et al. (1991), Pérez and Romero (1993, 1994)
	No effect	–	Sediment N_o+NO_3^-+P_i	Ceccherelli and Cinelli (1997)
C. aequorea (as *C. nodosa*)[c]	Decreased growth	Algal competition	Sediment N_o+NO_3^-+P_i	Ceccherelli and Cinelli (1997)
Cymodocea rotundata Ehrenberg et Hemprich ex Ascherson	Increased growth	N limitation	Sediment N_i+N_o+P_i	Agawin et al. (1996)
Cymodocea serrulata (R. Brown) Ascherson et Magnu	No effect	–	Sediment N_i or N_i+P_i	Udy and Dennison (1997a)
Diplanthera tridentata Steinheil (as *Halodule uninervis*)[c]	Increased growth	N limitation	Sediment N_i or N_i+P_i	Udy and Dennison (1997a), Udy et al. (1999)
Enhalus koenigi Rich. (as *Enhalus acoroides*)[c]	Increased growth	N or P limitation	Sediment N_i+N_o+P_i	Agawin et al. (1996)
	Increased growth	N limitation	Sediment N_i+N_o+P_i	Terrados et al. (1999)
Halodule wrightii Ascherson	Increased growth	N+P limitation	Sediment (guano) N+P_i	Powell et al. (1989)
	Increased growth	N limitation	Water-column NO_3^-	Burkholder et al. (1994)
	No effect	–	Sediment N_i	Kenworthy and Fonseca (1992)
Syringodium filiforme Kützing	Increased growth	N limitation	N (assumed, sediment)	Ferdie and Fourqurean (2004)
		P limitation	Sediment P_i+N_o	Short et al. (1990)
Syringodium isoetifolium (Ascherson) Dandy	Increased growth	N limitation	Sediment NH_4^++NO_3^- or sediment N_i+P_i	Udy et al. (1999)
Thalassia hemprichii (Ehrenberg) Ascherson	No effect	–	Sediment N_o+P_i	Erftmeijer et al. (1994)
	Increased growth	P limitation	Sediment N_i+N_o+P_i	Agawin et al. (1996)
Thalassia testudinum Banks ex König	Increased growth	N+P co-limitation	Sediment (guano) N+P_i	Powell et al. (1989)
	Increased growth	P limitation	Water-column P_i	Gallegos et al. (1993)
	Increased growth	N limitation (P — no effect)	Sediment N_i, offshore	Ferdie and Forqurean (2004)
	Increased growth	N+P co-limitation	Sediment N_i+P_i, nearshore	Ferdie and Forqurean (2004)
	Increased growth	N+P co-limitation (years 1–2)	Bird guano	Fourqurean et al. (1995)
	Die-off (years 3–8)	Replaced by *H. wrightii*	Bird guano	Fourqurean et al. (1995)
	Decreased abundance	Algal overgrowth/shading, herbivory of N-enriched plants	Sediment NH_4^++P_i	McGlathery (1995)
	Die-off	Algal overgrowth, shading	Water-column N_i+P_i	Tomasko and Lapointe (1991)
	Die-off	Light attenuation	Water-column N	Tomasko et al. (1996)
	Die-off	Algal overgrowth/shading	Water-column NH_4^+	Lapointe et al. (2002)

Data include seagrass species (temperate and tropical/subtropical), growth and/or survival response, possible mechanism for the observed response, nutrients involved, and source. Note that N_i indicates addition of both nitrate (NO_3^-) and ammonium (NH_4^+); P_i = phosphate (PO_4^{-3}); N_o = urea unless otherwise indicated.

[a] See taxonomic review by Moore and Short (2006).

[b] But note that Zimmerman et al. (1987), through statistical re-analyses of Harlin and Thorne-Millers' (1981) dataset, did not find significant positive effects of N additions on growth, plant density or biomass.

[c] See den Hartog and Kuo (2006).

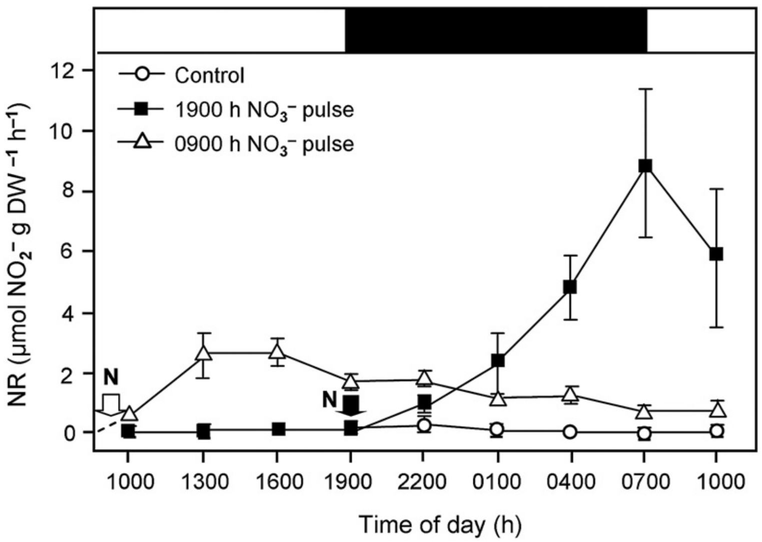

Fig. 3. The response of the seagrass, *Zostera marina*, to pulsed water-column nitrate enrichment in light and dark periods. Plant nitrate reduction is indicated as leaf activity of the enzyme (nitrate reductase [NR], the enzyme directly involved in nitrate assimilation) of previously un-enriched shoots. A spike of nitrate (7.86 µM, or 110 µg NO_3^- NL^{-1}) was added in the morning (white arrow) or, to a sub-sample of plants from the same population, at night (black arrow). NR activity (plotted as micromoles of nitrite product produced per gram dry weight of plant leaf tissue per hour) indicated that *Z. marina* reduced nitrate day or night, whenever a pulse was detected (means + 1 standard error). In fact, maximal NR activity was significantly higher when nitrate was added during the dark period. Reprinted from Touchette and Burkholder (2001), with permission from the publisher.

consortium, does not cause a similar effect (Burkholder et al., 1992) and can be mildly stimulatory (e.g. Kenworthy and Fonseca, 1992).

Other researchers (e.g. Burke et al., 1996; Touchette and Burkholder, 2001, 2002; Invers et al., 2004) have provided data in direct or indirect support of the water-column nitrate inhibition hypothesis for seagrasses, including seasonal differences in response depending upon mobilized carbon reserves. Touchette et al. (2003) found that sustained uptake of water-column nitrate (pulsed daily at 8 µM, with water-column concentrations <2 µM NO_3^-N for most of each diel cycle) promoted severe internal carbon imbalances in *Z. marina*, apparently from the need to shunt carbon skeletons for use in high amino acid synthesis to prevent internal accumulation of toxic products such as ammonia. Turpin et al. (1991) had earlier documented the physiological mechanism of an internal “carbon drain” from sustained nitrate uptake in algae. *Z. marina* shoots under excessive water-column nitrate enrichment can become structurally weakened in their meristematic regions (Burkholder et al., 1992). Excessive N_i enrichment has also promoted seagrass attack by pathogens such as the slime mold *Labyrnthula zosteroides*, hypothesized to occur because N and C are internally shunted to amino acid production rather than to production of alkaloids and other anti-microbial compounds (Short and Burdick, 1996).

Another seagrass examined for nitrate inhibition, *H. wrightii* showed depressed growth in response to nitrate enrichment (Burkholder et al., 1994), but at much higher N levels (ca. 100 µM, pulsed daily for 4–5 weeks) than for *Z. marina* (~3.5 to ~7.0 µM pulsed daily for 5–8 weeks; Burkholder et al., 1992, 1994; 8.0 µM pulsed daily for 14 weeks; Touchette et al., 2003). In contrast, *Ruppia maritima* was stimulated by high water-column nitrate but inhibited by elevated N_i as NH_4^+ (Burkholder et al., 1994). Ammonia toxicity is known for many vascular plants (Britto and Kronzucker, 2002). Growth of seagrasses *Ruppia drepanensis* and *Z. marina* was significantly depressed under NH_4^+ enrichment, unrelated to light attenuation from algal overgrowth (Santamaría et al., 1994; van Katwijk et al., 1997). Sediment and water-column NH_4^+ were high relative to levels typically found in seagrass habitats, with die-off occurring at 3–220 µM NH_4^+N in the water column, and at 500–1600 µM NH_4^+N in the sediment pore water. *Z. marina* plants growing in sandy sediments were more susceptible than plants grown in organic sediments (van Katwijk et al., 1997).

Duarte (1990) suggested that carbon limitation in seagrasses is probably rare. An alternate possibility is that carbon limitation may be a more frequent but overlooked effect for some seagrass species with increasing eutrophication of coastal waters worldwide. *Z. marina* is able to maintain nitrate reductase (NR) activity (the enzyme directly involved in nitrate assimilation) during dark periods, unlike most vascular plants, if adequate carbohydrate reserves and substrate are available (Touchette and Burkholder, 2002). This trait would provide a mechanism for response to pulsed nitrate enrichment whenever it occurred within a diel cycle. In contrast, *Halophila decipiens* and *H. stipulacea* lack inducible NR; for example, *H. stipulacea* showed little inducible in vivo nitrate reductase, unrelated to NH_4^+ suppression, even following substrate additions as high as 1 mM NO_3^- for 42 h, and apparently has lost the ability to reduce nitrate (Doddema and Howari, 1983).

Thus, while light limitation imposed by algal overgrowth is a common mechanism for seagrass declines under nutrient over-enrichment, the available evidence suggests that direct physiological responses of seagrasses can be contributing or major underlying factors. It is important to consider that seagrass species show substantial variation in their nutritional responses, so that inferences about one species in one geographic region should not be applied a priori to that species in other regions, or to seagrasses in general (Touchette and Burkholder, 2000).

3. Indirect stressors imposed by nutrient over-enrichment

Seagrass decline under increased nutrient loading involves, as Duarte (1995, p. 98) noted, "a cascade of direct and indirect effects interacting in a self-accelerating manner". Sudden shifts and step changes in submersed vegetation, rather than continuous, gradual changes in parallel with the rate of increased nutrient additions, often characterize seagrass response to eutrophication (Kemp et al., 1983; Nienhuis, 1983; Cambridge and McComb, 1984; Williams and Ruckelshaus, 1993; Duarte, 1995). Thus, the observed changes in submersed vegetation must be influenced in part by indirect and feedback mechanisms (Duarte, 1995). In addition to decreased available light, indirect effects can include sediment re-suspension from seagrass loss, increased system respiration, sediment anoxia, and internal nutrient loading from enhanced nutrient fluxes from sediments to the water column (Duarte, 1995 Fig. 4).

3.1. Light and temperature interactions

Eutrophication in seagrass ecosystems tends to proceed toward dominance of rapidly growing epiphytes and macroalgae that are considered superior competitors for light relative to seagrasses, and final dominance by phytoplankton at extremely high-nutrient loadings (reviewed in Duarte, 1995; Valiela et al., 1997). Macroalgal response to nutrient enrichment often translates into rapidly developing, thick canopies 0.75 to more than 2 m in height (e.g. Hauxwell et al., 2001; Sfriso et al., 1992), with substantial biomass — for example, in temperate areas, 650 g dry weight m^{-2} of *Ulva* sp., *Gracilaria tikvahiae* McLachlan and *Cladophora* sp. (Hog Island Bay, Virginia, USA — Havens et al., 2001); 750 g dry weight m^{-2} of *Ulva* and *Enteromorpha* (Coos Bay, Oregon, USA — Pregnall and Rudy, 1985); and 1800 g dry weight m^{-2} of *Ulva rigida* (Venice Lagoon, Italy — Sfriso et al., 1992).

In mesocosm experiments, light reduction has been shown to exacerbate the inhibitory effects of water-

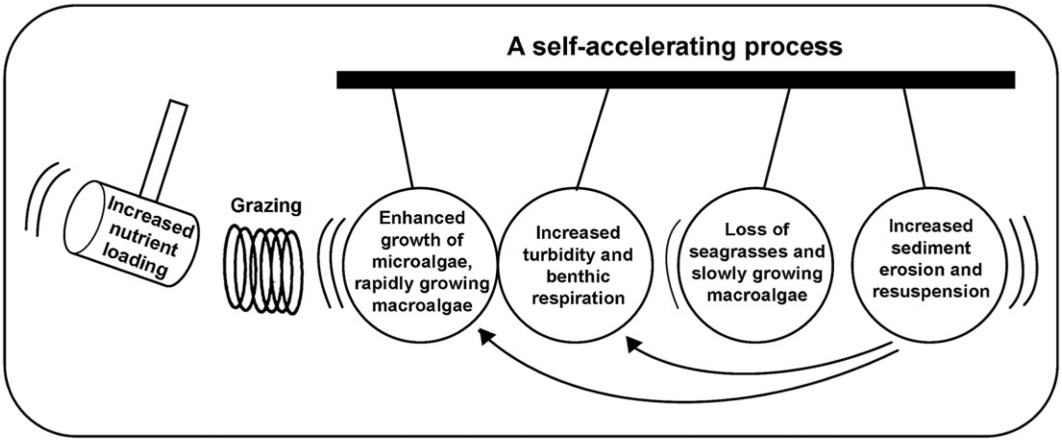

Fig. 4. Conceptual model (cartoon) of the effect of increased nutrient loading on seagrasses, stressing the self-accelerating nature of the process and operational buffer mechanisms. From Duarte (1995), with permission from the publisher.

column nitrate enrichment on shoot production in *Z. marina* (Burkholder, 2001; Fig. 5). Burke et al. (1996) reported that experimental shading of *Z. marina* for only 3 weeks in the spring growing season reduced non-structural carbohydrate concentrations in the leaves, rhizomes and roots by 40–51%, and reduced stored potential non-structural carbohydrate reserves by ~66%. Concomitant water-column nitrate enrichment under conditions of diminished carbon storage products would be expected to exacerbate seagrass decline, and to depress the ability of plants to survive dehiscence and dormancy periods.

Temperature effects on seagrasses under cultural eutrophication depend upon individual species' growth optima and tolerances. Temperatures elevated 3–4 °C above the mean, which is within the range of expected global mean temperature rise within this century (Levitus et al., 2001; Kerr, 2007), exacerbated inhibitory effects of water-column nitrate enrichment on root growth in the cold-optimal seagrass, *Z. marina*, growing in sandy sediments at the southernmost extension of its geographic range along the western Atlantic Ocean (Touchette et al., 2003, Touchette and Burkholder, 2007-this volume). The data suggest that warming trends in climate change may be expected to interact with eutrophication to promote the decline of this species in warmer areas of its range. Elevated temperatures also exacerbated the adverse response of *Z. marina* growing in sandy sediments to ammonium enrichment (van Katwijk et al., 1997). Sub-littoral meadows of *Z. marina* in the Dutch-Wadden Sea did not recover from wasting disease that was linked to elevated temperatures, and the beds gradually disappeared after the mid-1960s. The high, sustained incidence of wasting disease, in turn, was linked to increased turbidity from eutrophication (Giesen et al., 1990).

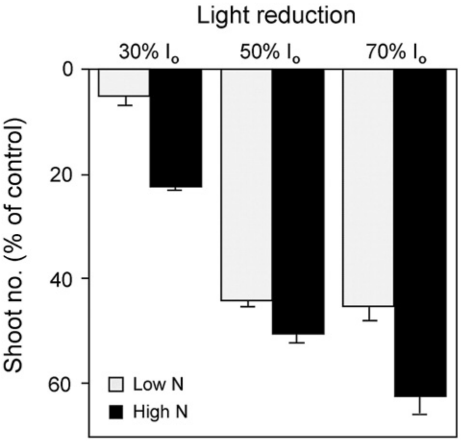

Fig. 5. The effects of water-column nitrate enrichment and light reduction on shoot production of the seagrass, *Zostera marina*. From first author's outdoor mesocosm experiments, indicated as the percent decrease from shoot production of control plants that did not receive water-column nitrate additions or light reduction (except that plants in controls and treatments all received an additional 30% light reduction for 3 h at 0900, 1200, and 1500 h on a 3-day rotation using neutral density screens to simulate conditions during high tide). Treatments were imposed for 10 weeks during the fall growing season for *Z. marina*. Controls were maintained at ambient natural light (except during simulated high tide) and nitrate (<2.15 µM or 30 µg NO_3^- NL^{-1}). Treatments included low N (at 3.57 µM or 50 µg NO_3^- L^{-1}), added daily as a pulse of enrichment and high N (at 7.14 µM or 100 µg NO_3^- NL^{-1}) at each of three imposed light levels as 30, 50, or 70% reduction at ambient surface light (I_o, accomplished using neutral density shades, with additional shading at simulated high tide as noted). *Z. marina* in all treatments with water-column nitrate enrichment declined in shoot production relative to shoot production of control plants, and the nitrate inhibition effect was exacerbated at lower irradiance (means+1 standard error; $P<0.05$, $n=3$). These effects were not caused by algal overgrowth, which was maintained at low levels in controls and all treatments throughout the experiment. From Burkholder (2001), with permission from the publisher.

3.2. Sediment loading and re-suspension

Nutrient enrichment is often accompanied by sediment loading and re-suspension. In addition to algal overgrowth, this sedimentation and turbidity can reduce the light available for photosynthesis and production of carbon reserves, and sedimentation has the added stress of inhibiting gas exchange (Ralph et al., 2006). High turbidity and nutrients associated with sediment re-suspension over denuded areas, in combination with erosion from waves and tidal currents, accelerate and maintain further seagrass loss (Bulthius et al., 1984; Clarke and Kirkman, 1989; Walker et al., 2006). Once the integrity of the meadow has been damaged, it can be repeatedly damaged by sediment re-suspension and siltation (e.g. Larkum and West, 1983). Thus, water quality conditions can reflect the effects of seagrass loss rather than indicating the cause of seagrass decline (Morris and Virnstein, 2004). For example, when seagrass cover decreases 25–50%, re-suspension has been shown to markedly increase (Moore et al., 1996). The suspended fine sediments can exacerbate anoxia and depress gas exchange (Ralph et al., 2006) by increasing the diffusion boundary layer for gas exchange across the leaf surface, thus decreasing photosynthetic rates (Ralph et al., 2006).

3.3. Water-column hypoxia and sediment anoxia

As rapidly growing macroalgae, epiphytes and phytoplankton respond to nutrient enrichment, oxygen production and respiration become increasingly uncoupled temporally, often leading to hypoxic and anoxic conditions (Sand-Jensen and Borum 1991). There is evidence that populations of at least some seagrass species can recover rapidly following short-term, extreme anoxia, through colonization by surviving seedlings (Churchill, 1992; Moore et al., 1993). For example, Plus et al. (2003) documented rapid recolonization (within three months) by *Z. marina* seedlings in the French Mediterranean Sea after a 5-day "anoxic crisis" caused mature shoot die-off. The authors noted, however, that consecutive anoxic events would be expected to deplete the annual seedbank and depress recovery.

Cultural eutrophication commonly promotes repeated, sustained anoxia. Anoxia during warm, calm periods occurs more frequently as eutrophication progresses (e.g. D'Avanzo and Kremer, 1994; Sfriso et al., 1992). Bottom-water and sediment anoxia impedes respiration, nutrient acquisition and other metabolic functions in seagrass roots (Smith et al., 1988). Prolonged anoxia increases the energy demand for translocating oxygen down to roots from photosynthetic shoot tissues, and inhibits root ammonium uptake (Pregnall et al., 1984), leading to reduced photosynthesis, smaller leaves, and reduced number of leaves per shoot (Holmer and Bondgaard, 2001). Meristematic aboveground tissues can be especially sensitive to anoxia (Greve et al., 2003).

Elevated nutrients, respiration and anoxia also promote increased sediment sulfide concentrations (Stumm and Morgan, 1996). Sulfide negatively affects seagrass photosynthesis, metabolism and growth (Goodman et al., 1995; Erskine and Koch, 2000; Holmer and Bondgaard, 2001; Ralph et al., 2006). In *Z. marina*, moderate sulfide levels (>400 μM) were related to depressed maximum rates of photosynthesis ($P_{\max}$), increased requirements for light, and decreased slope of the photosynthesis versus irradiance curve, which led to a 55% decrease in shoot-to-root ratios from shoot senescence/mortality within 6 days of exposure (Goodman et al., 1995; Holmer and Bondgaard, 2001). The authors reported that eutrophication effects through reduced light and increased sediment sulfide on $P_{\max}$ were additive, and suggested that elevated sulfide could contribute to *Z. marina* loss under low-light stress. Interactive effects of high salinity (55–60) and/or high temperature (35 °C) with high sulfide (6 mM), sometimes characteristic of its natural habitat, were linked to mortality of the tropical seagrass, *T. testudinum* (Koch and Erskine, 2001).

3.4. Architectural and biogeochemical changes imposed by macroalgae

Thick macroalgal drift assemblages can inhibit advective water exchange at the base of seagrass shoots and promote increased hypoxia/anoxia and hydrogen sulfide stress, exacerbated by high macroalgal respiration during the night (Hauxwell and Valiela 2004). Because of high respiration rates, macroalgal canopies commonly become hypoxic or anoxic at night, so that the sensitive basal shoot meristem of seagrasses such as *Z. marina* can be completely immersed in anoxic water (Greve et al., 2003; Hauxwell and Valiela, 2004). This effect can be compounded by excessive concentrations of ammonium (e.g. <2 μM in the overlying water column, versus up to ~260 μM within the macroalgal canopy) that can form through nitrogen regeneration by the macroalgae and underlying sediments, and through reduced advective losses imposed by the physical canopy structure (Bierzychudek et al., 1993; McGlathery, 1995; Hauxwell et al., 2001). For example, in a eutrophic area of Waquoit Bay, Hauxwell et al. (2001) documented that newly recruiting *Z. marina* shoots were entirely immersed in toxic concentrations of ammonium (>100 μM NH_4^+).

3.5. Trophic imbalances — "ghosts of grazing past", and grazers as "ecological engineers"

An excellent treatise by Valentine and Duffy (2006) compiled strong evidence in support of the premise that human fishing practices have caused severe reductions in large vertebrate grazers of seagrasses (green turtles — *Chelonia mydas* L., sirenians, waterfowl) and masked what was once a major influence of herbivory in seagrass food webs. Some tropical seagrass food webs have retained sufficient functional redundancy so that herbivores continue to have a major "top-down" influence in directly controlling aboveground seagrass biomass. In many areas, however – and in present-day temperate regions, in particular – Valentine and Duffy (2006) described the grazers as "ghosts", and their once-major influence on seagrasses as "now extremely episodic, negligible, or absent".

This profound alteration of seagrass ecosystems, an often-overlooked history, apparently and erroneously has led to an over-generalization that seagrass meadows are, and historically were, detritus-based — that direct consumption of seagrasses has "always" been negligible, so that the energy supplied by seagrasses to other trophic levels has been contributed through their decomposition (Domning, 2001; Heck and Valentine, 2006; Valentine and Duffy, 2006). Certain of the large herbivores that have been reduced or eliminated also would have consumed macroalgal overgrowth, and thus would have been expected to have exerted a complex suite of beneficial as well as detrimental, direct and indirect effects on seagrass populations — as true of herbivorous grazers of other aquatic macrophytes (reviewed in Wetzel, 2001; Valentine and Duffy, 2006). Those effects, in some cases, would have included a role of large grazers in mitigating macroalgal overgrowth from nutrient over-enrichment and, under some circumstances, helping to maintain balanced trophic structure in seagrass beds under eutrophication.

In many present-day seagrass meadows worldwide, the dominant primary consumers now are smaller herbivores or "mesograzers" (small grazing and detritivorous invertebrates, mostly amphipods, and isopod crustaceans, hermit crabs and gastropod molluscs (reviewed in Valentine and Duffy, 2006), as well as bivalve molluscs that can control phytoplankton through their filter-feeding activity) (Officer et al., 1982; Newell, 1988). Accumulating evidence from correlative field observations and experimental work supports the premise that mesograzers can play a central role as "ecological engineers" in indirectly maintaining seagrass populations by consuming algae that would otherwise overgrow and out-compete seagrasses for light and water-column nutrients (Jones et al., 1994; Valentine and Duffy, 2006).

The role of herbivores in controlling algal and seagrass biomass is highly variable, ranging from negligible to nearly 100% removal of primary producers (reviewed by Cebrián, 2004). Thus, grazing in seagrass meadows varies greatly in time and space, as in most other ecosystems (Heck and Valentine, 2006). Experimental data from greenhouse and outdoor mesocosm studies have shown that nutrient enrichment often negligibly affects the balance between seagrasses and algae if natural grazer populations are present to consume the algal biomass (e.g. Neckles et al., 1993; Williams and Ruckelshaus, 1993). As eutrophication and stimulation of rapidly growing algae progress, there appears to be a threshold point at which the grazers can no longer control algal biomass (Wetzel and Neckles, 1986); moreover, many mesograzers become stressed, reduced, or eliminated by the anoxic conditions that often accompany nutrient over-enrichment (Diaz and Rosenberg, 1995; Breitburg, 2002). Thus, as Hauxwell et al. (2001) noted, in estuaries the effects of herbivory on macroalgal biomass tend to decrease with increasing nitrogen loads.

The many interacting, indirect effects of eutrophication in stressing seagrass ecosystems are poorly understood; as Valentine and Duffy (2006, p. 487) pointed out, seagrass ecosystems under chronic eutrophication "may degrade as much as a consequence of altered food webs as of nutrient loading". These authors hypothesized that seagrass ecosystems exist as one of two alternate ecosystem states: seagrass-dominated, characterized by low biomass of epiphytes and macroalgae and abundant

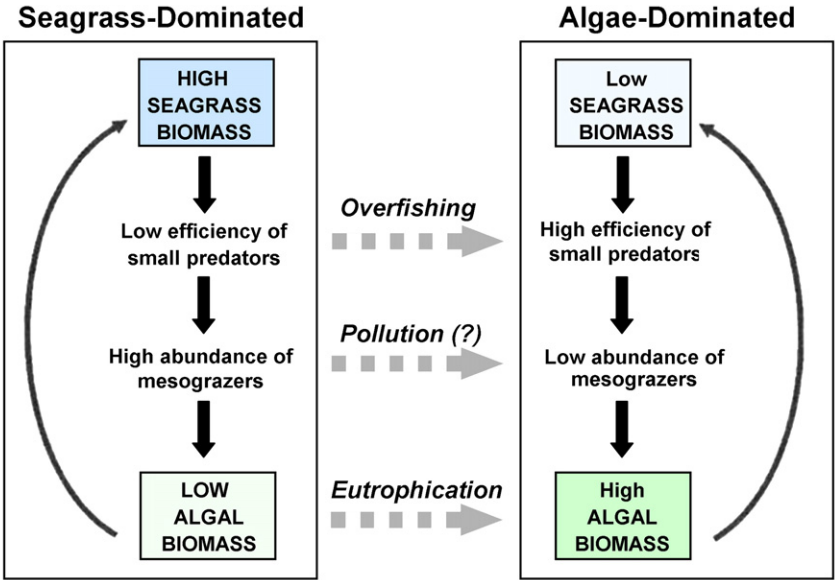

Fig. 6. Hypothesized alternate ecosystem states in benthic estuarine/marine ecosystems with non-limiting light for the dominant primary producers. In this hypothesis, both seagrass-dominated and algal-dominated states are maintained by positive feedback loops, mediated by food-web interactions, which make them resistant to perturbation. Once perturbed to the point of state change, however, these positive feedbacks similarly render the system resistant to restoration. Broken arrows denote anthropogenic perturbations impinging at different levels in the food web. Note that all anthropogenic perturbations drive the system in the direction of algal dominance and seagrass decline. From Valentine and Duffy (2006), with permission from the publisher.

seagrass that provides refuge habitat for mesograzers; or algal-dominated, characterized by abundant benthic or metaphytic algae that are competitively superior to seagrasses under high nutrient and light regimes, and therefore resistant to seagrass (re-)establishment (Fig. 6). Seagrass-dominated systems were described as relatively resistant to both eutrophication and increased densities of small predators because of removal of larger predators by overfishing.

Cultural eutrophication can also shift seagrass communities to favor exotic grazers that out-compete seagrasses for the critically important resource, space. For example, Reusch and Williams (1999) documented a negative feedback on *Z. marina* from nutrient enrichment in combination with an invasive Asian mussel (*Musculista senhousia* Benson). In healthy, unenriched meadows, the mussel was food-limited because of reduced water velocities and associated reduced food availability. In nutrient-enriched meadows, however, higher phytoplankton production resulted in depressed light penetration, stimulation of mussel growth, and seagrass decline.

4. Indicators of nutrient enrichment

The search for reliable early indicators of nutrient over-enriched seagrass meadows has been ongoing for several decades. Such indicators would benefit coastal resource managers in efforts to prevent “endpoints” of severe disruption of ecosystem function manifested by seagrass die-off and increased high-biomass algal blooms (Kemp et al., 1983; Orth and Moore, 1983; Short and Burdick, 1996). Direct measures of water-column nutrients are generally ineffective (e.g. Tomasko et al., 1996; Morris and Virnstein, 2004) since the nutrients typical of early enrichment are rapidly taken up by plants, adsorbed to particulate sediments, or otherwise removed from the water (e.g. Suttle and Harrison, 1988; Suttle et al., 1990). In addition, the rate of nutrient supply (i.e. recycling) can be important in controlling water-column concentrations (Howarth, 1988). The importance of considering watershed nutrient loads rather than water-column nutrient concentrations in assessing enriched conditions for seagrasses was shown by Tomasko et al. (1996): *T. testudinum* biomass and productivity in Sarasota Bay, FL, USA were negatively correlated with watershed N loads but not with water-column nutrient concentrations as assessed by routine monitoring programs, and water quality parameters did not clearly reflect differences in watershed nutrient inputs (Fig. 7; also see Fig. 2B for Waquoit Bay in New England, and Status of modeling efforts, below).

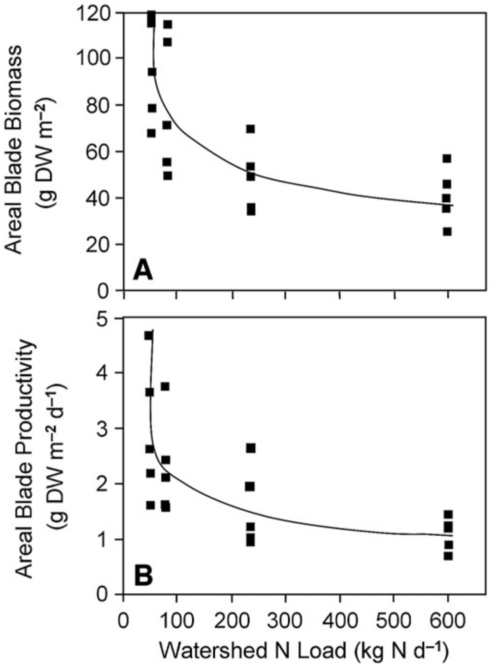

Fig. 7. (A) Areal blade biomass and (B) areal blade productivity plotted against watershed nitrogen loads for *Thalassia testudinum* from four sites in Sarasota Bay. Line is best-fit relationship. Modified from Tomasko et al. (1996).

Various biomarkers, used cautiously, have been successfully applied to indicate nutrient enrichment, sometimes even early onset (e.g. Maier and Pregnall, 1990), in seagrasses. A major area of emphasis has been to use seagrasses as “long-term” integrators (days to weeks) of variations in nutrient availability through analyses of their morphology and, especially, their tissue content (Gerloff and Krombholtz 1966; Atkinson and Smith, 1984; Fourqurean et al., 1992a,b). Spatial and temporal variations in C:N:P ratios or in C:N ratios have been used, with some success, as indicators of seagrass nutritional status, and in tracking nutrient supply sources, especially N (Touchette and Burkholder, 2000) (Table 2, Fig. 8). They can be especially helpful in assessment of nutrient-enriched seagrasses. Increased tissue N as a result of N enrichment has commonly been reported for seagrasses, and seagrasses from low-nutrient habitats have significantly higher C:N and C:P ratios than plants from high-nutrient conditions (Atkinson and Smith, 1984; Duarte 1990; Lee and Dunton 1999b). Tissue nutrient content of seagrasses reflects spatial and seasonal patterns in environmental nutrient regimes (Burkholder et al., 1992, 1994; Fourqurean et al., 1997). Although leaf nutrient content decreases with tissue age due to loss of N (e.g. Pirc, 1985; Pedersen and Borum, 1992), total shoot N content is dependent upon relative

Table 2
Parameters examined for use as nutrient enrichment indicators in seagrasses and seagrass ecosystems, and comments on efficacy

Parameter	Species examined	Comments on efficacy	Reference
Environmental			
Water-column N, P	–	Not consistently related to proximity of nutrient sources; do not reflect differences in modeled watershed N loads.	Tomasko et al. (1996)
		Poor indicators of trophic status of marine and estuarine ecosystems.	Smith et al. (1981), Valiela et al. (1990)
Sediment NH_4^+, PO_4^{-3}	–	Not related to proximity of nutrient sources.	Udy and Dennison (1997b)
Watershed N loads	*Thalassia testudinum*	Significant negative correlation with biomass, productivity.	Tomasko et al. (1996)
Morphological (canopy height, shoot density, biomass, growth)			
Leaf length, width	*Zostera marina*	Strong correlation with sediment N: short, narrow leaves in low-N sediment; long, wide leaves in high-N sediment (holding other factors such as light, flow and tidal exposure constant).	Short (1983a)
All factors	*Zostera capricorni*[a]	Not related to proximity of nutrient sources.	Udy and Dennison (1997b)
Number of leaves per shoot, blade width, leaf length, sheath length	*Z. marina*	Only weakly correlated with leaf tissue N (can be influenced by many other factors).	Lee et al. (2004)
Physiological			
Tissue C, N, P concentrations, ratios	27 temperate and tropical species	N and P are highly variable within species whereas C is relatively constant; median values of 1.8% N (dry weight) and 0.2% P can be used to infer nutrient limitation.	Duarte (1990)
Tissue C, N, P	Many species	Leaf C:N and C:P ratios higher, and/or C reserves higher, in un-enriched than enriched environments.	Atkinson and Smith (1984), Duarte (1990 and references therein), Burkholder et al. (1992, 1994), Invers et al. (2004), Fourqurean et al. (1997), Lee and Dunton (1999b), Touchette and Burkholder (2000, 2001, 2002), Touchette et al. (2003)
Comparison of above- versus belowground C, N and P contents	*Z. marina*	Can provide insights about effects of N enrichment or high temperature on C storage and plant survival (weeks to months).	Burkholder et al. (1992, 1994); Burke et al. (1996); Touchette and Burkholder (2000, 2001, 2002)
Tissue N content	*Z. marina*	Reflected environmental N gradients, but highly variable, limiting its utility to detect early stages of eutrophication.	Lee et al. (2004)
Glutamine, asparagine levels	*Z. capricorni*[a]	Can indicate elevated N assimilation/storage at nutrient-enriched sites.	Udy and Dennison (1997b), Touchette and Burkholder (2000)
	Posidonia oceanica	Used to indicate elevated N assimilation.	Invers et al. (2004)
Nitrate reductase	*Z. marina*	Indicated localized areas of groundwater nitrate enrichment into the water column of shallow nearshore meadows.	Roth and Pregnall (1988), Maier and Pregnall (1990)
		Indicated pulsed water-column nitrate enrichment in experimental mesocosms.	Roth and Pregnall (1988), Touchette and Burkholder (2000, 2001, 2002, 2007-this volume), Touchette et al. (2003)
Alkaline phosphatase	*Z. marina*	Can indicate phosphate limitation; recommended use together with other biomarkers.	Pérez and Romero (1993), Hernández et al. (1994a,b), Martinez-Crego et al. (2006), Touchette and Burkholder (2007)

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Table 2 (*continued*)

Parameter	Species examined	Comments on efficacy	Reference
δ^{15}N values	*Z. marina*	Can indicate nutrient sources (natural and anthropogenic).	Fourqurean et al. (1997), Udy and Dennison (1997b)
Area-normalized leaf mass (mg dry weight cm^{-2})	*Z. marina*	Strong, consistent negative relationship with leaf tissue N; significantly responded to estuarine nutrient gradients.	Lee et al. (2004)
Ratio of leaf N:leaf mass	*Z. marina*	More sensitive, consistent indicator of early eutrophication than either characteristic alone; recommended for use as a nutrient pollution indicator (NPI).	Lee et al. (2004)

[a] See taxonomic review in Moore and Short (2006).

nutrient availability (Fourqurean et al., 1997). It should be noted that the C:N ratio of seagrass leaves is a function of light as well as nutrient availability, since high photosynthetic rates at high light can lead to depletion of nutrient supplies (Zimmerman et al., 1987; Abal et al., 1994; Grice et al., 1996). In addition, under field conditions the time of year and both intra- and inter-specific differences should be considered in interpreting tissue nutrient content and other physiological variables (Agawin et al., 1996; Fourqurean et al., 1997; Udy and Dennison, 1997a,b). Even so, the variability in the content of nutrients in seagrass tissue can be a “potentially a superior indicator of nutrient availability when compared to measures of nutrient standing stocks in the water column and sediments” (Fourqurean et al., 1997, p. 154).

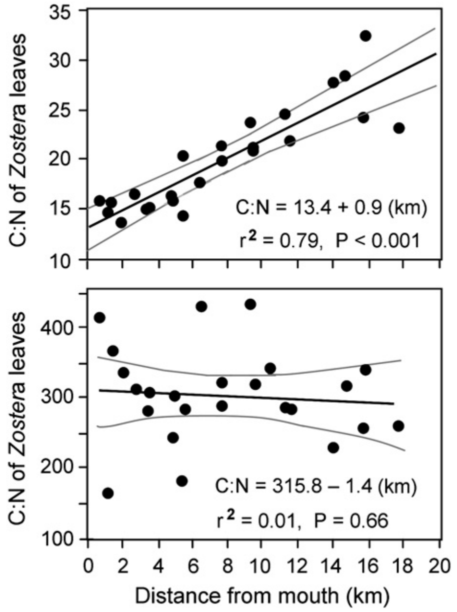

Fig. 8. Spatial pattern in (upper panel) the C:N ratio and (lower panel) the C:P ratio of green leaves from *Zostera marina* in Tomales Bay, California, USA, August 1992, also showing the least squares linear regression and 95% confidence interval for the regression. Position is represented by the distance (km) from the mouth of the bay, where nutrient enrichment was lowest. From Fourqurean et al. (1997), with permission from the publisher.

Data on C, N and P contents and nutrient ratios from 27 seagrass species at 30 locations were compiled by Duarte (1990) to examine the ranges and variability of C, N and P contents within and among species. N and P varied considerably within species, but C was relatively constant, likely reflecting the influence of structural carbon and, thus, C:N and C:P ratios were inversely related to changes in N and P contents. Duarte (1990) observed that the rate of change in C:N and C:P ratios with increasing N or P content in seagrass tissues should shift from large to small as nutrient supplies meet the plant’s demand. He suggested that the median N and P contents from the data (1.8% N and 0.2% P as percent dry weight) can be useful to predict the likelihood of nutrient limitation of seagrass meadows. In Tomales Bay, CA, USA, Fourqurean et al. (1997) found a strong spatial trend in tissue N content, but not P content, of *Z. marina*, and concluded that analysis of patterns in variation of C, N and P contents and isotopic composition (below) can be a powerful tool for investigating ecosystem-scale processes in coastal marine systems.

Morphological traits have been used with less success in assessing seagrass enrichment (but see use of the ratio of leaf N:leaf mass in Lee et al., 2004, discussed below), probably because they are also strongly influenced by other environmental factors such as light availability, current and wave strength, and tidal exposure. Short (1983a) reported a strong correlation between sediment N and eelgrass leaf morphology: Plants with short, narrow leaves grew in low-N sediment, whereas plants with long, wide leaves were found in high-N sediments (Table 1). Udy and Dennison (1997b) examined morphological and physiological characteristics (canopy height, shoot density, biomass, growth, tissue nutrient content, amino acid concentrations, δ^{15}N values of *Zostera capricorni* in Moreton Bay, Australia, near versus remote from nutrient sources (sewage, septic or prawn farm effluent, or river discharge) in an attempt to identify parameters that could be used to indicate anthropogenic nutrient inputs. Sediment nutrient concentrations (NH_4^+, PO_4^{-3}) and plant morphology and growth were not related to proximity to nutrient sources. On the other hand, certain physiological parameters, especially tissue nutrient content (C, N, P) and amino acid concentrations (notably glutamine and asparagine), were considered useful in identifying nutrient-enriched areas or seagrass "nutrient saturation", since these parameters consistently were higher at nutrient-enriched sites. Nutrient contents of *Z. capricorni* leaves at sites remote from anthropogenic nutrient sources approximated the critical values for limitation by N and P that were suggested by Duarte (1990). Increases in glutamine, a critical amino acid involved in N assimilation, logically may reflect elevated N assimilation at nutrient-enriched sites; asparagine and glutamine may also be used for N storage (Udy and Dennison, 1997b; Touchette and Burkholder, 2000).

The activities of certain enzymes, notably NR and alkaline phosphatases (APAs), have been suggested as indicators of nutritional status for some seagrasses. While sediment NH_4^+ regeneration in *Z. marina* meadows is sufficient to provide most or all of the N required for *Z. marina* growth (Iizumi and Hattori, 1982; Short, 1983b; Dennison et al., 1987), plants growing near the edge of a meadow can have much lower interstitial N available to them (Short, 1983b). In elegant work, Roth and Pregnall (1988) found that plants growing at the shallow nearshore edge of a *Z. marina* meadow had high NR activity (up to 1500 nmol nitrite produced g^{-1} fresh weight h^{-1}). Their suggestion that high NR in *Z. marina* might result from chronic or episodic exposure to nitrate via groundwater inputs was borne out by subsequent research which showed that in areas where the plants had high NR, pore water NO_3^- concentrations were 100–400 μM within a few meters inland from the waterline; and that there was high, variable nitrate efflux into the nearshore water column (2160+660 μmol m^{-2} h^{-1}) when associated with rapid percolation (37+11 L m^{-2} h^{-1}) of nitrate-enriched pore water (Maier and Pregnall, 1990). Turbulent wave mixing rapidly diluted the nitrate. Maier and Pregnall (1990) also showed that macroalgae and *Z. marina* growing adjacent to a beach with high-nitrate efflux had NR activities 3- to 7-fold higher than those of algae and *Z. marina* growing along a beach section with low-nitrate efflux. NR of *Z. marina* increased in response to low daily nitrate additions (10–25 μM) as less dramatic, but still detectable responses. The high variation in flux rates that Maier and Pregnall (1990) observed over small distances was believed to reflect heterogeneity both of nitrate enrichment and of groundwater discharge through sandy beaches into nearshore water columns (e.g. Johannes, 1980). Thus, unless intensive surveying is performed, high-nitrate locations could easily be missed, and elevated *Z. marina* NR could be useful in detecting such areas.

The ecological role of phosphatases, especially APAs, has been linked to P deprivation in seagrasses as well as many other aquatic plants (Pérez and Romero, 1993; Touchette and Burkholder, 2000, 2007). Martinez-Crego et al. (2006) combined correlational (across-sites comparison) and experimental approaches to evaluate the utility of APA as a eutrophication marker in *Posidonia oceanica*. APA decreased immediately following (nitrate, phosphate and ammonium) additions and the response was maintained except during the winter season. APA also varied across natural meadows under different levels of nutrient discharges at scales relevant for monitoring purposes. The authors considered APA as an "optimal physiological biomarker" that responds quickly to pulsed nutrient enrichment. They suggested, however, that because of its seasonal limitations and its variation with depth and sediment characteristics (e.g. carbonate content), APA should be used together with other bioindicators in assessment of eutrophication. Factors such as light, temperature, pH, salinity, epiphytic development, and (under non-P_i limited conditions) ambient metabolic conditions for C and N assimilation are also known to influence seagrass APA activity (Pérez and Romero, 1993; Hernández et al., 1994a,b; Lapointe et al., 1994; Touchette and Burkholder, 2007). Moreover, in some studies, significant correlations between seagrass APA activity and low P_i have been lacking (e.g., Hernández et al., 1994a; Touchette and Burkholder, 2007), possibly because growth was not P-limited or because other environmental and developmental variables more strongly influenced APA activities.

In areas with few major N sources, seagrass tissue $\delta^{15}N$ values can be used to help identify the N source, although this parameter cannot be used to infer the nutrient load (Fourqurean et al., 1997). A nice illustration of the utility of $\delta^{15}N$ values was provided by Udy and Dennison (1997b), who reported that in Moreton Bay, Australia, $\delta^{15}N$ values were 9.2‰ for treated sewage particulates, 5.1‰ for raw sewage, and (by definition) ~0‰ for N from N_2 fixation. High tissue values for *Z. capricorni* (9.3‰) suggested sewage discharge sources of N, whereas *Z. capricorni* in sites remote from anthropogenic sources had low $\delta^{15}N$ values (~2.4‰) indicating N_2 fixation as a major source. Similar work that suggested the importance of natural processes as a source of N for seagrasses in unenriched areas was contributed by Fourqurean et al. (1997) from a study of *Z. marina* in Tomales Bay, CA, USA.

While tissue nutrient content, concentrations of certain amino acids, activities of certain enzymes, δ^{15}N values and, sometimes, morphological features have been used to assess seagrass enrichment, at present there are no available, reliable tools for *early* assessment of nutrient over-enrichment in seagrasses. In an attempt to address this need, Lee et al. (2004) assessed the morphology and leaf tissue N content of *Z. marina* along estuarine N gradients in three New England estuaries. Leaf N content reflected environmental N gradients but was highly variable, limiting its use in detecting early stages of eutrophication. Plant morphological characteristics (number of leaves per shoot, blade width, leaf length, sheath length) were only weakly correlated with leaf tissue N. Area-normalized leaf mass (mg dry wt cm^{-2}) exhibited a strong and consistently negative relationship with leaf tissue N, and a significant response to the estuarine nutrient gradients (Fig. 9). The ratio of leaf N:leaf mass was a more sensitive and consistent indicator of early eutrophication than either characteristic alone. Lee et al. (2004) suggested use of this ratio as a nutrient pollution indicator (NPI) for *Z. marina*, with potential application to other seagrasses. This NPI may hold promise for some seagrass species, although its sometimes-substantial variability across nutrient enrichment gradients (Fig. 10) suggests that it should not be used alone but, rather, in consideration with other environmental and physiological parameters to indicate seagrass meadows under early eutrophication.

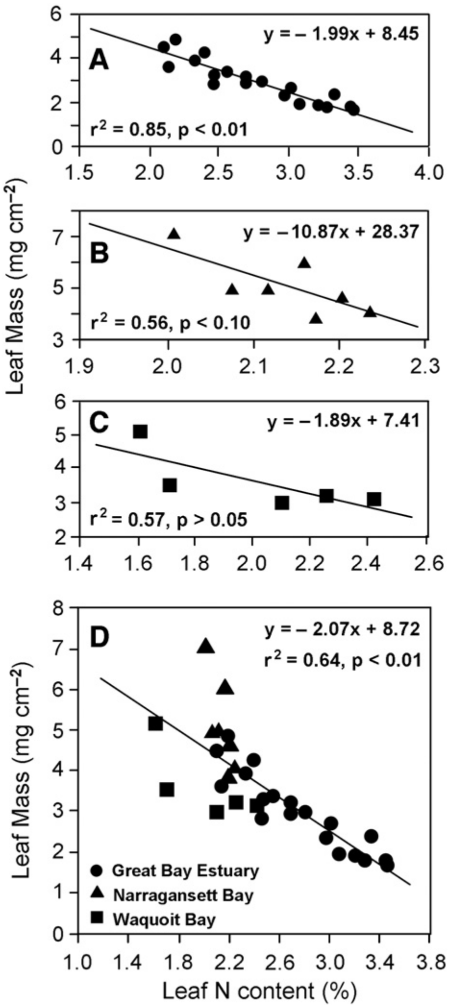

Fig. 9. Relationship between leaf N content and leaf mass in (A) Great Bay Estuary, (B) Narragansett Bay, (C) Waquoit Bay, and (D) all three ecosystems collectively. From Lee et al. (2004), with permission from the publisher.

5. Oligotrophication and seagrasses: recovery, replacement, or sustained declines

Given the global expansion of the human population with high density especially in coastal areas (Nicholls and Small, 2002), there are few examples of seagrass meadow recovery following nutrient reductions (e.g. Duarte et al., 2006; Walker et al., 2006). One of the best documented recoveries was in Tampa Bay, Florida, USA: Estimates of localized seagrass declines (*T. testudinum*, *H. wrightii*) in parts of Tampa Bay (i.e., Hillsborough Bay) exceeded 90% between 1948 and 1982. Following a 57% reduction in N loading between the 1980s and 2002, lower phytoplankton biomass and improved water clarity have coincided with recovery of more extensive seagrass coverage (Johansson and Greening, 2000; Tomasko et al., 2005; Ralph et al., 2006). Seagrasses in nearby Sarasota Bay, Florida responded similarly to a 46% reduction in N loading (Tomasko et al., 2005).

As an example of declines, partial recovery (by another species) and further declines, major loss of *T. testudinum* in Florida Bay in 1987, initially replaced by *H. wrightii*, was attributed to various factors including a protistan pathogen (*Labyrinthula*), increased turbidity, sulfide toxicity and elevated water temperatures; in addition (although in debate), a possible role was suggested for increased salinity from a drought, altered hydrology, and reduced flow of rivers to the bay (see reviews in NRC, 2002; Lapointe and Barile, 2004; Walker et al., 2006). Eutrophication was also an important contributing factor (NRC, 2002), evidenced by increased nutrient loads to the shallow, N-limited western and central regions of the bay, and high phytoplankton, epiphytic and macroalgal biomass (Lapointe and Clark, 1992; Boesch et al., 1993; Lapointe and Barile, 2004). When the drought lifted in the early 1990s, the sustained nutrient enrichment

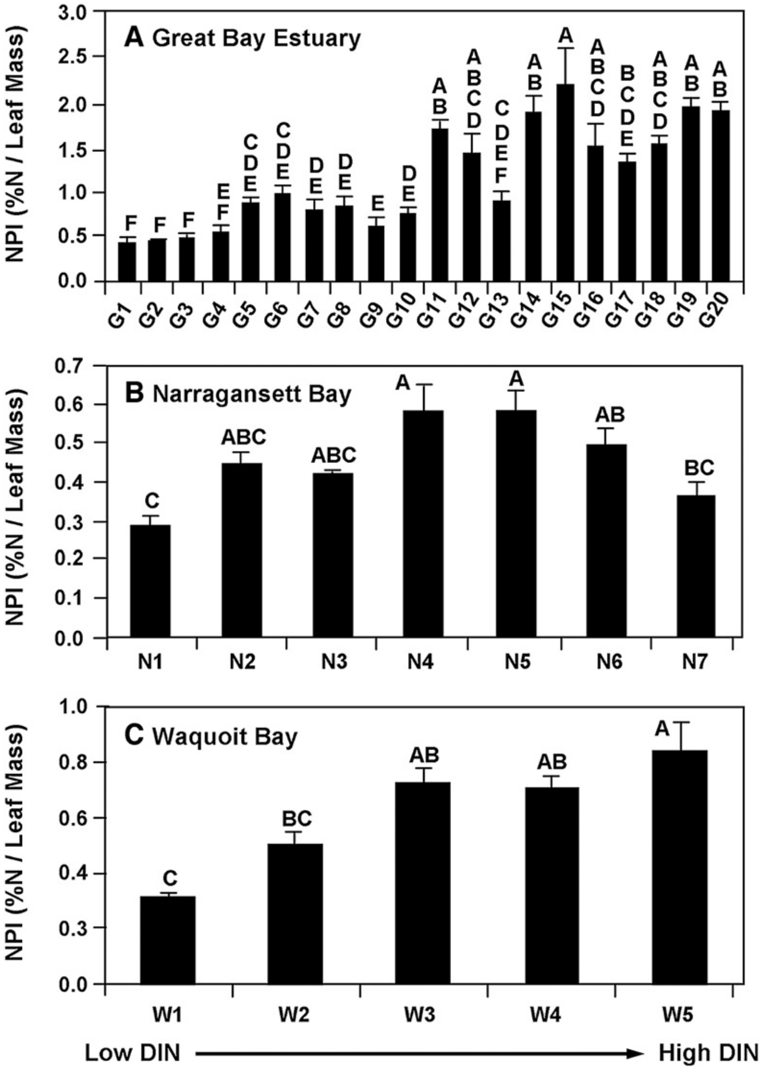

Fig. 10. The nutrient pollution indicator (NPI), defined as the ratio of leaf nitrogen content (%N) to leaf mass, along dissolved inorganic nitrogen (DIN) gradients in (A) Great Bay Estuary, (B) Narragansett Bay, and (C) Waquoit Bay. Values within the same letter (listed above each bar) are not significantly different among sampling stations ($P<0.05$). Modified from Lee et al. (2004).

and light reduction from phytoplankton blooms and other algal growth led to additional losses not only of *T. testudinum*, but also of *H. wrightii* and *Syringodium filiforme* (Hall et al., 1999). The more troubling case – of seagrass loss, nutrient reductions, and lack of seagrass recovery – is exemplified in Cockburn Sound, Western Australia. *Posidonia sinuosa*, *P. angustifolia* Cambridge and Kao, and *P. australis* Hooker substantially declined from 1976 to 1981 in apparent response to eutrophication, but did not recover in the 1980s following reductions in nutrient loads (Silberstein et al., 1986; Walker et al., 2006).

Rates of seagrass loss and recovery have been linked to species-specific differences in lifespan, extent of clonal growth, and seedling recruitment (Walker et al., 2006). The relationship between reproductive effort and patch formation is complex because generally a low percentage of shoots, germinated from seeds that must drift into the area, survives to develop patches (reviewed in Duarte 1995). Relatively rapid re-growth (months to years) has been documented for *Zostera japonica* Ascherson et Graebner (Baldwin and Lovvorn, 1994), *H. decipiens* Ostenfeld (Williams, 1988), and *H. wrightii* (Robbins and Bell, 2000). In contrast, *Posidonia* spp. only re-grow at the scale of decades (Meehan and West, 2000; Gobert et al., 2006; Walker et al., 2006). Thus, for more long-lived genera such as *Posidonia* and *Thalassia*, the time scale of recovery appears to be very slow, ranging from several years to centuries

(Birch and Birch, 1984; Duarte, 1995; Walker et al., 2006). Researchers who assessed recovery of *P. oceanica* beds in Cabrera Archipelago National Park (Spain) concluded that while regulation has improved the status of seagrasses in the park, full recovery may even require centuries, and will be threatened by organic inputs from visitors to the park (Marbà et al., 1996).

It has been suggested that seagrass declines and recoveries in poorly flushed bays and lagoons follow a natural cycle (Morris and Virnstein, 2004). Declines have been hypothesized to occur after long-term accumulation of dead seagrass and mats of drift algae form a thick (10–15 cm) layer of organic detritus and ooze in which the poorly rooted plants are stressed by high sulfide concentrations (Zimmerman and Montgomery, 1984). The loss of plants through mass mortality and the removal of the ooze layer by storm events “resets” the area so that recolonization, by seeds from populations in adjacent areas, begins on primarily mineral sediments. The frequency of this natural cycle is not known, but given the fact that there are few examples of seagrass meadow recovery following nutrient reductions (e.g. Duarte et al., 2006; Walker et al., 2006), it seems clear that the natural cycle is disrupted, slowed or indefinitely blocked by cultural eutrophication.

6. Status of modeling efforts

Physiological models of seagrass response to nutrient resources mostly have remained at the level of conceptual models showing various metabolic pathways, or of quantitative models using classical Michaelis–Menten and Monod equations for uptake and growth, respectively, and sometimes using Droop equations for cell quota with nutrient forms considered separately (see reviews in Zimmerman et al., 1987; Touchette and Burkholder, 2000). Zimmerman et al. (1987) developed a mathematical model for *Z. marina* response to N_i (both NO_3^- and NH_4^+) × availability of photosynthetically active radiation. The model was applied to make steady-state predictions about the relative effects of light and N_i availability on N_i uptake and partitioning between above- and belowground tissues. It was also applied to estimate the N_i concentrations in the sediment and water column that would be required to saturate growth. The following assumptions were used: Nutrients and light were donor-controlled, non-depletable resources; NH_4^+ uptake occurred in above- and belowground tissues, whereas nitrate uptake occurred only in aboveground tissues; and NH_4^+ uptake by roots and nitrate uptake by leaves was feedback-inhibited depending upon the degree of saturation of NH_4^+ uptake by aboveground tissues. The model indicated that roots may be more important in overall N acquisition in most natural habitats, but that leaves become increasingly important in supplying N to this species under low-light regimes characteristic of eutrophic conditions. N_i levels required to saturate growth were estimated to be less than commonly reported concentrations, leading the authors to suggest that N limitation of *Z. marina* historically was rare.

A more complex model was developed to simulate photosynthesis and growth of *Z. marina* in lower Chesapeake Bay (Wetzel and Neckles, 1986). The model was based upon theoretical non-linear functions to simulate biologically controlled processes, and empirical or statistical relationships to include physical/chemical interactions and environmental forcing functions. Model runs over 1, 4 and 10 yr identified light and temperature as the major physical factors that controlled *Z. marina* photosynthesis and growth, but typical conditions were suboptimal for growth so that small changes in light and/or temperature predicted decline and eventual loss of *Z. marina* habitat. Model simulations also suggested that a major factor controlling the growth and long-term survival of *Z. marina* was the interaction between epiphytic grazing intensity and ambient light levels, with nutrient enrichment affecting epiphyte abundance and light availability.

Models that reliably predict population response to cultural eutrophication are lacking for most seagrass species, or limited to localized areas (e.g. Tomasko et al., 2001; Fourqurean et al., 2003; Hauxwell and Valiela, 2004). Seagrass response to nutrient loadings has proven difficult to quantify beyond localized areas because long-term data consistent in quality are generally lacking, and seagrass abundance and productivity in natural habitats are often highly variable from year to year because of stochastic meteorological and hydrographic conditions (Valiela et al., 1992; Tomasko et al., 2001; Howarth et al., 2002; Burkholder et al., 2006). Early empirical models by Nielsen et al. (1989) and Duarte (1991) enabled simple predictions of areal loss of seagrass meadows following reduced water transparency from eutrophication. Nielsen et al. (1989), for example, reported a positive linear relationship between phytoplankton and TN concentrations, and a negative relationship between TN and depth distribution of seagrasses, based on a study of 20 estuaries across a gradient of N loading in Denmark. Beyond these efforts, quantitative or semi-quantitative relationships rarely have been developed for nutrient loads or concentrations and algal versus seagrass abundance (Sand-Jensen and Borum, 1991; Valiela et al., 1992; Johansson and Greening, 2000; Tomasko et al., 2001; Hauxwell et al.,

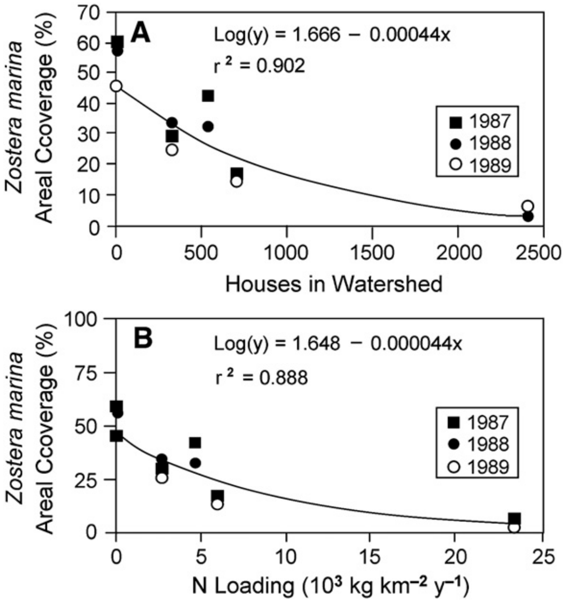

Fig. 11. Relationship between seagrass areal coverage (log of eelgrass area) in Waquoit Bay during 1987–1989 and (A) the number of houses in the sub-watersheds, and (B) nitrogen loading. From Short and Burdick (1996), with permission from the publisher; note that loading was estimated based on watershed area.

2003; Hauxwell and Valiela, 2004). Two examples are highlighted as follows:

In Lemon Bay, Florida, USA, Tomasko et al. (2001) were able to predict depth limits for seagrass meadows based upon water clarity alone ($r^2 = 0.46$ for predicted versus observed depth limits). They modeled area-normalized N loads (with area referring to the size of the watershed) for pre-development conditions (~1850–5.3 kg TN ha^{-1} yr^{-1}) versus in 1995 (8.4 kg TN ha^{-1} yr^{-1}), and targeted stormwater runoff as responsible for about three-fourths of the annual N load, followed by septic tank systems (14%), rainfall (10%), and a negligible contribution from baseflow. An empirically derived ratio of N load: phytoplankton chlorophyll *a*, developed for Tampa Bay waters, was used to estimate that a 45% increase in N loads to Lemon Bay could cause a ~29% increase in annual average chlorophyll *a* concentrations. That chlorophyll *a* increase was applied to an empirically derived optical model for Lemon Bay to estimate that light attenuation coefficients in Lemon Bay would, in turn, increase ~9%, leading to a ~24% decrease in the average depth limit for the dominant seagrass, *T. testudinum*. The authors cautioned, however, that more uncertainty should be expected in attempting to predict changes in biomass and productivity in remaining seagrass meadows under further reduction in water clarity. Considering a previously described inverse relationship between watershed N loads and seagrass biomass and productivity in nearby Sarasota Bay (Tomasko et al., 1996; Fig. 7), Tomasko et al. (2001) hypothesized that decreased water clarity from further urbanization and increased N loads would lead to fewer acres of seagrass meadows, as well as sparse, less productive remaining beds.

Waquoit Bay of New England in the USA has been the focus of intensive research on *Z. marina* response to cultural eutrophication (e.g. Fig. 2). Short and Burdick (1996) retrospectively related housing development and N loading to loss of *Z. marina* over a 3-yr period (Fig. 11). Hauxwell and Valiela (2004) summarized relationships between area-normalized annual N loads (with "area" referring to the size of the open water [not the watershed]) to changes in *Z. marina* and algal abundance in small, shallow sub-estuaries of Waquoit Bay, based on a decadal dataset. The range of N loads to these estuaries

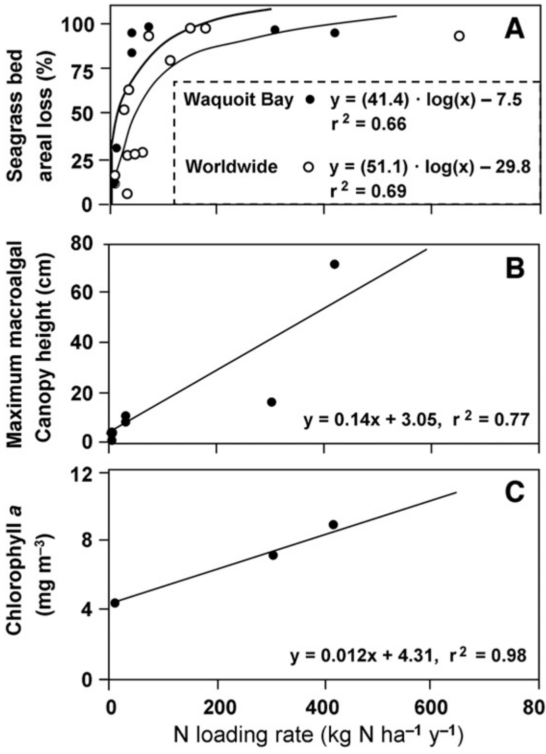

Fig. 12. (A) Relationship between seagrass loss (*Zostera marina* for the Waquoit estuaries between 1987 and 1997, adapted from Hauxwell et al. (2003); other reports from around the world adapted from Valiela and Cole 2002). (B) Maximum macroalgal canopy height (annual maximum from monthly sampling, adapted from Hauxwell et al., 2003). (C) Phytoplankton production (4-yr annual average between 1990 and 1994, based upon monthly sampling) and nitrogen loading rates to the Waquoit Bay estuaries. From Hauxwell and Valiela (2004), with permission from the publisher; note that loading was estimated based on area of open water.

encompassed ~75% of the range of reported N loads to various estuaries worldwide. The inputs occurred mostly in groundwater, and resulted in gradual fragmentation and loss of the meadows. Seagrass loss rapidly followed increased N loads, and the same logarithmic relationship held for various estuaries throughout the world considered collectively (Fig. 12). Seagrasses remained only in estuaries that received N loading within the lowest 12% of N loads worldwide. Concomitantly, macroalgal canopy height and phytoplankton increased linearly with N loading in Waquoit Bay estuaries, as reported for other systems (Nielsen et al., 1989).

Various authors have been careful to clarify, however, that physical factors such as water residence time can alter or completely mask these relationships, making it difficult, at best, to observe temporal patterns even at relatively small scales. In addition, fringe buffer wetland areas can reduce nutrient loading to seagrass meadows and mute predicted effects. For example, larger areas of fringing salt marsh or mangrove swamps around seagrass meadows resulted in higher seagrass production and smaller losses as N loads increased (Valiela and Cole, 2002). Overall, concerted efforts have led, for certain ecosystems, to development of models that retrospectively quantify the levels of nutrient loading that cause seagrass decline, but for many systems, the diversity of processes involved in seagrass response – and the fact that the response may not be proportional to the changes in nutrient loading – renders quantitative prediction of these changes difficult (Duarte 1995). As Hauxwell and Valiela (2004, p. 85) summarized, "While in certain settings, the relationship between nutrient loading and seagrass loss seems relatively straightforward, we must emphasize that additional ecological complexities may alter processes and patterns."

In consideration of future efforts to improve protection of remaining seagrass meadows and reduce nutrient loading, Duarte (1995) noted that reversing the eutrophication process suggests replacement of rapidly growing algal species by more slowly growing seagrasses, with the expectation of increased lag periods between nutrient reductions and seagrass recovery. Duarte (1995) developed a simulation model for seagrass recovery from eutrophication as a non-linear process best represented by a logistic growth curve ($r^2=0.92$, $P<0.0001$):

$$\begin{aligned}\log \% \,\text{cover} = 2{*}[1.5\cdot\log \text{time (years)} + 1.24\\ \cdot \log \text{patch elongation}\left(\text{m}^{-2}\,\text{yr}^{-1}\right) + 0.61\\ \cdot \log \text{patch formation}\left(\text{patches m}^{-2}\,\text{yr}^{-1}\right)].\end{aligned}$$

This model projects that seagrass recovery will be more rapid with increasing rate of patch elongation than with increasing rate of patch formation. It predicts that the time scale needed for meadow re-formation (95% cover) would range from less than 1 yr (small, rapidly growing species, e.g. *Cymodocea nodosa*) to about four centuries (large, slowly growing species, e.g. *P. oceanica*). Thus, although seagrass loss can be rapid, the ecosystem consequences may last for decades or centuries after nutrient reductions are imposed (Duarte 1995).

7. Further needs

Most available information on seagrass physiological responses to nutrient over-enrichment has been obtained from relatively few species. The species that have been examined in detail have sometimes varied substantially in nutritional response and the basic nutritional physiology of many seagrasses remains to be examined and compared across geographic regions (Touchette and Burkholder, 2000). Certain physiological variables show promise as indicators of nutrient conditions and anthropogenic nutrient enrichment, and should continue to be rigorously developed at the species level, together with growth and other metrics, for potential value in assessing seagrass nutritional status and seagrass population health.

Seagrasses have been described as "'coastal canaries', global biological sentinels of increasing anthropogenic influences in coastal ecosystems" (Orth et al., 2006). An important advance in addressing a fundamental monitoring need to improve documentation of changes in seagrass meadows worldwide was recently achieved with the advent of SeagrassNet in 2001 (Short et al., 2006). SeagrassNet, the first program developed to monitor seagrasses on a global basis, uses a consistent protocol for both seagrass parameters and environmental parameters to assess change in seagrass habitat over relatively short periods (1- to 2-yr intervals), through quarterly monitoring of permanent transects and plots. At present, SeagrassNet has been established at 48 sites in 18 countries, with data collection by a team of trained local people, and maintenance of a central database. As it continues to develop and strengthen, this program should provide a powerful tool for coastal resource managers through improved tracking of seagrass populations over time. Such exemplary programs should be coupled with improved models to enable reliable, quantitative forecasts of the cumulative effects of eutrophication from adjacent watersheds and other stressors on seagrass growth and survival (Orth et al., 2006).

Additional research should emphasize experimental studies to assess the response of a wider range of species to chronic, low-level as well as acute, pulsed nutrient enrichment. These experiments should extend over the growing season for the species. They should be conducted in the field or in large-scale mesocosms following appropriate acclimation, and should emphasize factor interactions (N, P, C; turbidity; temperature; flushing rate; herbivory) to more closely simulate reality in seagrass ecosystems (Moore et al., 1996; Short and Neckles, 1999). They should also "scale up" to address processes that occur over larger scales, including food-web dynamics that involve highly mobile predators and herbivores (Valentine and Duffy, 2006).

More than a decade ago, Tomasko et al. (1996, p. 448) wrote, "Despite the well-documented relationship between increased water-column nutrient input and degradation of seagrass habitats, seagrass losses continue at unprecedented rates on a global scale." A decade later there has been no change in that trend; catastrophic losses of seagrass meadows continue worldwide along rapidly urbanizing coastal zones (Walker et al., 2006), despite recognition of the enormous ecological and economic value of seagrass meadows (Orth et al., 2006). A global conservation effort to protect seagrass habitat is critically needed (Orth et al., 2006). Thus far, efforts to protect remaining seagrass meadows from damage and loss under cultural eutrophication, within countries and across regions, are generally lacking or weak, ineffective and even sometimes counter-productive. As a result, globally about 20% of documented seagrass area had disappeared because of direct and indirect human impacts, and many remaining beds are increasingly stressed and fragmented (Green and Short, 2003). While additional research can help to strengthen insights about seagrasses under cultural eutrophication, without any further research one point is presently very clear: Concerted, strengthened local and national policies and actions, thus far mostly lacking, are needed worldwide to protect remaining seagrass meadows from accelerating cultural eutrophication in rapidly urbanizing coastal zones.

Acknowledgments

Funding for this research was provided by the Park Foundation and the North Carolina General Assembly. **[SS]**

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