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Nitrogen loading alters seagrass ecosystem structure and support of higher trophic levels

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ABSTRACT

1. Anthropogenic-derived nutrient inputs to coastal environments have increased dramatically worldwide in the latter half of the 20th century and are altering coastal ecosystems. We evaluated the effects of nitrogen loading on changes in macrophyte community structure and the associated fauna of a north temperate estuary. We found that a shift in primary producers from eelgrass to macroalgae in response to increased nutrient loading alters habitat physical and chemical structure and food webs. As nitrogen load increased we found increased macroalgal biomass, decreased eelgrass shoot density and biomass, decreased fish and decapod abundance and biomass, and decreased fish diversity.

2. The central importance of macroalgae in altering eelgrass ecosystem support of higher trophic levels is evident in the response of the ecosystem when this component was manipulated. Removal of macroalgae increased eelgrass abundance and water column and benthic boundary layer O_2 concentrations. These changes in the physical and chemical structure of the ecosystem with lower macroalgal biomass resulted in higher fish and decapod abundance and biomass.

3. Both a ^{15}N tracer experiment and the growth of fishes indicated that little of the macroalgal production was immediately transferred to secondary consumers. $\delta^{15}N$ values indicated that the most abundant fishes were not using a grazing food web based on macroalgae. Fish tended to grow better and have a greater survivorship in eelgrass compared to macroalgal habitats.

4. Watershed-derived nutrient loading has caused increased macroalgal biomass and degradation and loss of eelgrass habitat, thus reducing the capacity of estuaries to support nekton.

KEY WORDS: ecosystem alteration; nutrient loading; estuarine; seagrass; food webs; eutrophication; fish; decapods

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INTRODUCTION

Anthropogenic-derived nutrient inputs to coastal environments have increased dramatically worldwide in the latter half of the 20th century and are altering coastal ecosystems (Valiela *et al.,* 1992; Nixon, 1995; Howarth *et al.,* 1996). The increased delivery of nutrients to coastal waters has been driven primarily by local changes in the watersheds (Howarth *et al.,* 1996; National Research Council, 2000), although global changes in atmospheric deposition also contribute (Vitousek *et al.,* 1997). The concern over nitrogen loading is particularly acute in estuaries because loading is increasing and because primary production in coastal waters is limited by nitrogen (Howarth, 1988). This increase in nitrogen loading is having large but only partially understood effects on estuarine ecosystem function. One of the prominent functions of estuaries is their support of higher trophic levels such as fish, decapod crustaceans and shellfish. Over 50% of all U.S. economically important and over 25% of all east-coast shelf fish species use estuaries at some stage in their life history (Houde and Rutherford, 1993; Ray, 1997).

Eutrophication can result in changes in ecosystem level dynamics such as productivity, dissolved oxygen concentrations, nutrient cycling, trophic structure and energy flow which have the potential to affect fish production (Nixon *et al.,* 1986; Breitberg *et al.,* 1997; Valiela *et al.,* 1997a,b). Although there are links between changes in estuarine ecosystem dynamics and fish production, in most cases these linkages have not been quantified.

In many temperate coastal systems, eelgrass (*Zostera marina*) is a dominant primary producer and is an important habitat for fish and invertebrates (Adams, 1976; Orth *et al.,* 1984; Virnstein, 1987; Deegan *et al.,* 1997). In many ecosystems, increased loading of the limiting nutrient alters the assemblage of primary producers (Rapport and Whitford, 1999). In coastal systems, nitrogen enrichment enhances the proliferation of faster growing phytoplankton, epiphytic algae and macroalgae that compete with seagrass for light and space (Valiela *et al.,* 1997b; Raffaelli *et al.,* 1998; Hauxwell *et al.,* 2001). This interaction is thought to be a major factor contributing to the widely observed declines in seagrass abundance worldwide (Orth and Moore, 1983; Cambridge *et al.,* 1986; Duarte, 1995; Raffaelli *et al.,* 1998).

Although the shift in primary producers in response to nitrogen loading has been well documented, the impacts of this change on estuarine food webs and the production of fishes and invertebrates are not well known. Many studies have suggested that the high productivity of seagrass ecosystems provides abundant food while their structural complexity benefits fish and invertebrates by providing protection from predation (Heck and Thoman, 1981; Leber, 1985; Bell and Westoby, 1986; Heck and Crowder, 1991; Heck *et al.,* 1997). In some estuaries, such as the Chesapeake Bay, competition with phytoplankton and epiphytes eliminates eelgrass and the resulting bare substratum does not support a diverse or abundant fish assemblage (Orth and Moore, 1983; Wyda *et al.,* in press). In shallow, low energy estuaries, macroalgae replace eelgrass (Harlin and Thorne-Miller, 1981; Duarte, 1995; Valiela *et al.,* 1997b; Hughes *et al*., in press). The replacement of eelgrass by macroalgae potentially changes the structural complexity, food webs and the chemical suitability of the habitat for nekton. The competition between macroalgae and eelgrass leads to diminished eelgrass growth and stature, and declines in shoot density and total habitat area (Short *et al.,* 1995; Short and Burdick, 1996; Hauxwell *et al*., 2001). Low dissolved oxygen levels and anoxia often occur in macroalgal mats due to the respiration of the algae and the decomposition of the accumulated macroalgal detritus (Johnson and Welsh, 1985; Hull, 1987; D'Avanzo and Kremer, 1994; D'Avanzo *et al*., 1996). The loss and degradation of seagrass habitats has undoubtedly affected the distribution and productivity of the animals that use them, however, for the most part, these changes have not been demonstrated (Dennison *et al.,* 1993; Hoss and Thayer, 1993).

The present study examines the effects of increased nutrient delivery from watersheds on eelgrass habitat plant communities, ecosystem physical and chemical structure, and fish and invertebrate abundance and diversity. We hypothesize that increased nutrient loading alters primary producers, replacing eelgrass with macroalgae. This replacement decreases ecosystem physical structure by replacing the tall, dense eelgrass

canopy with low lying, finely branched macroalgae mats, decreases chemical suitability by the development of low dissolved oxygen at the benthic boundary and alters food webs. We hypothesize that as macroalgal biomass increases the abundance of small fish and decapod abundance decreases because the changes in ecosystem physical and chemical structure will make these animals more susceptible to predation. We also hypothesize that these ecosystem structure changes will lead to little macroalgal primary production being passed up the food web to secondary consumers. Thus, as macroalgal biomass increases in response to nitrogen loading, the areal extent and suitability of eelgrass habitats as a nursery area for fish and decapods declines.

We used several approaches to test these hypotheses. We examined natural fish, invertebrate and macrophyte abundance in three sub-estuaries of Waquoit Bay that are subject to different nitrogen loading rates. This was a space-for-time substitution (Pickett, 1989), where different nitrogen loading rates in similar estuaries simulated changes over time due to increased anthropogenic nutrient loading (Valiela *et al.,* 1992). We conducted a macroalgal biomass experiment creating decreased and increased macroalgal biomass and examined the response of plants, invertebrates and fish. We conducted a macroalgal ^{15}N-enrichment experiment to trace macroalgal organic matter through the food web and examined the growth of fishes in eelgrass and macroalgal-dominated habitats.

METHODS

General site description

This study was conducted in 3 of the 9 interconnected sub-watersheds that make up the Waquoit Bay estuarine system (Figure 1). Hamblin, Timms and Sage Lot Ponds, are shallow sub-embayments with fringing salt marsh. The physical characteristics of these ponds are similar, with salinity ranging seasonally between 20‰ and 32‰ and temperature between 9°C and 30°C. The maximum depth at mean low water is between 1.1 and 1.3 m, with a tidal range of 0.2–0.3 m. Although eelgrass was historically abundant throughout Waquoit Bay, it has been declining in extent and abundance since the middle 1970s (Valiela *et al.,* 1992; Short and Burdick, 1996).

Nitrogen loading gradient comparison

To compare eelgrass ecosystems under differing nitrogen loading rates, we sampled the nekton (fish and decapod crustacea species), eelgrass and macroalgal communities of Hamblin, Sage Lot and Timms Ponds during the summers of 1992 and 1993. The variation in watershed development determines the amount of nitrogen a pond receives (Valiela *et al.,* 1992, 1997a, 2000). Upland development and nitrogen loading varies (Valiela *et al.,* 2000) from very low for Timms Pond (0 houses ha^{-1}, 16 kg N yr^{-1}) to light for Sage Lot Pond (0.12 houses ha^{-1}, 534 kg N yr^{-1}) to moderately high in Hamblin Pond (1.3 houses ha^{-1}, 1679 kg N yr^{-1}). We sampled two eelgrass areas in Sage Lot and Timms Ponds, and four areas in Hamblin Pond. Areas were sub-divided into quarters to facilitate distribution of samples around the site.

Eelgrass and macroalgae were measured in June and August 1992 and August 1993. We measured eelgrass shoot density in 0.063 m^2 quadrats haphazardly placed in each quarter of a site ($n = 8$ for a site at a sampling period). Macroalgal and eelgrass biomass was sampled by lowering a 0.073 m^2 cylinder, with a mesh bag (500 μm mesh) attached, over the eelgrass down to the sediment and using a thin plate at the sediment surface to cut and remove the enclosed eelgrass, algae and detritus. Samples ($n = 6$ per site per sampling period) were washed on a steel screen and algae were sorted to species. Plants were oven-dried at 60°C for (at least) 24 h and weighed (Cowper, 1978).

Nekton were sampled in June, July and August in 1992, and in June and August 1993. Nekton (fish and larger individuals of decapod crustacea species) abundance and species composition were sampled using

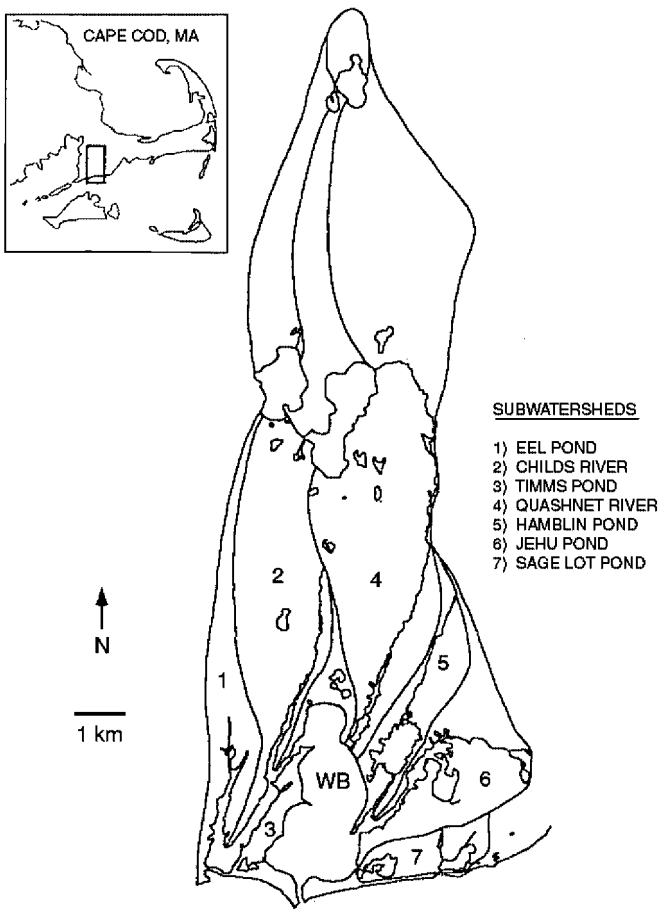

Figure 1. Waquoit Bay with sub-estuaries and sub-watersheds indicated. The comparison of ecosystem response to a nitrogen loading gradient was conducted in Hamblin (1679 kg N y^{-1}), Sage Lot (534 kg N yr^{-1}), and Timms (16 kg N yr^{-1}) Ponds. The macroalgal removal study was conducted in Hamblin Pond.

1 m × 1 m throw nets in each site ($n = 4$ site per sampling period). Small mesh (3 mm mesh) throw nets were used because they effectively sample nekton in high densities of aquatic vegetation (Heck and Wetstone, 1977; Gore *et al.,* 1981; Kushlan, 1981). As the boat drifted towards a site, the nets were tossed away from the boat into a site. Nekton were removed from the enclosed area by a bar seine made from PVC pipe and netting that fit closely to the inside of the throw net. We swept the bar seine through the throw net eight times, and all fish and decapods were removed after each sweep. The traps were then allowed to sit undisturbed for 10 min (allowing animals to re-emerge from hiding), after which four more sweeps were conducted. We continued the pattern until no more animals were caught by four consecutive sweeps. Initial tests using marked fish indicated a 90–95% recapture rate with this procedure. Animals were frozen and later identified to species and counted.

Macroalgal biomass experiment

To evaluate how the development of a thick macroalgae mat affected both eelgrass and the animal community, we manipulated macroalgal biomass. The macroalgal biomass experiment was conducted

during April–August in Hamblin Pond because this pond had high macroalgae biomass and still had eelgrass. The high ambient biomass of macroalgae allowed us to create the greatest differences between control plots (ambient macroalgal biomass) and experimental plots (lower or higher macroalgae biomass). The macroalgae were primarily *Cladophora vagabunda,* and *Gracilaria tikvahiae* with some blooms of *Ulva lactuca, Enteromorpha plumosa* in the spring and fall. To measure the effects in the higher trophic levels, and to avoid edge effects, treatment areas were four 25 m × 25 m blocks; each block was further divided into four 10 m × 10 m plots with an approximately 1 m buffer zone between both the edge and another plot. Each treatment was randomly assigned to a block prior to any sampling or manipulation.

Scuba divers created four macroalgal biomass treatments: (1) control — plots in this treatment were not manipulated and had ambient macroalgal biomass; (2) low macroalgal biomass — macroalgae were removed; (3) high macroalgal biomass — macroalgal biomass was doubled (2 ×) over control levels; (4) disturbance control — macroalgal biomass was not altered, however, macroalgae were removed and then replaced in the same plots as a control for disturbance. To create the low macroalgal biomass treatment, divers on snorkel or scuba removed noticeable macroalgae by hand from around the eelgrass and placed it in bags. Large invertebrates (primarily hairy sea cucumbers, *Sclerodactyla briareus*) and fish, but not small invertebrates such as amphipods, were removed from the macroalgae and returned to their original plots. The removed macroalgae were added to the high macroalgal biomass treatments to increase macroalgal biomass levels to approximately two times the control levels. In the disturbance control plots, divers removed the algae and then returned the algae to the same plot without removing invertebrates or fish. Macroalgae in the control plots were not disturbed. A crew of 8–10 divers completed the experimental setup in about 3 weeks in late May and early June. Experimental treatments were maintained weekly until the end of September.

Once prior to macroalgal manipulation (pre-treatment: April–May 1990) and monthly after manipulation (post-treatment: June–August), we assessed the eelgrass, macroalgae, nekton and macro-epifauna according to the methods previously described in the 'Nitrogen loading gradient comparison' section with some minor modifications. To assess how the chemical environment might have changed we measured temperature, salinity and oxygen periodically throughout the experiment. Vertical profiles of dissolved oxygen (Yellow Springs Instrument, Dissolved Oxygen Meter) were taken at dawn on 26–27 July and 2–3 August. Vertical dissolved oxygen profiles were taken from 6–7 AM in the four treatments, in the order: low, control, disturbance, and high. Sampling treatments in this order gave us a conservative estimate of differences in dissolved oxygen between treatments. One 1 m wide transect along the diagonal (14 m long) of the plot was counted each month to produce an integrated estimate of eelgrass shoot density for each plot. We measured macroalgal biomass and macro-epifauna abundance from 2 cylinder samples in 3 of the 4 plots of each treatment each month. All animals retained on a steel screen (0.5 mm mesh; Thrush, 1986) were preserved in 95% ethanol, and later identified to the lowest possible taxon (usually species) using a dissecting microscope. Nekton were sampled using throw nets. Because throw net sampling disturbed approximately a 1.5 m × 1.5 m area, and we were concerned about the cumulative disturbance this would cause over the course of the experiment, we took only one throw net sample in each of the 4 plots of a treatment each month. Areas that had been sampled were marked and not re-sampled. Although this limits our ability to detect differences because of small sample size, we felt that to sample more intensively would cause excessive sampling disturbance.

^{15}N macroalgal labelling experiment

To understand the contribution of the macroalgae to the food web that supported fishes, we labelled macroalgae with ^{15}N and traced the propagation of this label through the food web within a cage in Hamblin Pond. On 13 July 1992, a 2 m × 2 m cage constructed of PVC pipe and netting (10 mm mesh) was placed in the pond, and the macroalgae (7605 g wet weight) were removed. Macroalgae were placed in a

302 litres tub under ambient conditions. Beginning 13 July 1992, the ^{15}N label was added as 99% enriched $(^{15}NH_4)_2SO_4$ in aqueous solution (8.03×10^{-4} M) in daily increments for 6 d (total $(^{15}NH_4)_2SO_4 = 0.4074$ g). On 23 July the ^{15}N-labelled algae and representative species of animals were placed in the cage at abundances approximating those found during our sampling in Hamblin Pond.

Measurements of $\delta^{15}N$ in algae, invertebrates, and fish were performed on reference samples and at weekly intervals for three weeks (30 July, 6 August, and 13–15 August). Macroalgae (aggregates of all species), invertebrates and fish (1–5 whole individuals) were dried at 60°C for 24 h and ground with a mortar and pestle. $\delta^{15}N$ was analyzed at the Ecosystems Center's Stable Isotope Facility using an automated elemental analyzer with a cryogenic purification system coupled to a Finnigan Delta S isotope ratio mass spectrometer. Stable isotope ratios are expressed using δ notation defined thus: $\delta^{15}N\ (‰) = [(^{15}N{:}^{14}N_{sample} / ^{15}N{:}^{14}N_{standard}) - 1] \times 1000$. Air was used as the standard. Analytical precision was $\pm$ 0.1‰.

Fish growth experiment

To determine if macroalgae affected the growth rate of fishes, fourspine stickleback (*Apeltes quadracus*) and rainwater killifish (*Lucania parva*) growth rates were examined in eelgrass and macroalgal habitats in Timms and Sage Lot Ponds. We placed cages (2 m high $\times$ 1 m^2, 10 mm mesh) in each habitat in each pond. Individual fish were anaesthetized using phenoxyethanol and marked mid-dorsally with one of 6 colors of acrylic paint using a 26-guage hypodermic needle. Five marked fish of each species were placed in each cage on 21 August 1995, and collected approximately 21 d later. We measured individual fish wet weight ($\pm$ 0.01 g) and total length ($\pm$ 1 mm) at the beginning and end of the experiment, and calculated growth as the difference between these two measurements. Fish used in this experiment were mid-sized juveniles (initial length ranged between 26 and 30 mm for both species; initial weight 0.16 g for fourspine stickleback and $\sim$0.25 g for rainwater killifish).

Statistical analysis

Data were analysed using either factorial analysis of variance or repeated measures analysis of variance (ANOVA; SAS Institute Inc., 1998). Means are presented with one standard error. The Tukey–Kramer *post-hoc* test was used to test differences in means if a significant main effect was found. Significance level was $\alpha = 0.05$ for all analyses. Data were transformed as needed to meet the assumptions of ANOVA.

In the macroalgal biomass experiment the treatment plots were considered the experimental unit ($n = 4$). Factorial ANOVA was used on pre-treatment samples when one sample was taken per plot in the pre-treatment period (eelgrass shoot density and fish and decapod abundance). For macroalgal biomass and macro-epifauna pre-treatment samples in which more than one sample was taken in a plot, the replicate samples were considered repeated measures ($n = 3$ because only three of the 4 plots were sampled). Repeated measures ANOVA was used for all post-treatment measurements. Multiple samples taken in a plot in the same month were replicate measures within that month. Monthly samples were treated as repeated measures for the plot. For the nitrogen loading comparison, we used a nested factorial ANOVA with plots nested with ponds. To meet the assumptions of ANOVA, macroalgal biomass, macro-epifauna, and fish and decapods abundances were log-transformed (ln $(x+1)$) before analysis. Analysis of fish and decapods was restricted to small-sized individuals (<25 g individual total weight), because larger, more mobile fish are not effectively sampled with throw nets. Eelgrass shoot density did not need to be transformed. Equality of variance F-tests and K–S Normality tests indicated that the transformed variables were normally distributed and more closely met the equality of variance assumption; therefore we used ANOVA. Growth (mm or g wet weight per 21 d) of individual fish in the cage experiments was compared between macroalgal and eelgrass habitats using ANOVA.

RESULTS

Nitrogen loading gradient comparison

Macroalgal biomass increased, and eelgrass biomass and shoot density decreased with increased nitrogen loading (Figure 2, Table 1). The response to nitrogen load was similar between years, although there were some year-to-year differences in absolute abundances. The plant community was dominated by eelgrass with little macroalgae at the lowest nitrogen loading (Timms Pond) and changed to thin stands of eelgrass surrounded by a deep (4–14 cm) macroalgal mat at the highest nitrogen load (Hamblin Pond). Eelgrass comprised 99% (Timms Pond), 35% (Sage Lot Pond), and 1% (Hamblin Pond) of the total plant (algae + eelgrass) biomass. Eelgrass biomass (156 g m^{-2}) and shoot density (300–600 shoots m^2) were highest in Timms Pond and declined with increasing nitrogen load. Three species of macroalgae, *Gracilaria tikvahiae, Cladophora vagabunda,* and *Chaetomorpha* sp. accounted for greater than 95% of the total macroalgal biomass. Macroalgal biomass was positively related to nitrogen loading and was highest in Hamblin Pond (171 dry g m^{-2}). Hamblin Pond also had a thick (~70 cm deep) layer of black, anoxic mud, while Timms Pond had a thinner (5 cm) layer of organic matter overlying the sand layer.

Fish abundance and average number of fish species were negatively related to increasing nitrogen loading (Figure 2, Table 1). The pattern of fish community response to nitrogen loading was similar between years, although year-to-year variation in community characteristics was also apparent. In both years, the mean

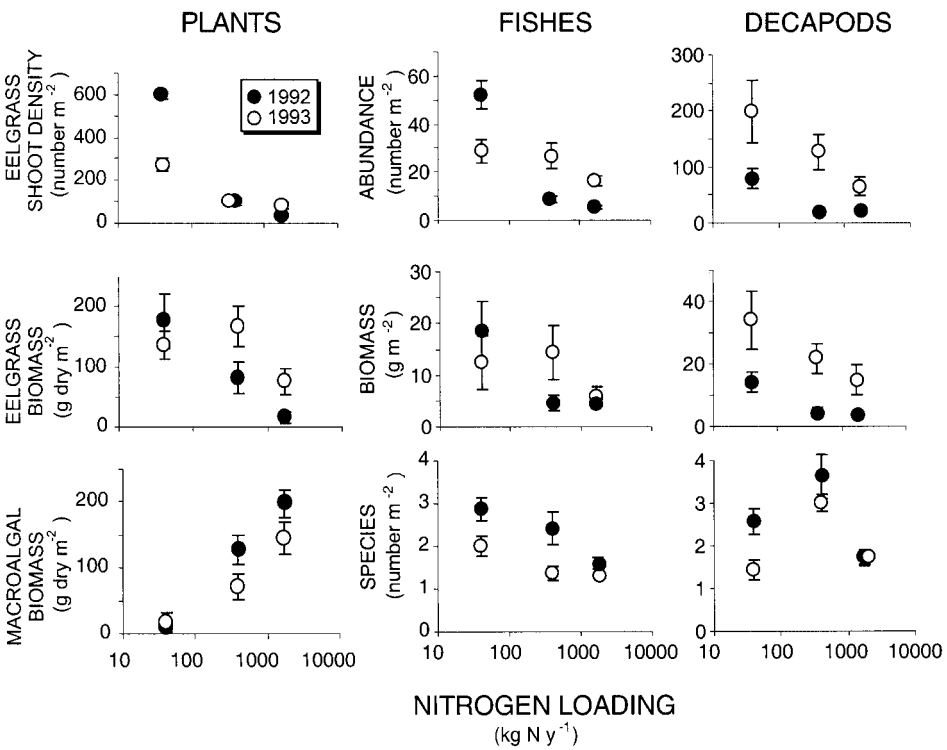

Figure 2. Mean biomass (g dry wt m^{-2}) of eelgrass and macroalgae, eelgrass shoot density (number shoots m^{-2}), and biomass (g dry wt m^{-2}), abundance (number m^{-2}) and number of species (number m^{-2}) of fishes and decapods during June–August, 1992 and 1993 in Hamblin, Sage Lot and Timms Ponds. Data are mean ± standard error for all plots within a pond. Some standard errors are smaller than the symbol size.

Table 1. F-values for ANOVAs comparing eelgrass community characteristics of Timms, Sage Lot and Hamblin Ponds[a]

		Abundance (number m^{-2})	Biomass ($g\,m^{-2}$)	Species (number m^{-2})
PLANTS				
Eelgrass	Pond(plot)	30.7***	6.3***	—
	Year	2.9	22.3**	—
	Year*Pond(plot)	6.4**	2.3	—
Macroalgae	Pond(plot)	—	21.8***	—
	Year	—	2.4	—
	Year*Pond(plot)	—	0.52	—
ANIMALS				
Fish	Pond(plot)	10.7***	5.6***	4.1***
	Year	19.7***	18.7***	0.9
	Year*Pond(plot)	6.2***	3.2**	0.7
Decapod	Pond(plot)	1.8	2.2**	2.1*
	Year	13.2**	18.3***	11.2**
	Year*Pond(plot)	1.2	1.0	3.1**

[a] Data collected June-August 1992 and 1993; ANOVAs were on $\ln(x+1)$ transformed data for macroalgae, fish and decapods. For each variable the degrees of freedom were: Pond(plot), 7; Year, 1; Year*Pond(plot), 7. Residual degrees of freedom were: Eelgrass density (number shoots m^{-2}), 384; Eelgrass biomass (dry wt g m^{-2}), 78; Macroalgal biomass (dry wt g m^{-2}), 76; Fish and decapods (Density, number m^{-2}; Biomass, g wet wt. m^{-2}; number of species), 128. Significance levels are: *** = 0.001; ** = 0.01; * = 0.05.

number of species per m^2 declined with increased nitrogen loading (Figure 2). Total number of species captured did not differ appreciably in the 2 years of our study (12 species in 1992 and 13 in 1993). Sage Lot Pond had the highest number of total fish species (13), while Timms and Hamblin had the same number of total species (8). Six species were common to all three ponds: fourspine stickleback, American eel (*Anguilla rostrata*), northern pipefish (*Sygnathus fuscus*), mummichog (*Fundulus heteroclitus*), rainwater killifish (*Lucania parva*) and Atlantic silverside (*Menidia menidia*). Fourspine stickleback was the most abundant and most frequently caught fish species in all three ponds, and accounted for 82% of the total number of fish caught in 1992 and 73% in 1993. In 1992 oyster toadfish (*Opsanus tau*) accounted for 56% of total biomass, due to the capture of one 735 g individual in Hamblin Pond. American eel accounted for 42% of total biomass, due to the capture of six large individuals (20–137 g). Large toadfish and eels were found exclusively in Hamblin Pond. Because 1 m^2 throw nets do not accurately sample large mobile fish such as these, we excluded these larger fish from our analysis. With the exclusion of these fish, mean fish biomass was highest in the pond with the lowest nitrogen load and was negatively related to increasing nitrogen load.

Although abundance of decapods was highly variable, we found a general decline with increasing nitrogen loading (Figure 2, Table 1). Total number of species caught in 1992 ($n = 8$) was greater than in 1993 ($n = 5$). The daggerblade grass shrimp (*Palaemonetes pugio*) was the dominant species in both abundance and biomass during both years. This species accounted for 67% of the total catch in 1992 and 95% in 1993.

Macroalgal biomass experiment

The distribution of plants and most animals did not differ among treatments prior to the macroalgal biomass manipulation (Figure 3, Table 2). Eelgrass shoot density was low (5 shoots m^2) and macroalgae

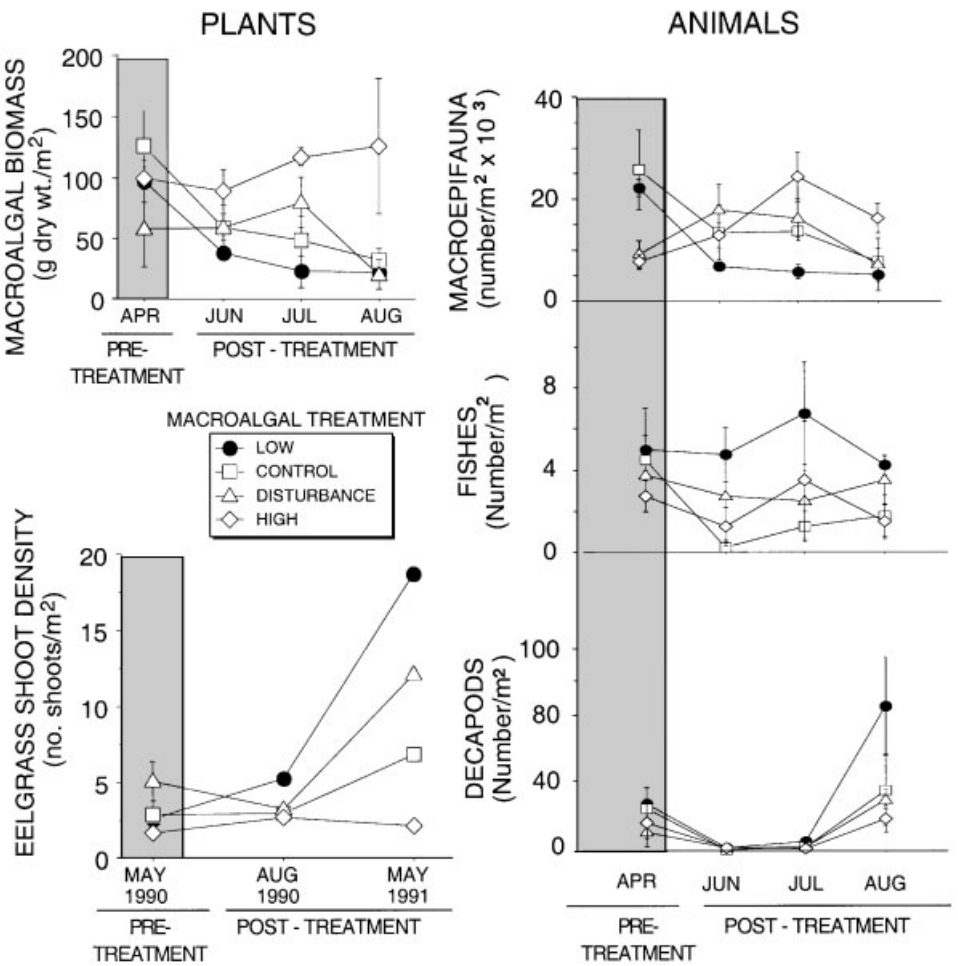

Figure 3. Mean ($\pm$ standard error) macroalgal biomass (g dry wt m^{-2}) and eelgrass shoot density (number of shoots m^{-2}) and macroepifauna, fish and decapod abundance (number m^{-2}) over time in the macroalgal manipulation experiment. Some standard errors are smaller than the symbol size.

biomass high (~ 100 g dry wt m^{-2}) with no significant differences across the plots. *Gracilaria tikvahiae, Cladophora vagabunda* and *Chaetomorpha* sp. were the dominant species of algae, representing approximately 50%, 20% and 5% of total macroalgal dry mass, respectively. Initial fish abundance did not differ among the treatments (4 ± 0.4 individuals m^{-2}), although the low treatment had the highest ($5 \pm 0.4\,m^{-2}$) and the high treatment the lowest (3 ± 0.3 individuals m^{-2}) pre-treatment mean abundances. Abundance of decapods did not differ among the treatments (20 ± 2.9 individuals m^{-2}). Only the initial abundance of macro-epifauna differed among treatments (Figure 3), with the control and low treatments having similar and higher abundance (2.5×10^4 individuals m^{-2}) compared to the high and disturbance treatments (1.0×10^4 individuals m^{-2}) (Tukey–Kramer test, $p < 0.05$).

Altering the biomass of macroalgae changed many aspects of the eelgrass ecosystem, including relative plant and animal abundance and biomass and chemical suitability. Macroalgal biomass was highest in the spring and declined over the summer except in the High macroalgal biomass treatment (Figure 3, Table 2).

Table 2. Pre- and post-macroalgal manipulation experiment ANOVA analyses of algal biomass, eelgrass density, and fish and invertebrate abundance[a]

Pre-treatment		DF	F-value
Plants			
Algal biomass (in dry wt m^{-2})	Treatment	3	2.47
	Replicate	1	0.02
	Replicate × treatment	3	1.78
Eelgrass density (number of shoots m^{-2})	Treatment	3	1.98
Animals			
Macro-epifauna abundance (in number m^{-2})	Treatment	3	4.86*
	Replicate	1	3.26
	Replicate × treatment	3	0.17
Fish abundance (in number m^{-2})	Treatment	3	1.01
Decapod abundance (in number m^{-2})	Treatment	3	1.08
Post-treatment			
Plants			
Algal biomass (in dry wt m^{-2})	Treatment	3	21.75***
	Month	2	4.70*
	Month × treatment	6	2.53
	Replicate	1	1.23
	Replicate × treatment	3	0.03
	Month × replicate	2	2.16
	Month × replicate × treatment	6	0.62
Eelgrass density (number shoots m^{-2})	Treatment	3	487.0***
	Month	1	1018.0***
	Month × treatment	3	225.0***
Animals			
Macro-epifauna abundance (in number m^{-2})	Treatment	3	4.75*
	Month	2	8.82**
	Month × treatment	6	2.94*
	Replicate	1	0.43
	Replicate × treatment	3	0.42
	Month × replicate	2	3.17
	Month × replicate × treatment	6	0.38
Fish abundance (in number m^{-2})	Treatment	3	4.75**
	Month	2	0.73
	Month × treatment	6	0.15
Decapod abundance (in number m^{-2})	Treatment	3	2.77
	Month	2	37.2***
	Month × treatment	6	1.08

[a] Treatments are: reference, removal, disturbance, addition. Pre-treatment samples were collected in April–May 1990, before experimental manipulation of macroalgae. Pre-manipulation algal biomass and macro-epifauna were analyzed with repeated measures ANOVA, with replicate samples treated as the repeated measure; Eelgrass, fish and decapod abundance were factorial ANOVA as no replicate within plots samples were taken. Pre-treatment residual degrees of freedom were: Algal biomass and macro-epifauna, 8; Fish and decapods, 12; Eelgrass density, 8. Post-treatment samples were collected in June-August, 1990, after macroalgal manipulation, and analyzed with repeated measures ANOVA. Significance levels are: *** = 0.001; ** = 0.01; * = 0.05.

Macroalgal biomass was lowest in the low and highest in the high treatments compared to the other treatments. Throughout the course of the experiment, macroalgal samples were collected the day before the weekly maintenance of the macroalgal biomass treatments. The doubling time of macroalgae in these systems is less than a week (Peckol *et al.*, 1994; Peckol and Rivers, 1996) indicating macroalgae could grow substantially between treatments and sampling. Thus, our estimates of macroalgal biomass represent the peak biomass of the macroalgae in the treatments for a week and are a conservative estimate of the effectiveness of experimental manipulations on macroalgal biomass. We found no differences in macroalgal species composition over the course of the experiment (data not shown).

The removal of macroalgal biomass resulted in a denser cover of eelgrass that persisted into the following spring. Eelgrass shoot density was highest in the low macroalgal biomass treatment compared to all other treatments by August of the same year and was over $4\times$ higher the following spring (Figure 3). We observed small plant sprouts as well as more leaves per shoot at the end of the season in the low macroalgal biomass treatments, but not in the other treatments. The greatest difference among treatments was seen the following spring when eelgrass shoot density in the low macroalgal biomass treatment was $4\times$ higher than in the control, $2\times$ higher than in the disturbance control, and $10\times$ higher than the high macroalgal biomass treatment.

High macroalgal biomass reduced O_2 concentrations and removal of macroalgae increased O_2 near the sediment–water interface (Figure 4). Vertical profiles indicated higher oxygen content in the water and near the bottom in the low macroalgal biomass treatment compared to the other treatments. On 26 July, after several warm cloudy days and with a high tide at 3–4 AM the low macroalgal biomass treatment had twice as much oxygen at the surface as did the other treatments. On 3 August, the low macroalgal biomass treatment had consistently higher dissolved oxygen levels throughout the profile than did the other treatments. The higher O_2 content in the low macroalgal biomass treatment is probably due to the combination of less respiration because of low macroalgal biomass and higher oxygen production by the more abundant eelgrass blades. The dissolved oxygen in the high macroalgal biomass treatment was consistently lower than in the other treatments. We found no vertical stratification of either temperature or salinity during the experiment (data not shown).

Macro-epifauna showed no consistent pattern of response to the macroalgal biomass manipulation. Macro-epifauna abundance declined over the summer in the control and low macroalgal biomass treatments, while they increased in the disturbance control and high macroalgal biomass treatments (Figure 3, Table 2). The higher levels of macro-epifauna in the high macroalgal treatment could be attributed to transferring the animals with the macroalgae. A total of 53 species were caught; of these 10 genera/species comprised 90% of the small invertebrates caught. These were amphipods (*Microdeutopus* sp., *Corophium* sp., *Ampithoe* sp, *Lysianopsis alba*), tunicates (sea grape *Molgula* sp.), polychaetes (*Haploscoloplos robustus, Podarke obscura*), isopods (*Erichsonella attenuata*), burrowing sea cucumbers (*Leptosynapta* sp.) and a small snail (*Hydrobia minuta*). The total number of species present in the low (43) and high (42) macroalgal biomass treatments was higher than in the control (39) or disturbance (36) treatments.

Removal of macroalgae created a more suitable habitat for fishes as evidenced by the higher fish abundance and diversity in the low macroalgal biomass treatment compared to all other treatments (Figure 3, Table 2). A total of 10 fish species were collected: threespine stickleback (*Gasterosteus aculeatus*), Atlantic silverside, winter flounder, northern pipefish, mummichog, oyster toadfish, American eel, inland silverside (*Menidia beryllina*), rainwater killifish and northern searobin (*Prionotus carolinus*). More fish species were found in the low macroalgal biomass treatment (8) than in any of the other treatments (5 species in the control; 6 in the disturbance; and 4 in the high macroalgal biomass treatments). Some benthic-associated fish, such as winter flounder, were found only in the low macroalgal biomass treatment. In June, July and August the fish were primarily young-of-the-year fish from adults that spawned earlier.

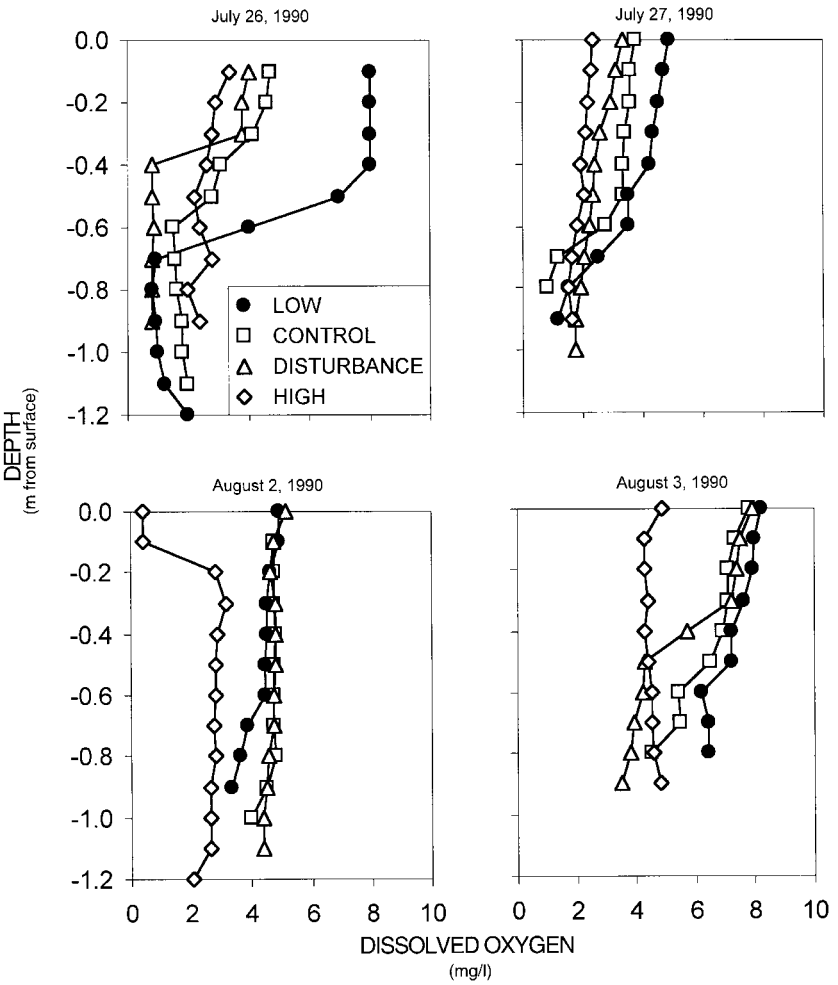

Figure 4. Water column dissolved oxygen profiles from the macroalgal manipulation experiment. Measurements were taken in the early morning (6–7 AM) after a 3–4 AM high tide.

We observed a seasonal pattern in the response of decapods to manipulation of macroalgal biomass (Figure 3, Table 2). Decapod abundance was highest in April and declined to near zero in all treatments during June and July. In August the low macroalgal biomass treatment had a higher decapod abundance compared to all other treatments (Tukey–Kramer test, $p < 0.05$), due primarily to the recruitment of newly hatched daggerblade grass shrimp. Decapods were primarily adults and young-of-the-year of shrimp and crabs: daggerblade grass shrimp (the dominant species), sand shrimp (*Crangon septemspinosa*), spider crab (*Libinia dubia*), blue crab (*Callinectes sapidus*), and mud crabs (*Rhithropanopeus harrisii, Neopanope sayi* and *Panopeus herbsteii*).

^{15}N Macroalgal labelling experiment

Changes in the δ^{15}N of primary producers and consumers in the ^{15}N enrichment experiment indicated that very little of the macroalgal production was transferred up the food web to the common fish species

Table 3. The $\delta^{15}N$ of primary producers and consumers in the ^{15}N enrichment of macroalgae experiment in Hamblin Pond, July 1992

Trophic level	Species	Date			
		Initial	1 week	2 weeks	3 weeks
		23 July	30 July	6 August	13 August
Primary producers					
Macroalgae	*Gracilaria sp.* *Cladophora sp.*	979		456	
Eelgrass	*Zostera marina*	7.3		10.1	
Primary consumers					
Herbivores					
Amphipod	*Gammarus mucronatus*	5.5	1370	411	
Isopod	*Ericsonella filiformis*	4.3	30.2		10.1
Isopod	*Idotea baltica*	6.3			32.9
Detritivores					
Hairy cucumber	*Sclerodactyla briareus*	7.1	13.5		9.7
Polychaete	*Nereis sp.*	7.3	89.1		
Secondary consumers					
Oyster toadfish	*Opsanus tau*	10.5	24		31.7
Atlantic Silverside	*Menidia menidia*	10.0		10.5	11.1
Threespine stickleback	*Gasterosteous aculatus*	10.6	11.2	9.7	10.5
Sand Shrimp	*Crangon septimspinosa*	9.5		9.4	9.4

(Table 3). The $\delta^{15}N$ of macroalgae was enriched 160 × (970‰) over baseline values (6‰) when it was placed in the enclosure. After 2 weeks, the algae had lost about 50% of the initial ^{15}N enrichment, but remained 80 × as enriched as baseline (450‰). These enriched values clearly distinguish the macroalgae from all other primary producers ($\delta^{15}N$ values of around 6–10‰) in the estuary.

The $\delta^{15}N$ values of some primary consumers (invertebrate herbivores and detritivores) were enriched 2–250 × that of natural values indicating they were consuming enriched macroalgae. Background variation in natural $\delta^{15}N$ value for a consumer species may be 1–2‰, therefore we considered any increase above 5‰ to indicate enrichment over natural abundance levels (Hughes *et al.,* 2000). Within 1 week, the herbivorous amphipod *Gammarus mucronatus* was enriched with $\delta^{15}N$ values 250 × its natural abundance $\delta^{15}N$ value. The $\delta^{15}N$ value for *Gammarus* (1370‰) after 1 week was higher than that of the algae mix (979‰), possibly due to preferential consumption of epiphytic diatoms on the macroalgae. Although it was not possible to determine a separate $\delta^{15}N$ value of the epiphytic diatoms and macroalgae, it is likely that because of its fast turnover this diatom film would be enriched relative to the bulk macroalgae. The rate of the decline in the $\delta^{15}N$ value of *Gammarus* during the experiment was proportional to the decline in the macroalgae suggesting macroalgae was its primary food source. Two other herbivores, the isopods *Ericsonella* and *Idotea*, were slightly enriched in $\delta^{15}N$ indicating minimal consumption of the enriched algae. The degree of enrichment in the detritivores was highly variable. *Nereis* was rapidly enriched (15 × baseline; 89‰ after 2 weeks) indicating direct consumption of the macroalgae. In contrast, the hairy cucumber showed little to no enrichment after 3 weeks.

The most abundant secondary consumers, Atlantic silverside and three-spine stickleback, showed little to no enrichment indicating they were not depending on a macroalgal based grazing food web. Of the three fish species sampled only a benthic predator, a juvenile oyster toadfish (10 g wet weight), was found to have

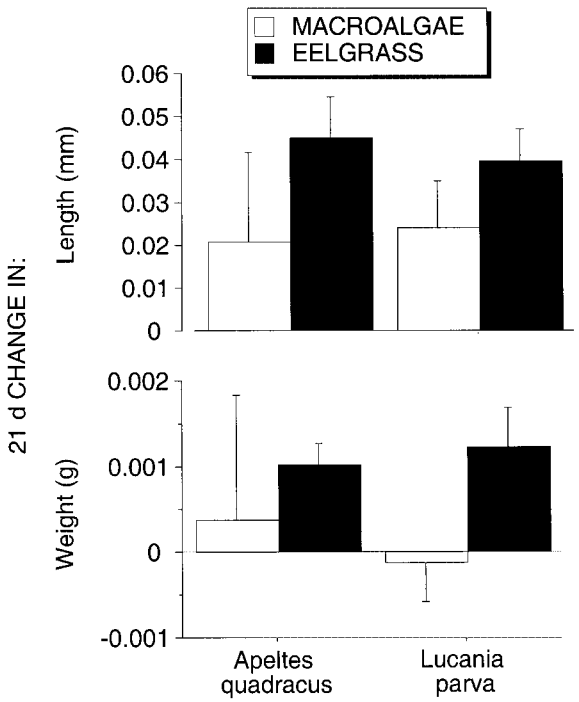

Figure 5. Mean growth (± standard error) of fourspine stickleback (*Apeltes quadracus*) and rainwater killifish (*Lucania parva*) in eelgrass and macroalgae-dominated habitats.

an enriched δ^{15}N value (31.7‰ versus a baseline of 10.5‰). The predatory sand shrimp did not show an increase over natural abundance δ^{15}N values.

Fish growth experiment

There was a tendency for growth of fish to be higher in eelgrass compared to in macroalgal habitats (Figure 5), however, the difference was significant for rainwater killifish ($p = 0.03$ for weight; $p = 0.3$ for length; $n = 17$) but not fourspine stickleback ($p = 0.6$ for weight; $p = 0.4$ for length; $n = 21$). Individuals of both species lost weight in macroalgal but not in eelgrass habitats. Fewer fourspine sticklebacks were recovered from macroalgal habitats ($n = 4$) compared to eelgrass habitats ($n = 17$), indicating poorer survival in macroalgal areas. The low survival of fourspine stickleback in macroalgal areas made it difficult to determine if the growth difference between the habitats was statistically important. In both habitats, fish growth rates were relatively low (range: 0.06–0.08 g 21 d^{-1}; 0.0–2.2 mm 21 d^{-1}). This was perhaps because the experiment was run late in the growing season (late August to September).

DISCUSSION

These results indicate that anthropogenic nutrient enrichment is causing a shift in primary producers and altering fish and invertebrate communities and food webs in estuarine ecosystems. We found both

watershed nutrient loading induced and experimentally manipulated alteration in macroalgal biomass resulted in changes in fish and invertebrate abundance and diversity, as well as fish survival and growth. Several aspects of our study indicate that the finely branched, filamentous macroalgal mats that replaced eelgrass do not provide support to fish equivalent to that of eelgrass ecosystems.

We observed an increase in macroalgae and a decline in eelgrass biomass in response to increased nutrient loading as has been found worldwide (Duarte, 1995; Valiela *et al.,* 1997b; Raffaelli *et al.,* 1998). Macroalgae are a natural component of eelgrass ecosystems, but in pristine watersheds, such as Timms Pond, with low nutrient conditions macroalgal biomass is low relative to eelgrass (Short and Burdick, 1996; Duarte, 1995). Nutrient loading enhances the growth of macroalgae leading to increased biomass as we observed in Hamblin Pond. Removal of macroalgae increased abundance of eelgrass suggesting that excessive macroalgae interferes with eelgrass growth through light or space competition (Short *et al.,* 1995; Short and Burdick, 1996; Hauxwell *et al.,* 2001).

Fish abundance and diversity and decapod abundance decreased with increased nitrogen load and macroalgal dominance. Other studies have found a greater abundance and diversity of fish and invertebrates in seagrass habitats compared to bare areas (Orth *et al.,* 1984; Virnstein, 1987) or macroalgal habitats (Phil *et al.,* 1994). Increased biomass of macroalgae as a result of eutrophication has been suggested to reduce juvenile plaice (*Pleuronectes platessa*) recruitment in Sweden (Phil and van der Veer, 1992) and juvenile cod (*Gadus morhua*) recruitment in Norway (Tveite, 1984). Sogard and Able (1991) found higher densities of fish and invertebrates in macroalgae (*Ulva lactuca*) compared to unvegetated habitats, but macroalgae did not provide fishes with an equivalent substitute for eelgrass.

The central importance of macroalgae in altering eelgrass ecosystem support of fish and decapod crustacea is evident when macroalgal biomass was manipulated. When macroalgal biomass was lowered, eelgrass shoot density increased 2–10-fold and fish and decapod abundances were higher compared to other areas with high macroalgal biomass. Interestingly, despite the higher abundance of invertebrate prey in the high macroalgal biomass treatment, fish and decapods preferentially occupied the low macroalgal biomass treatment.

Excessive macroalgae may alter food webs by changing the physical or chemical structure of the habitat. Both the ^{15}N enrichment experiment and the growth of fishes in eelgrass and macroalgal habitats indicated that little of the macroalgal production was transferred to the dominant fishes. We found that ^{15}N enriched macroalgae could be traced to herbivorous and detritivorous invertebrates, but that little was found in the most abundant fishes. The high ^{15}N value for *Gammarus mucronatus* is consistent with feeding trials that have shown that *Gammarus* is an important herbivore on macroalgae in Waquoit Bay (Hauxwell *et al.,* 1998). The lack of ^{15}N enrichment in the dominant fishes and the lower growth of fishes suggest that macroalgal habitats are not providing the same food web support as eelgrass habitats.

Alteration of the physical structure of the habitat resulting from the increase in compact, filamentous and finely branched algal species may prevent fish from feeding on benthic invertebrates. Macroalgae hinders predator efficiency and provides a refuge for invertebrates (Heck and Thoman, 1981; Kulczycki *et al.,* 1981; Wilson *et al.,* 1990; Isaksson *et al.,* 1994; Dorf and Powell, 1997). Algal biomass and morphology have been shown to be important in preventing fish from feeding on amphipods, crabs and shrimp (Holmhund *et al.,* 1990). Both laboratory and field studies have confirmed that fish have more difficulty capturing prey in fine, filamentous algae compared to broad bladed algae, eelgrass or bare substrate (Dean and Connell, 1987; Isaksson *et al.,* 1994; Preisser and Deegan, 1995).

Low dissolved oxygen near the bottom associated with high levels of macroalgal biomass may be a chemical barrier that prevents fish from feeding on the benthos. Many fish will not cross a dissolved oxygen cline to feed on the bottom if dissolved oxygen concentrations are less than 2–3 ppm (Nestlerode and Diaz, 1998). O_2 concentrations of 2 ppm or lower are typical in high macroalgal biomass areas (this study, D'Avanzo *et al.,* 1996). Oyster toadfish are very tolerant of low dissolved oxygen levels and burrow in even

anoxic substrate for shelter (Bigelow and Schroeder, 1953). These characteristics of oyster toadfish may explain why this fish can exist and feed in macroalgal mats while other fish species do not. The alteration of the physical and chemical structure of the habitat by macroalgae results in fewer and weaker connections between in the benthic food web and the dominant fish species.

The change in the physical and chemical structure of the habitat due to macroalgae may also affect survival of small fishes. Fish and invertebrate survival is suggested to be higher in seagrass because of the structural complexity of seagrass ecosystems compared to adjacent open areas (Heck *et al.,* 1997). The species of macroalgae that proliferate in response to nutrient enrichment tend to form unattached low-lying mats that do not provide the same degree of structural complexity as a tall eelgrass canopy. Some fish have impaired escape behaviors in hypoxic environments resulting in increased predation (Breitburg *et al.,* 1997). In severe cases short-term anoxic events (<24 h) in eutrophied areas with high macroalgal biomass can kill an entire year-class of fish, especially those with high site fidelity such as winter flounder (Deegan and Buchsbaum, 2001).

There are at least three implications of our work relevant to the conservation of eelgrass habitats and managing ecosystems to prevent biotic impoverishment. Biologists agree that the major proximate causes of biotic impoverishment today are habitat loss, degradation, and fragmentation (Soulé, 1991). First, our work suggests that all plants do not provide equal ecosystem function and that conversion of eelgrass habitats to macroalgal dominated areas is the equivalent of habitat loss. Breitburg (1998) suggested that the negative effects of nutrient loading on fish communities might be weaker if nutrient additions caused changes in which species provides structure rather than a complete decline in macrophyte biomass. In addition, compensatory replacement (some species increase while others decrease) has been suggested as a mechanism that buffers the effects of stress at the ecosystem level resulting in similar levels of production and biomass despite a change in species composition (Fogarty and Murawski, 1998; Rapport and Whitford, 1999). Macroalgae, despite their high primary productivity and biomass, did not provide a suitable alternative habitat for most eelgrass-dependent fishes and compensatory replacement by other species did not occur.

Second, our work suggests that we need to consider both the quality of the remaining habitat and the surrounding ecosystem when applying habitat fragmentation theory to the conservation of eelgrass habitats (Robbins and Bell, 1994). The study of declining or fragmenting habitats has been dominated by two classical paradigms, island biogeography and metapopulation dynamics. Both of these approaches invoke a habitat patch–matrix model where number of species is considered a function of habitat area (species–area relationship) and isolation is measured as the distance across the matrix between habitat patches (Rosenzweig, 1995; Ricketts, 2001). The habitat patch–matrix model has been successfully used in conservation biology, however recent work has indicated that fuller development of this theory is needed (Ney-Nifle and Mangel, 2000; Vos *et al.,* 2001). An underlying assumption of the species-area relationship is that the habitat remaining is equivalent in function to the original habitat (i.e., that patch carrying capacity is constant; Vos *et al.,* 2001). In this paper and others (Hughes *et al.,* 2001; Wyda *et al.,* 2001), we have shown that that the loss of an ecosystem can be qualitative and involve a change or degradation in the structure, function, or composition of an ecosystem (Noss, 1990). Increasing biomass of macroalgae in an area that would still be considered eelgrass habitat decreases the capacity of the ecosystem to support species even if the area of the habitat does not change. An additional consideration is that seagrass patches maybe more isolated than simple distance would indicate depending on the intervening matrix (Ricketts, 2001). Our work suggests that the physical and chemical structure of the surrounding macroalgal matrix causes patches to be more effectively isolated than if they are surrounded by sand or bare bottom (Robbins and Bell, 1994; Frost *et al*., 1999). For example, fish will tend to remain in an eelgrass patch because the oxygen content of the eelgrass patch is more favourable to their survival than the oxygen content of the surrounding macroalgal matrix. Thus, nutrient loading leading to the conversion of eelgrass habitat to macroalgal dominated areas, degradation by increasing

biomass of macroalgae within existing eelgrass habitat and fragmentation of eelgrass habitat into small patches surrounded by high macroalgal biomass, has reduced the overall capacity of estuaries to support animal populations.

Third, our work suggests that macroalgal removal offers a potential short-term management option for conservation of eelgrass habitat. The macroalgal biomass experiment shows that some of the effects of macroalgae on plant, animal and chemical components of the ecosystem can be reversed by removal of the macroalgae and that the results persist beyond a single growing season. This sustained response, at least at the plant level, has also been seen in another study that examined the effect of macroalgae canopy height on eelgrass (Hauxwell *et al.,* 2001). While it is clear that survival of seagrass ecosystems ultimately depends on reductions in nutrient loading, removal of macroalgae provides a short-term approach to maintaining seagrass habitats while long-term controls on nutrient loading are developed.

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