EPA Public Access

Author manuscript

Ecol Indic. Author manuscript; available in PMC 2018 September 12.

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Published in final edited form as:

Ecol Indic. 2017 August ; 79: 207–227. doi:10.1016/j.ecolind.2017.04.034.

Development of an epiphyte indicator of nutrient enrichment: a critical evaluation of observational and experimental studies

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Abstract

An extensive review of the literature describing epiphytes on submerged aquatic vegetation (SAV), especially seagrasses, was conducted in order to evaluate the evidence for response of epiphyte metrics to increased nutrients. Evidence from field observational studies, together with laboratory and field mesocosm experiments, was assembled from the literature and evaluated for a hypothesized positive response to nutrient addition. There was general consistency in the results to confirm that elevated nutrients tended to increase the load of epiphytes on the surface of SAV, in the absence of other limiting factors. In spite of multiple sources of uncontrolled variation, positive relationships of epiphyte load to nutrient concentration or load (either nitrogen or phosphorus) often were observed along strong anthropogenic or natural nutrient gradients in coastal regions. Such response patterns may only be evident for parts of the year. Results from both mesocosm and field experiments also generally support the increase of epiphytes with increased nutrients, although outcomes from field experiments tended to be more variable. Relatively few studies with nutrient addition in mesocosms have been done with tropical or subtropical species, and more such controlled experiments would be helpful. Experimental duration influenced results, with more positive responses of epiphytes to nutrients at shorter durations in mesocosm experiments versus more positive responses at longer durations in field experiments. In the field, response of epiphyte biomass to nutrient additions was independent of climate zone. Mesograzers activity was a critical covariate for epiphyte response under experimental nutrient elevation, but the epiphyte response was highly dependent on factors such as grazer identity and density, as well as nutrient and ambient light levels. The balance of evidence suggests that epiphytes on SAV will be a useful indicator of persistent nutrient enhancement in many situations. Careful selection of appropriate temporal and spatial constraints for data collection, and concurrent evaluation of confounding factors will help increase the signal to noise ratio for this indicator.

Keywords

Nutrient indicator; water quality indicator; nutrient additions; seagrass; rooted macrophytes

1. Introduction

Opportunistic algal growth resulting from elevated nutrients may result in significant negative impacts for biological substrata such as seagrasses, freshwater macrophytes, or macroalgae. A variety of studies have reviewed epiphytes with varying degrees of focus on

the response to elevated nutrient levels (Borowitzka and Lethbridge, 1989; Harlin, 1995; Jernakoff et al., 1996; Hillebrand, 2002; Hughes et al., 2004; Borowitzka et al., 2006; Burkholder et al., 2007; Michael et al., 2008; Nelson, 2009; US EPA [Appendix B.5], 2010; Sutula et al., 2011; Thomsen et al., 2012). One of the first conceptual models of macrophyte decline under increased nutrient input from human activity was derived from lakes by Phillips et al. (1978). This model highlighted the role of increased growth of epiphytes under nutrient addition, which reduced macrophyte growth and survival through shading. The dominant effect of heavy epiphytic cover on macrophyte substrata appears to be decreased growth and a reduced potential for survival caused by reduced light availability (Sand-Jensen, 1977; Borum and Wium-Anderson, 1980; Bulthuis and Woelkerling, 1983; Sand-Jensen and Borum, 1984; Cambridge et al., 1986; Silberstein et al., 1986; Sand-Jensen and Revsbech, 1987), especially at lower ambient light levels (Morgan and Kitting, 1984; Twilley et al., 1985; Wetzel and Neckles, 1986). Depression of photosynthesis by epiphyte loads also may be caused by a reduction in the rate of diffusion of HCO_3^- across the seagrass blade surface (Sand-Jensen, 1977). Increased physical drag from epiphytes may result in increased loss of leaves or plants under high wave or current conditions (Borowitzka and Lethbridge, 1989). Exposure to elevated levels of nitrogen has been shown to decrease the tensile breaking strength of some seagrass species (Kopp, 1999; Nafie et al., 2012), which might further increase loss of leaves under nutrient enhanced epiphyte loads.

Among water body types, epiphyte increases in response to increased nutrients have been observed in lakes (Moss, 1976; Phillips et al., 1978; Sand-Jensen and Søndergaard, 1981; Sand-Jensen, 1990; Vermaat and Hootsmans, 1994; Strand and Weisner, 1996; Bécarea et al., 2008), rivers (Köhler et al., 2010), and estuaries. In estuarine systems, responses have been documented from northern European estuaries (Borum, 1985; Borum and Wium-Andersen, 1980), Baltic brackish waters (Rönnberg et al., 1992), US estuarine waters (Tomasko and Lapointe, 1991; Frankovich and Fourqurean, 1997; Tomasko et al. 1996), Australian estuaries (Bulthuis and Woelkerling, 1983; Silberstein et al., 1986; Neverauskas, 1987a, 1987b; Bryars et al., 2011), Mediterranean estuaries (Balata et al., 2008; Giovannetti et al., 2010), and tropical Atlantic waters (McGlathery, 1995; Stoner et al., 2014), among other locations.

Observations and experiments have demonstrated that elevated levels of water column nutrients can result in increased levels of epiphytic algal material on submerged aquatic vegetation within relatively short time periods. (Bulthuis and Woelkerling, 1983; Borum, 1985; Twilley et al., 1985; Silberstein et al., 1986; Jensen and Gibson, 1986; Neverauskas, 1987a; Dunton, 1990; Tomasko and Lapointe, 1991; Frankovich and Fourqurean, 1997; Neckles et al., 1993; Williams and Ruckelshaus, 1993; Lapointe et al., 1994; Murray et al., 2000). Since macrophyte substrata tend to remain in place long enough to integrate local nutrient loads, the use of epiphyte metrics as indicators of system response to nutrient levels has appeared promising (Gobert et al., 2009; Balata et al., 2010; Giovannetti et al., 2010; Castejón-Silvo and Terrados, 2012; Marbà et al., 2013; McMahon, 2013). However, there are also cautionary notes. Wood and Lavery (2000) assessed the role of perception in determining the assessment of coastal condition, and found that while Best Professional Judgement rated epiphyte biomass as an important indicator of seagrass ecosystem condition, the metric failed to distinguish between sites designated “healthy” or “unhealthy”.

Fourqurean et al. (2010) suggested that epiphyte load is not a reliable nutrient indicator for oligotrophic ecosystems, and US EPA (2010) evaluated epiphyte indicators as not yet useful for setting water quality criteria in the state of Florida.

However, quantitative reviews of epiphytes include a meta-analysis of periphyton responses to increased nutrients and grazing from lakes, streams and a few coastal studies (Hillebrand, 2002), and a similar meta-analysis (Hughes et al., 2004) assessing effects of grazing and nutrients on seagrasses and their epiphytes. Both concluded that nutrients significantly increased and grazers significantly reduced epiphytes/periphyton. The available studies addressing macrophyte epiphytes and nutrients have greatly increased since these meta-analyses were conducted. Therefore, an extensive review of the literature on epiphyte responses to elevated nutrients was conducted based primarily on seagrasses or other rooted aquatic species from coastal systems as the macrophyte host. The review included field observational studies, and both laboratory and field mesocosm experiments that manipulated nutrient levels and observed epiphyte responses. Where feasible, quantitative analyses were used to determine the conditions under which epiphyte responses occurred. The ultimate goal of the review is to provide the weight of evidence to support establishment of threshold levels for use of epiphyte indicators in coastal waters (Nelson, 2017) that may have application in protection of water quality.

2. Methods

2.1 General Methods

The literature on seagrass epiphytes, but also including some brackish and freshwater rooted macrophytes, was reviewed to categorize response patterns to excess nutrients. The assessment sought to evaluate whether there was clear, quantitative evidence that excess nutrients lead to negative impacts on host plants. The objective was to determine whether metrics of epiphytic load on seagrasses can be used as quantitative biological indicators for nutrient impacts in estuarine waters. In excess of 400 publications were examined, including peer reviewed literature, theses and dissertations, and “gray” literature technical reports. The focus included observational studies in the field, which included data on seagrass epiphyte responses to nutrient inputs (e.g. waste water, fish farms, bird guano), and experimental studies in both the field and laboratory, which manipulated nutrient levels and recorded epiphyte responses. Searches for relevant studies relied on previous reviews of seagrass epiphytes (e.g. Hughes et al., 2004; Burkholder et al., 2007; Michael et al., 2008; Nelson 2009; Thomsen et al., 2012), and included bibliographic searches for relevant terms using Google Scholar, Web of Science, and search engines for web sites for scientific journals.

If it was necessary to acquire data from scatter plots or bar graphs, data were digitized with Grab It!™ software (Datatrend Software). Images of graphs from PDF files of publications were copied with the Microsoft Snipping Tool app, saved to JPG format image files, and imported into Grab It!™, which operates within Microsoft Excel. Repeated measurements of the same data points with the software gave a measurement precision of less than 0.1%. Comparison of the values extracted via software to values for the same data points that were given in the publication gave a measurement accuracy on the order of 3%. Reanalysis of data digitized from the original publications provided a Quality Assurance check for analyses

provided in the original papers. In a relatively few cases, authors were ambiguous with regard to units for data presented, and such data sets were excluded.

2.2 Field Observation Assessment

Evaluation of results from field observational studies generally relied on data collected along nutrient gradients, but results varied so greatly in terms of study sites and conditions, including type and magnitude of nutrient sources, that comparisons were primarily qualitative. However, in a number of cases, data were extracted as described above and regression relationships (linear, nonlinear) were examined between epiphyte responses and nutrient conditions. Regression analyses were conducted with Sigmaplot 13.

2.3 Mesocosm Experiments Assessment

Results were compiled from a total of 22 laboratory microcosm and mesocosm studies (Supplemental Table 1) that enriched either nitrogen (N), phosphorus (P) or both, and also assessed epiphyte responses for rooted macrophytes (7 species), primarily seagrasses (5 species). A total of 35 separate experiments reported either qualitative (n=4) or quantitative (n=31) results, which are summarized in Table 1. Biomass (dry weight (DW), ash free dry weight (AFDW), cell volumes) and chlorophyll-*a* (chl *a*, $\mu\text{g cm}^{-2}$) responses were categorized as Increase (I), Decrease (D) or No Response (NR), if measured. Change in community composition of epiphytes was scored as “Yes” if the paper reported any marked shift in taxonomic composition or relative proportions of pigment types, and “No” for no apparent change. Study locations were characterized in terms of climate zone as Temperate, Subtropical, and Tropical. Mesograzers density in experimental mesocosms was qualitatively estimated as High, Medium, or Low, or specified as Unknown (Table 1) where information was not given. A G-test of independence was conducted to determine whether there was a significant association of response of epiphyte biomass to nutrient addition with mesograzers abundance.

2.4 Field Experiments Assessment

A literature search provided 47 field experiments (Table 2) of nutrient addition where epiphyte response was qualitatively (n=6) or quantitatively (n=41) assessed. Experiments consisted of 32 water column addition studies and 15 sediment addition studies. Experimental results from a study were treated separately where 1) multiple seagrass species were independently evaluated, 2) separate physical locations were reported, or 3) separate water column and sediment addition experiments were performed. Responses of biomass, chl *a* and community composition of epiphytes were categorized as described above (section 2.3). Study locations were characterized in terms of climate zone as Temperate (T=16), Subtropical (ST=16), and Tropical (TR=15).

Studies included 14 seagrass species, experimental plot sizes from 0.16 – 7 m², durations from 15 d to 7 yr, various response metrics, and a range of nutrient addition sources, application methods, and loading rates (Supplementary Table 2). Some 51% (24 of 47) of experiments inferred nutrient elevation but did not measure it directly in experimental plots, while 64% (30 of 47) of the studies did not control for or measure mesograzers. Therefore, definitive interpretation of results is difficult. As Hughes et al. (2004) point out, meeting the

requirements for formal meta-analysis tends to greatly reduce the number of studies in an assessment. Therefore, the decision was made to examine a wider span of studies for general trends rather than do a meta-analysis.

G-tests of independence were conducted to determine whether there were significant associations of epiphyte biomass or chl a response with fertilization location (Water Column, Sediment) or with climate zone. G-tests of goodness of fit were used to test the distribution of outcomes (i.e. I, D, or NR) from laboratory and field experiments in terms of null hypotheses of equal probability of outcomes.

3. Results and Discussion

3.1 Evidence for the Role of Nutrient Loads in Determining Epiphytic Load

Evidence for the importance of nutrient loads in determining epiphyte response comes from field observations, mesocosm experiments at a variety of scales, and from field experimental manipulations of nutrient concentrations. Previous reviews of studies from freshwater and marine systems (Orth and Van Montfrans, 1984; Harlin, 1995; Smith et al., 1999; Ralph et al., 2006; Burkholder et al., 2007; Leoni et al., 2008; Östman et al., 2016) have found relationships between nutrient enrichment, epiphyte increases, and decrease or loss of macrophytes.

3.2. Review of Field Observations

Field observational studies along strong nutrient gradients have tended to support the connection between elevated nutrients, increased epiphytes, and negative impacts to macrophytes. However, such studies may also have unmeasured covariates and other issues that may complicate interpretation of results.

Among early studies from Australia, Bulthuis and Woelkerling (1983) found that highest epiphyte levels on *Heterozostera tasmanica* were found at the site with the highest nutrient input, and epiphyte loads were estimated to diminish light sufficiently to impact seagrass. Silberstein et al. (1986) found higher epiphyte loads and lower seagrass (*Posidonia australis*) standing stock, shoot density, flower number, leaf production, and growth near a sewage outfall at a location with significantly elevated water column phosphate. Epiphyte accumulation rates on artificial substrata were also 4.5× times greater. However, the study did not sample prior to the introduction of sewage, and it is possible that some site differences were present before the sampling, nor were site differences in grazer impacts assessed. Seagrass (*Posidonia* and *Amphibolis*) largely disappeared near a sewage outfall in Western Australia within 4 years of initial discharge, while seagrass at greater distances showed high epiphyte loads (Neverauskas, 1987a). Highest epiphyte recruitment rates were on artificial substrata placed closest to the outfall (Neverauskas, 1987b). However, reanalysis of the data shows only a non-significant, slightly negative relation of epiphyte biomass to distance from the outfall (Supplemental Figure 1, $r^2 = 0.07$, n.s.). Distance may not accurately reflect relative nutrient exposures due to variable flow patterns, but no nutrient data were provided for the study sites.

Other early studies from Denmark found that epiphyte biomass on *Zostera marina* during summer increased exponentially with increasing water column total nitrogen (TN) concentration along a gradient of nutrient concentrations (Borum, 1985). Reanalysis of the Borum data to plot summer mean epiphyte load versus mean TN at the four study locations emphasizes the highly significant exponential relationship (Figure 9, $r^2 = 0.99$, $p = 0.0059$). The maximum depth for the distribution of the freshwater macrophyte (*Littorella uniflora*) in Danish lakes was inversely related to epiphyte load (Sand-Jensen, 1990). Epiphytes accounted for 62% of the total water column light attenuation at the leaf surface in a eutrophic lake, versus only 5% in the most oligotrophic lake.

Accumulation rates of epiphytes on both natural and artificial substrata have been shown to be positively related to nutrient loads in several field studies in temperate estuaries. In short term (12 d) deployments in Maryland, 14× greater biomass of epiphytes had accumulated after 7 d in a high nutrient estuary compared to a low nutrient system (Brandt and Koch, 2003). Epiphyte accumulation rate, both as biomass and chl *a*, significantly increased with increased N load across subsystems in Waquoit Bay, MA (Wright et al., 1995). While epiphyte accumulation rates on artificial substrata showed only weak associations with TN or dissolved inorganic phosphorus (DIP) along a section of the Patuxent river estuary (Stankelis, 2001; Fig. 4–14), reanalysis of the data indicates a highly significant relationship to the N/P ratio, reflecting a transition associated with the salinity gradient moving from upstream to downstream stations (Figure 2, $r^2 = 0.95$, $p = 0.001$). In this case the N/P ratio also mirrors the water clarity pattern, which may more directly affect accumulation rate of epiphytes.

In field studies from generally more oligotrophic waters in Florida, the Bahamas, and the Caribbean, epiphytes responded variably to both P and N. Jensen and Gibson (1986) found that the highest epiphyte biomass on seagrasses (*Thalassia*, *Syringodium*, *Halodule*) was at sites with the highest concentrations of P, N and silicate. Elevated epiphyte biomass, low shoot numbers, and low seagrass biomass were found near concentrated nutrient sources such as septic tanks, bird rookery islands, and seabird roost sites (Tomasko and Lapointe, 1991; Frankovich and Fourqurean, 1997; Herbert and Fourqurean, 2008). Richardson (2006) found alterations in epiphytic foraminiferan communities on seagrass along a presumed gradient of nutrient enrichment near a bird nesting island in Belize. At sites along a nutrient gradient in the Florida Keys, eutrophic and hypereutrophic areas had high levels of epiphytes and low seagrass shoot densities and production rates (Lapointe et al., 1994). Reanalysis of data indicates that there were highly significant relationships of epiphyte load to both TN (Figure 3A, $r^2 = 0.96$, $P < 0.0001$) and total phosphorus (TP) (Figure 3B, $r^2 = 0.79$, $P < 0.0001$) during the winter period, but there were no significant relationships during the summer (TN, $p = 0.7$; TP, $p = 0.13$).

In contrast, across Florida Bay as a whole, Frankovich and Fourqurean (1997) found that epiphyte load was weakly correlated with TP availability, and concluded that epiphyte levels were not very sensitive to moderate nutrient enrichment in this environment. They suggested that epiphyte load may be only a late indication of nutrient enrichment, and thus is not a sensitive indicator of nutrient condition. Tomasko et al. (1996) found that the spatial pattern of epiphyte biomass showed little relation to N loadings within Sarasota Bay, Florida. The

observation most at odds with the general hypothesis of a positive response of epiphyte load to increased nutrients is the significant, negative relationship observed by Fourqurean et al. (2010) between epiphyte load and water column TN. Data were mean values of quarterly samples collected over 7 years from sites across Florida Bay, and thus contained both intraannual and interannual variability, but these variance sources do not readily explain the negative relationship.

Spatial variation in average annual epiphyte load was observed among sites within Charlotte Harbor, FL for samples collected during 1997–98 by Dixon and Kirkpatrick (1999). This study did not collect nutrient data, but information on nutrient loads across segments of the estuary for 1992–94 are available in Squires et al. (1998). Epiphyte load was positively associated with load estimates for both N (Figure 4A, $r^2 = 0.9$, $p=0.0004$) and P. For samples taken in 1995–96 in the same estuary, Tomasko and Hall (1999) found only limited quantities of epiphytic algae on seagrass. However, there was poor water clarity in that period due to high freshwater flow during the wet season that may have reduced epiphyte cover.

For *Halodule wrightii* in the Indian River Lagoon, FL, Miller-Myers (1997) found no relation between epiphyte biomass and nutrients (total Kjeldahl nitrogen (TKN), TP) along a spatial gradient integrated over the 5 month (summer) sample period. In multispecies seagrass beds (*Thalassia*, *Syringodium*, *Halodule*) in the same estuary, Hanisak (2001) found that above-ground seagrass biomass (site means) decreased with increased epiphyte load, but he found no consistent spatial relation of epiphyte load and nutrient concentration. However, reanalysis of data from Hanisak (2001) for mean values at the 6 study sites showed a positive relationship of epiphyte load to P concentration (Figure 4B, $r^2 = 0.5$, $p=0.07$), but there was little relationship to N. Hanisak (2001) also found relationships of epiphyte load and seagrass biomass with grazer abundance, and suggested that grazers might be responsible for the epiphyte patterns. While this explanation cannot be excluded, the findings that epiphyte biomass correlates to P concentration and that the epiphyte loads reported by Hanisak (2001) are extremely high at all stations, suggest a more important role for nutrients than grazers.

In one of the few observations from the tropical Indo-Pacific, Christianen et al. (2012, Fig. 4c) found a strong relation ($r^2 = 0.7$, $P<0.01$) of epiphyte biomass on *Halodule uninervis* with water column P concentrations. Reanalysis of data from the coast of Kenya (Uku and Björk, 2005) indicated a positive, but not significant, ($r^2 = 0.65$, $p=0.19$), relationship of epiphyte percent cover on *Thalassodendron ciliatum* to nitrate concentrations during the monsoon.

Among Mediterranean studies, Pergent-Martini et al. (2006) found that in 5 of 6 studies reviewed, epiphyte biomass on *Posidonia oceanica* increased with proximity to fish farms in coastal waters. Among these studies, data from Dimech et al. (2000) indicated an increase in epiphyte load near a fish farm (reanalysis of data from Fig. 1.c, $r^2 = 0.93$, $p=0.0017$), with an associated significant negative relationship between epiphyte biomass and shoot density over this transect (Dimech et al., 2000, data from Figs. 1.c, 1.d; $r^2 = 0.73$, $p=0.029$). The exception (Ruiz et al. 2001) occurred when large numbers of sea urchin herbivores close to a

fish farm grazed the tips of seagrass blades, removing the densest region of epiphytes. Experiments with urchin exclosures (Ruiz et al., 2010) resulted in an increase in epiphyte biomass to reference levels in the region with elevated urchin abundance. Balata et al. (2008) found higher epiphyte load and changes in community composition of epiphytes on *P. oceanica* at sites with elevated nutrients off Italy. Reanalysis indicated a highly significant regression relationship between nitrate concentration and epiphyte load (Supplemental Figure 2, $r^2 = 0.91$, $p = 0.003$). Although the regression is strongly affected by one station, removal of which generates a non-significant relationship ($r^2 = 0.2$, $p = 0.43$), there is no a priori justification for exclusion. Castejón-Silvo et al. (2012a) found significant differences in epiphyte load on *P. oceanica* among 10 sites around Palma Bay, Majorca, Spain. Reanalysis of data indicated a negative relationship between epiphyte load and seagrass leaf biomass (Castejón-Silvo et al. 2012a, data from Figs. 2a, 2b; $r^2 = 0.37$, $p = 0.06$).

A number of studies have compared epiphyte loads among sites that were assumed to differ in exposure to nutrients, but authors did not actually confirm exposure levels. Comparing a site near a sewage outfall with two controls sites off the coast of Italy, Piazzini et al. (2004) found no effects attributable to the outfall. There were no measurements of nutrients, and filamentous algal epiphyte coverage was very low at all sites (<5%). Terrados and Medina-Pons (2008) compared a disturbed bed of *P. oceanica* with two control locations in the Balearic Islands, and found no difference in epiphyte loads, but nutrients were not measured. At regional scale, Bryars (2009) found no relation of epiphyte load to an assumed gradient in nutrient loads in South Australia, but the authors did not actually measure nutrient exposure. At a smaller spatial scale, York (2010) compared multiple estuarine sites classified as either high or low nutrient sites based on land use patterns, and found a significantly elevated epiphyte load on *Zostera capricorni* within sites presumed to have high nutrients.

3.2.1. Field Observation Conclusions—In spite of the multiple sources of uncontrolled variation, there are numerous examples of clear positive relationships of epiphyte load to nutrient level, particularly along strong gradients in temperate coastal regions. In some cases, observed relationships are to anthropogenic sources of nutrients, but responses have also been observed to natural nutrient sources. Patterns in responses in the field may only be evident in particular periods of the year, e.g. summer at north temperate locations, or winter at tropical locations. Detection of responses of epiphytes to nutrients tended to be more difficult in oligotrophic waters. Responses of epiphytes to nutrients varied depending on the locally limiting nutrient, which may shift over the spatial scale of some studies. In some cases, relationships may be better described by comparison to N/P ratio than to either N or P separately. Use of surrogates (e.g. land use, estimated degree of anthropogenic disturbance) in place of direct assessment of nutrient elevation appears to be highly tenuous, and renders only very weak evidence concerning effects of nutrients on epiphytes.

3.3 Review of nutrient addition experiments – Mesocosms

Conditions of the various mesocosm experiments conducted to examine the effects of nutrient additions on seagrass epiphytes are summarized in Table 1, with additional experimental details provided in Supplemental Table 1. Unfortunately, as first noted by

Murray et al. (2000), the extreme variation in mesocosm size, flow rates, type of nutrient addition (pulse versus continuous addition), and presence or absence of grazers within experimental systems make generalizations difficult. Variation in experimental protocols has not decreased with the addition of newer studies; experimental volumes ranged from 10 to 340,000 l, rates of water replacement ranged from 0.05 to 61 d⁻¹, and included a wide range of nutrient loads and experimental treatment combinations (Supplemental Table 1). Most experiments applied dissolved nutrient solutions either as pulse or continuous application (n=38), while a few studies (n=8) added nutrients as solid fertilizer, which was allowed to dissolve.

Examination of geographical distribution indicated that there was a strong bias in mesocosm studies, with the large majority conducted with temperate zone species (37), while only 5 were conducted in either subtropical (2) or tropical locations (3) (Table 1). Investigators measured nutrient concentrations only in 72% of experiments (30). Therefore, evaluating the effectiveness of nutrient inputs in the remaining experiments is difficult (Table 1).

Mesograzers were quantified in 42% of 42 experiments (Table 1). In some cases, methods descriptions indicated grazers were present but not quantified, or that specified initial grazing densities were established but were not subsequently measured. In other cases, experimental descriptions indicated that mesograzers were most likely low or absent. The types of mesograzers utilized included amphipods, isopods, grazing gastropods, shrimp and hermit crabs. Overall, there was no significant association of estimated relative abundance level of grazers (high, medium, low) with the observed response (Increase, No Response) of epiphyte biomass in mesocosm experiments (G test of independence, n=24, G = 0.55, p>0.05).

Epiphyte biomass increased in response to nutrient additions in mesocosms in 27 cases, with no biomass response in 9 cases. Epiphyte load (as chl *a*) increased in 10 cases, with 1 instance of No Response. The combined outcomes of biomass and chl *a* metrics (37,10) differed significantly from that of an equal expectation of outcome (G-test for goodness of fit, G= 16.5, p<0.001). Epiphyte taxonomic changes in response to nutrient additions were observed in all five measurements of this metric. Examination of results of the specific experiments provides additional insights.

Potamogeton perfoliatus and *Ruppia maritima* were exposed to pulse additions of N and P in large mesocosm ponds (Twilley et al. 1985, Supplemental Table 1), and developed dense epiphytes in all nutrient addition treatments, with decreases of >80 % of light at the leaf surface at highest levels of nutrients, and decreases of macrophyte photosynthesis. There were significant positive exponential relationships of epiphyte load to both total P (TP) ($r^2 = 0.55$, $p = 0.006$, Figure 5A) and TN ($r^2 = 0.42$, $p = 0.02$) concentration in the mesocosms, as well as a significant negative linear relationship to N/P ratio ($r^2 = 0.79$, $p = 0.002$, Figure 5B) (Twilley et al., 1982, 1985). In smaller mesocosm experiments with *P. perfoliatus* (Sturgis and Murray, 1997), epiphyte abundance increased significantly within most treatments using either pulse or continuous nutrient addition.

Epiphytes on eelgrass *Zostera marina* have received the most attention in mesocosm experiments. Epiphyte biomass was positively correlated with continuous elevation of ammonium concentration in the water column in laboratory microcosms (Williams and Ruckelshaus, 1993, $r^2 = 0.37$, $p = 0.007$, Fig. 5, Supplemental Table 1), and eelgrass growth also decreased significantly with increased epiphyte biomass (Williams and Ruckelshaus, 1993, $r^2 = 0.36$, $p = 0.002$, Fig. 6.). In a reanalysis combining both laboratory and field experimental data, there was a highly significant, negative-exponential relationship of *Zostera* growth rate with epiphyte load ($r^2 = 0.92$, $p < 0.0001$, Figure 6A). At loads above $100 \text{ mg shoot}^{-1}$, growth rates were reduced by approximately 50%. Short et al. (1995, Supplemental Table 1) found that low light levels generally inhibited the epiphyte response to elevated nutrients in larger mesocosm tanks with high water turnover and grazers present. With full light levels, there was a $12 \times$ increase of epiphytes relative to controls, although for intermediate light reduction, there was a much larger epiphyte biomass at ambient nutrient level. Kaldy (1992) confirmed the limiting effects of low light levels on epiphyte responses to nutrients with experiments in the same mesocosms. However, although there was no significant treatment effect for nutrients analyzed with ANOVA, reanalysis of the data indicates that there was a highly significant negative regression relationship of seagrass biomass to epiphyte biomass (Figure 6B, $r^2 = 0.71$, $p < 0.0001$).

Responses of *Z. marina* and associated epiphytes to nutrient additions were examined by multiple authors using the same large mesocosm system (Taylor et al., 1995; Lin et al., 1996; Bintz et al., 2003). Tanks included a variety of fish and invertebrates, but apparently lacked significant numbers of grazers. Two papers reported results from the same experiment but used substantially different epiphyte metrics (mg cm^{-2} DW, second and third youngest leaves, (Taylor et al., 1995), versus mg cm^{-2} AFDW, fourth and fifth youngest leaves, (Lin et al., 1996)). Both studies found no significant effects of nutrient treatments on epiphytes, *Z. marina*, or drift algae. However, supplemental N caused brown tide blooms that suppressed other primary producers. Switching from pulse to continuous addition of nutrients in the same systems, Bintz et al. (2003) showed that epiphyte loads increased in warm, nutrient addition treatments as compared to cool, ambient or enriched nutrient treatments. Both phytoplankton and macroalgal blooms developed in the mesocosm tanks. More recently, Blake and Duffy (2012) also demonstrated temperature effects, with both increased nutrients and temperature increasing epiphytes on *Z. marina*, while negatively affecting the eelgrass.

Also using large mesocosms, Burkholder et al. (1992 Supplemental Table 1), showed no effect of nutrient additions on epiphyte loads on *Z. marina* and *Halodule wrightii* (measured as cell counts rather than biomass). Grazer densities in all treatments were quite high, and may explain the lack of epiphyte response. Moore and Wetzel (2000) tested effects of elevated nutrients and reduced light levels on *Z. marina* in mesocosms with moderate densities of gastropod grazers. Epiphyte responses were highly dependent on treatment. Only the spring treatment with high light level showed major ($10 \times$) elevation in epiphyte biomass, principally due to macroepiphytes rather than microepiphytes. The combination of low light, high nutrients, and low grazers was not examined. Jaschinski and Sommer (2008) found that increased nutrients significantly increased both epiphyte biomass and productivity. While grazers significantly decreased both parameters compared to non-grazer

treatments, nutrient effects were larger than grazer effects. Reanalysis of data shows a significant exponential relationship of epiphyte biomass to nutrient concentration for all treatments pooled ($r^2 = 0.94$, $p = 0.0015$, Figure 7). Examining the effects of both light and nutrients in tanks with *Z. marina*, Kopp (1999) found no statistically significant nutrient effect on epiphyte loads at either light level. Reanalysis of these data by regression showed positive relationships of epiphyte load and dissolved inorganic nitrogen (DIN) concentration for the 4 nutrient addition treatments in both light treatments (100% light, $r^2 = 0.78$, $p=0.14$; 55% light, $r^2 = 0.73$, $p=0.11$), but slopes were not significant due to small sample sizes.

Mesocosm studies of nutrient impacts are more limited for *Thalassia testudinum* and *Halodule wrightii*. Virnstein et al. (1987) applied pulse addition of nutrients on *H. wrightii* at a reduced light level. Greatly decreased growth of new blades was observed with increased epiphyte load in all nutrient addition treatments ($r^2 = 0.31$, $p = 0.01$, Figure 8). In mesocosm experiments in the Florida Keys (Tomasko and Lapointe, 1991; Lapointe et al., 1994; Supplemental Table 1), both N and P additions increased epiphyte biomass (as a percent of seagrass biomass) versus controls, and decreased rhizome growth rates in both *H. wrightii* and *T. testudinum*. Light reduction with nutrient addition reduced the relative increase in epiphyte biomass. Hays (2005) examined both grazer and nutrient effects on *T. testudinum* and its epiphytes, and found no significant nutrient effect on epiphytes over the 40 d experiment.

3.3.1. Mesocosm Conclusions—Relatively few mesocosms studies measuring epiphyte response from nutrient additions have been done with tropical or subtropical species, and more such controlled experiments would be helpful. While epiphyte responses to nutrient additions in mesocosms have varied considerably, some generalities emerge in understanding response patterns. Where responses were observed, epiphytes increased with increases in nutrient levels. Higher temperatures tend to enhance epiphyte growth. Increases in epiphytes can result in negative impacts on the host plants. Light reduction may greatly inhibit development of epiphyte biomass in the presence of elevated nutrients, whether from the use of light-reduction screens simulating increased depth, or as a result of phytoplankton or macroalgal blooms that reduced light penetration in the tanks (Short et al., 1995; Kopp, 1999; Moore and Wetzel, 2000). Elevated nutrient levels are not necessarily predictive of an epiphyte increase in mesocosms where alternate trophic pathways for nutrient uptake are possible. Grazers may also inhibit the development of epiphytes in spite of elevated nutrients in mesocosms, but the outcome is highly dependent on multiple factors in the experiment, such as grazer identity, density, nutrient level, and light levels available. Measurement or control of mesograzer densities is a critical covariate for nutrient addition in mesocosms, but this variate has often not been adequately addressed.

It is a reasonable assumption that increased duration of fertilization in mesocosm tanks would result in more instances of epiphyte increases. However, the mean duration of nutrient exposure for NR was significantly greater than that for I (87 vs. 52 d; two-tailed t-test, $p<0.015$; see Supplementary Figure 3 for frequency distributions). This suggests that longer mesocosm experiments have an increased risk that uncontrolled factors, e.g. increase in grazers, will influence experimental outcomes.

3.4 Review of nutrient addition experiments – Field Experiments

Based on meta-analyses that included the effects of experimental nutrient additions on seagrass epiphytes, Hughes et al. (2004) concluded that additions to the water column tended to increase epiphyte biomass, while sediment additions tended to decrease epiphyte biomass. While this analysis involved 5 “cases” of sediment additions, it appears to have been based on only one or two studies. In the present review, reduced epiphyte biomass was observed 5 times in experiments with nutrient addition in the field (Table 2), twice with sediment addition and three times with water column additions. Willams and Ruckelshaus (1993) found this unanticipated effect both in sediment addition treatments in an April experiment, and in water column additions in an August experiment, suggesting no relationship to placement of the nutrient addition. The authors discounted higher seagrass growth rates as leading to lower epiphyte loads from blade turnover because of the short duration (15 d) of the experiments. They also discounted grazer differences as explanatory since mean grazer abundances among treatments were not significantly different for either experiment. However, if data from both experiments are combined, there is a significant negative relationship between epiphyte biomass and grazer abundance (Figure 9) ($r^2 = 0.79$, $p = 0.003$), suggesting that grazer numbers may actually have been a factor influencing the results observed. York (2010) also reported decreased epiphyte load in enclosures with nutrient enrichment, but results are confounded by an experimental design that cannot separate nutrient and grazer effects.

There was no significant association of fertilization location (sediment, water column) with response of epiphyte biomass (+, -, NR) (G-test of independence, $G = 4.31$, $0.05 < p < 0.1$), although the number of “increase” responses was much larger for water column than sediment nutrient additions (16, 2). There was a significant association of the response (+, NR); insufficient data for -) of epiphyte load as chl a with location of experimental fertilization ($G = 4.34$, $p < 0.05$). There was no support for the result by Hughes et al. (2004) that sediment addition of nutrients resulted in decreases in epiphyte load.

There was no significant association of biomass response (+, -, NR) to experimental nutrient additions within the three climate zones compared ($G = 4.73$, $p > 0.05$). However, there was a significant association of chl a response with climate zone ($G = 14.0$, $p < 0.025$), reflecting high frequency of NR in the Temperate region. However, most of these results are experimental results from the *Zostera* Experimental Network (Duffy et al., 2015), and if these studies are omitted, there was no significant association of result with climate zone ($G = 2.7$, $p > 0.05$). These results are discussed further in section 3.4.1 below. Changes in epiphyte taxonomic composition in response to nutrient enhancement were observed about 3× as often as no change (14 vs. 4), which is significantly different from a random expectation (goodness of fit test, $G = 5.88$, $p < 0.025$).

Within the chl a data set, three studies from the Florida Keys (Armitage et al., 2005; 2006; 2011) evaluated responses at the same sites over time periods from 18 months to 7 years of bimonthly, sediment nutrient additions. Epiphyte responses were highly variable and site specific. Statistical analysis of epiphyte chl a over 7 years found significant date, site and date x P and site x P interaction terms, and no significance for treatments with N addition. All three studies found instances of both lower and higher epiphyte chl a with P addition.

However, the studies did not measure water column nutrients in experimental plots, and did not measure mesograzers, so it is difficult to evaluate potential causes for these observations.

In situ, nutrient enrichment in the water column has also been performed in the Mediterranean with *Posidonia oceanica*. Leoni et al. (2006) conducted +NP addition to the water column in caged and uncaged plots over a 13-month period, and observed a significant increase in the epiphyte load only in the period May – September and no effect at other times of year. During this period, epiphyte load was positively correlated with ammonium, nitrate and phosphate in the water column within the seagrass canopy. A similar experiment by Castejón-Silvo et al. (2012a) also found that epiphyte load increased by approximately 100% with +NP addition to the water column after a two-month period in summer, and the elevated epiphyte load persisted through the end of the experiment in October. An additional experiment in the summer period (Castejón-Silvo et al. 2012b) compared controls to +NP addition to the water column at four sites, two each with low and high initial epiphyte levels. In spite of some among-site variation in response, nutrient additions after 42 d had significantly increased epiphyte loads in both types of sites. Prado et al. (2008) added nitrate, ammonium and phosphate to the water column, but the placement of new nutrient release was re-randomized within plots monthly. Samples of epiphytes were taken from blades close to nutrient release points over 14 months, but samples would represent 1 month of close exposure to nutrient release. Significant increases in epiphyte load were found only in August and September. Despite the differences in experimental protocols, results were relatively consistent in that effects of local nutrient enhancement were only observed in the summer and early fall months. Exposures of only 30–40 d to nutrient enhancement were sufficient to generate significant elevations in epiphyte load.

An extended experiment (27 months) with two Australian seagrass species in an oligotrophic region found elevation of epiphyte load after approximately 4 months, with negative impacts on seagrasses being recorded in the period <4 to 8 months (Bryars et al., 2011). Seagrass did not recover to control level after a 12-month recovery period. In both species, epiphyte loads at approximately 1.5 g DW g⁻¹ DW seagrass were associated with the seagrass impacts.

Field experiments that have combined nutrient addition with grazer manipulation and have also provided data on epiphyte responses are discussed in Section 3.5.1 below.

3.4.1 Field Experiments Conclusions—Until recently, the preponderance of experiments with nutrient addition in the field with data on SAV-epiphyte response had been performed in Florida and the Mediterranean, with little data for *Zostera* species in the temperate zone. Duffy et al. (2015) have now provided data on 15 temperate, Northern Hemisphere, *Zostera* sites so that balance among climatic regions in results has improved. Analysis of the composite data set (Table 1) shows no evidence to support the suggestion that experimental addition of nutrients to sediments tends to result in SAV epiphyte decreases. The response of epiphyte biomass to experimental, nutrient additions in the field appears independent of climate zone. However, the relationship of epiphyte chl a response to climate zone is currently uncertain. If results from the ZEN project (Duffy et al., 2015) are included in the analysis, there was a significant association of NR of epiphytes and the Temperate zone; whereas if they are excluded, no association of outcome and climate zone

was seen. There is some uncertainty about the influence of sediment versus water column addition of nutrients on epiphytes. For biomass, there was a tendency for water column addition to elicit more epiphyte increases, while for chl a, there was a significant association of NR with water column additions if the ZEN experiments are included, and no association if they are excluded. Experimental additions of nutrients in the water column generally confirm observational data that epiphyte responses to increased nutrients may only occur during limited periods within the year. While significant effects on epiphytes were first observed after nutrient exposure periods as short as 1 month, initial response could also take as long as 4 months. The relative role of differences in experimental methodology versus local variability is extremely difficult to resolve with regard to reported results.

In terms of the effects of treatment duration, as with the mesocosm assessment, longer fertilization would be assumed to increase the likelihood of observing an increase in epiphytes, either measured as biomass or chl a. Examining the subset of experiments in Table 2 that provided quantitative results, and also used water column additions of nutrients, the duration of experiments that recorded increases in either epiphyte load metric was 4× greater than those recording no epiphyte response (two tailed t-test, $t=3.58$, $df=40$, $p<0.01$). The protocol for the ZEN project sites (Duffy et al., 2015) was for an experimental duration of only 28 days. While epiphyte increases have certainly been recorded at equivalent experimental durations, (Supplemental Figure 4), this analysis of duration suggests that longer exposures may be required in field experiments to insure adequate time for epiphyte responses to manifest.

3.5. Factors Modifying the Expression of Epiphyte Response to Nutrients

3.5.1. Grazers—There is a host of evidence that complex interactions among trophic components in seagrass systems may determine the ultimate extent of the effect of epiphytes on their seagrass substrate. A full review of the interactions between grazers and seagrasses is beyond the scope of this paper. However, since variability in grazer abundance may clearly affect epiphyte indicator values, (Jernakoff et al., 1996), it is important to consider how grazing might relate to epiphytes as an indicator.

Seminal reviews (van Montfrans et al., 1984; Orth and van Montfrans, 1984; Jernakoff et al., 1996) have indicated that across a wide variety of seagrass species either a) decreased epiphyte load caused by grazer removal tends to improve seagrass growth (e.g. Hootsmans and Vermaat, 1985; Howard, 1982; Howard and Short, 1986; Philippart, 1995) or b) that increased epiphyte loads inhibit seagrass growth.

Early mesocosm work by Williams and Ruckelshaus (1993) examined both the effects of grazers and nutrient additions on seagrass and epiphyte production in separate experiments. Treatments without the isopod grazer *Idotea ressecata* had epiphyte biomass almost 300% higher than in grazed tanks (at field grazer densities). However, there was no significant effect on *Z. marina* growth after about two months, presumably because total epiphyte load in the ungrazed treatment was still below the critical threshold for impacts on seagrass growth.

In a series of mesocosm experiments, Neckles et al. (1993) varied season, nutrient concentrations and densities of epiphyte grazers to examine the relative effects of these factors on growth of *Z. marina*. Epiphyte biomass increased with nutrient enrichment in all experiments, although marginally so in the fall experiment. Grazer densities and impacts were greatest in the two summer treatments. The effects of nutrient enrichment were never large enough to overwhelm the impact of grazers. In all experiments, the ungrazed, ambient nutrient treatments always possessed epiphyte biomass greater than or equal to the grazed, nutrient enriched treatments. As the authors pointed out, absolute nutrient level, water temperature, grazer density, and grazer composition all varied simultaneously among the experiments, which makes it impossible to identify the causes of the observed differential seasonal responses of epiphytes to grazers.

Grazing impacts on epiphytes in mesocosms may be influenced by a variety of hydrodynamic factors, including the frequency and timing of nutrient additions, the residence time of water, and relative trophic complexity of food chains (Murray et al., 2000). Grazing had the largest relative effect on *Potamogeton perfoliatus* growth, except under high nutrient loads, when changes both from pulsed to continuous nutrient addition, and from high to low water exchange rates, led to larger relative responses in macrophyte growth than grazing. Grazers reduced the magnitude of the epiphyte response under high nutrient loads (+63% for grazed versus +112% for ungrazed), but the grazing impact had no ameliorating effect on the macrophyte response, which was -88% for macrophyte growth in both grazed and ungrazed treatments (Murray et al., 2000).

Interest in determining the relative effects of top-down versus bottom-up control of trophic structure in seagrass systems has led to numerous field experimental studies manipulating nutrients and grazer densities that also provide data on epiphyte response. In the Baltic region, Moksnes et al. (2008) found nutrient enrichment caused initial (3 weeks) algal blooms, including filamentous epiphytes, but the effect was not significant at 6 weeks, suggesting control by mesograzers. In a multi-site regional comparison, Baden et al. (2010) found spatially variable outcomes of treatments, ranging from large algal increases to no epiphytic response, and proposed variation in grazer control as a prime explanatory factor.

Other recent field experiments have used pesticide application in situ to avoid cage artifacts, in combination with nutrient enhancement, to examine effects of mesograzer decreases on seagrasses and epiphytes, again with variation in results. Cook et al. (2011) decreased amphipod abundance in plots in Australian seagrasses, and found increased epiphyte load on *Posidonia sinuosa* but not *Amphibolis* spp., and no effect on seagrass biomass over the 7-week experiment. Myers and Heck (2013) showed a significant elevation in epiphyte load only at protected sites, and only with both nutrient addition and mesograzer reduction. Reanalysis of their data showed no significant impact on seagrass biomass after 10 weeks, consistent with epiphyte loads reaching the relatively low maximum value of 0.4 g g^{-1} seagrass (Nelson, 2017). McSkimming et al. (2015) found significant interactions among nutrient and grazer effects on epiphyte load and percent cover, generally showing increases in both measures with decreased grazers or with increased nutrients after 162 d. Effects on the seagrass *Posidonia angustifolia* were not clear cut, with no effect on leaf density and only a significant increase in above-ground biomass in plots with a minor level of nutrient

increase and reduced grazers. Reanalysis of data from Fig. 4 of McSkimming et al. (2015) did not show a relation of epiphyte response to seagrass response ($r^2 = 0.06$, $p = 0.65$).

A series of experiments in Chesapeake Bay also found variable effects of nutrient addition on epiphytes while concurrently using a chemical deterrence for grazers. Douglass et al. (2007) observed a rapid, initial increase of epiphytes to nutrients (9 d), which had disappeared by day 23, apparently due to grazing response. A reverse pattern was seen by Whalen et al. (2013), where for an experiment in the fall, epiphytes increased significantly due to fertilization only after 24 d when mesograzers had decreased. During a parallel summer experiment there was no significant effect of nutrients out to 38 d, with epiphyte chl *a* showing a negative relationship to density of crustacean mesograzers (Whalen et al., 2013). In similar field experiments, Reynolds et al. (2014) found no significant effects of added nutrients on epiphytes. However, in 4 weeks, while epiphyte load responded with an estimated 500% increase to reduced grazer numbers, there was a nearly 1300% increase to increased nutrients along with reduced grazers, relative to respective controls, suggesting an important influence of nutrients. Seagrass biomass at 8 weeks was significantly reduced in reduced grazer treatments. The nutrient and grazer manipulation protocols developed in Chesapeake Bay were extended to 15 sites with *Z. marina* (Duffy et al., 2015), and composite results found significant effects of grazers but not fertilization on epiphyte chl *a*.

Recent reviews and meta-analyses (Hughes et al., 2004; Burkepile and Hay, 2006; Heck and Valentine, 2007; Duffy et al., 2013, 2015; Östman et al., 2016) have proposed the primacy, or at least a closely equivalent importance, of grazer effects to those of nutrients in generating declines of seagrass beds. The “mutualistic mesograzer” model (Duffy et al., 2013) posits that mesograzers prevent overgrowth of seagrasses by algal epiphytes, promoting seagrass growth and providing a significant buffer for seagrass under nutrient pollution. This viewpoint has also been promoted by Heck and Valentine (2007), although they provide the significant caution that most experiments on effects of mesograzers are conducted at scales of meters or less for weeks or months (Supplementary Table 2, Figures 3, 4), while nutrient exposures may occur at scales of tens to hundreds of square kilometers over years to decades.

Given the wide range of consumption rates for different epiphyte grazers (Jernakoff et al., 1996), composition of the grazer community and relative abundance will clearly be critical factors in determining the ultimate level of effect on seagrass epiphytes. Most controlled experiments combining both nutrient elevation and grazer density manipulations have been at very small scale, and the question of whether results can be applied to large scale, temporally persistent cases of nutrient elevation remains unclear. An additional question is whether there is any specific level for elevation of nutrients where grazer controls will likely be overwhelmed. Hughes et al. (2013, 2016) described the recovery of seagrass cover in the context of processes operating at time and space scales affecting an entire estuary. The recovery of a top predator, the sea otter, in Elkhorn Slough CA appears to have helped mitigate the effects of nutrient loading on seagrass through a trophic cascade. Seagrass has expanded greatly since the reintroduction of otters, while loading levels for nitrate have continued to rise.

3.5.2. Leaf turnover—Epiphytes are typically more abundant on the distal (oldest) portions of all blades, and most abundant on the oldest blades within a plant, and thus the mean life span of leaves will influence epiphyte build-up on seagrass blades. As a result, selection of a standardized measurement approach by defining which blade(s) to measure for a given SAV species would be essential for development of a formal metric of epiphyte load. Typical life spans are 27–63 d (Borowitzka and Lethbridge, 1989), but rates of blade turnover can vary widely across seasons, and for *Z. marina* may range from 50–70 d in summer to 200 d in winter (Borum et al., 1984). Dixon and Kirkpatrick (1995) observed that light attenuation by epiphytes was highest during winter months when leaf turnover rates were reduced and higher epiphyte loads accumulated. Experimental work (Orbita and Mukai, 2009) has suggested that some, but not all, macrophyte species increased leaf turnover rates with increased epiphyte loads, a response that would partly offset epiphyte impacts. However, the publication includes questionable data plots (identical data for experimental and control treatments), such that it is difficult to ascertain whether the paper's conclusions are valid. Ruesink (2016), following manipulation of epiphyte loads in situ on *Z. marina*, suggested that the seagrass is able to tolerate current epiphyte loads because rapid production of new leaves corresponds to highest periods of epiphyte occurrence. The contribution of fresh leaves to total plant photosynthesis before they are fouled appears to offset the light reduction of epiphytes. Ruesink (2016) also suggested that any alteration of the balance of top-down and bottom-up controls might shift plants beyond a tipping point where this growth mechanism is insufficient to prevent negative impacts of epiphytes.

3.5.3. Wave Energy—In areas of high wave energy, the physical movement of the macrophyte substrate may limit the accumulation of epiphytes (Strand and Weisner, 1996; Lavery et al., 2007; Bryars, 2009) by as much as an order of magnitude (Lavery et al., 2007). As a result, high-energy locations may not be appropriate as monitoring sites for epiphyte metrics in that impacts of nutrients may tend to be underestimated. Hydrodynamic conditions may also interact with grazing efficiency. For example, biomass of epiphytes on *Z. marina* was highest in sites exposed to water movement (Schanz et al., 2002), while there was little epiphyte coverage in sheltered areas with extremely high abundance of the grazing snail *Hydrobia ulvae* ($151 \times 10^3 \text{ m}^{-2}$). In situ, flume experiments showed that snail density was negatively and epiphyte cover was positively correlated with current velocity. A trophic cascade effect was proposed, caused where fast currents removed or inhibited feeding of micrograzers, thus releasing epiphytes from grazing pressure. Myers and Heck (2013) also found lower crustacean mesograzers at an exposed compared to a protected seagrass bed. There was no significant effect of experimentally increased nutrients or decreased grazers at the exposed site, while there was a significant interaction between nutrient increase and grazer reduction at the protected site. No values for epiphyte load were reported for the exposed site, so it cannot be determined if there were site differences in load along with the difference in responses. In contrast, Caine (1980) found both higher epiphyte biomass and abundance of amphipod grazers in quiet water versus high wave action sites, and the two factors were also positively correlated on individual seagrass blades. Thus, the influence of hydrodynamics may be determined by species-specific differences in dominant grazer response.

3.5.4. Spatial Variability—Scales of variance have been examined for a variety of epiphyte metrics (Vanderklift and Lavery, 2000; Lavery and Vanderklift, 2002; Saunders et al., 2003; Piazzini et al., 2004; Johnson et al., 2005; Pardi et al., 2006; Balata et al., 2007; Castejón-Silvo and Terrados, 2012), but not typically in relation to nutrient gradients. Studies that examined epiphyte variation in relation to nutrient patterns (e.g. Frankovich and Fourqurean, 1997; Bryars et al., 2009), have not tended to include formal, nested analysis of variation at a hierarchy of scales. Variation in metrics used as epiphyte indicators at multiple spatial scales is likely, and consideration of such variability will be useful in design of monitoring programs. At scales likely to be relevant to assessment of nutrient impacts, regional calibration to reflect variation in the natural background for nutrient conditions may be required (Frankovich and Fourqurean, 1997; Bryars et al., 2011).

3.5.5. Temporal Variability—Temporal variability in epiphyte abundance is a standard feature in virtually any location, driven by seasonality in environmental factors such as light, temperature, and other drivers of primary production. Intra-annual variation may be large (Rice et al., 1983; Borum et al., 1984; Morgan and Kitting, 1984; Borum, 1985; Nelson, 1997; Nelson and Waalund, 1997; Frankovitch and Ziemann, 2005; *et alia*). For an epiphyte metric to be a useful indicator of nutrient effects, it will be necessary to select a temporal index period when epiphyte abundance is likely to be at the seasonal maximum. This index period may vary considerably among boreal, temperate and tropical (subtropical) locations, and will need to be regionally determined. Existing literature may be adequate to do so in many cases.

4. Summary Conclusions

There was general consistency in the results derived from field observations, laboratory experiments in mesocosms, and field experiments, in that elevated levels of nutrients tended to increase the level of epiphytes on the surface of SAV, in the absence of other limiting factors. Consistent with an increased difficulty in constraining multiple controlling factors, experimental outcomes tended to be more variable in field versus mesocosm experiments. For example, epiphyte D responses were never observed in mesocosm experiments. The limiting nutrient to which epiphytes respond may vary depending on local conditions, and may be either N or P. Combining the outcomes from both the laboratory and field experiments for both biomass and chl a metrics, frequency of I (77), NR (43), and D (7) differed significantly from an equal expectation for outcome (goodness of fit test, $G=70.8$, $p<0.001$). A comparison for only I and NR also differed significantly from an equal expectation for outcome (goodness of fit test, $G=9.8$, $p<0.005$). The experimental evidence, particularly for laboratory results, strongly supports the relation between increased nutrients and increased epiphytes. This conclusion is supported by Östman et al. (2016) who recently provided meta-analyses for experimental data from nutrient enrichment in the field with both seagrass (*Zostera*) and the seaweed *Fucus* within the North Atlantic region. Partial regression analysis indicated a positive relationship between the magnitude of response of filamentous algae and the magnitude of nutrient addition, again supporting the relation between increased nutrients and increased epiphytes.

Although there is some spatial bias in results, with mesocosm studies being more common for temperate regions, data from experimental manipulations was also helpful in defining conditions under which epiphytes tend to respond to nutrient addition. The variation in epiphyte response associated with experimental addition of nutrients via the water column, compared to sediment addition, merits further examination as to whether fertilization location influences epiphyte response. Mesograzers density is a critical covariate for experiments on nutrient addition, but grazer density has often not been adequately addressed in experimental designs. While grazers may inhibit the development of epiphytes in the presence of experimentally elevated nutrients, the outcome is highly dependent on factors such as grazer identity, density, nutrient level, and ambient light level.

The balance of evidence suggests that epiphytes on SAV will be a useful indicator of persistent nutrient enhancement in many situations. However, observational studies suggest that patterns of epiphyte response to nutrient load tend to be less evident as the spatial and temporal scale of data sets expand. For example, intra-annual seasonal variation may mask response patterns that are present primarily during the peak season of growth for epiphytes. Careful selection of an appropriate, annual index period for data collection, and selection of an appropriate spatial context to minimize influence of confounding factors (e.g. wave energy that may remove epiphytes) will help increase the signal to noise ratio for this indicator. Nelson (2017) has noted that development of a standardized approach for measurement of epiphyte load, analogous to the detailed guidance on measurement of epiphyte biomass provided by Kendrick and Lavery (2001), is critical. Agreement on use of artificial substrates may provide a better method of comparison across systems of loads among species with highly varying morphology (Nelson 2017). Development of standardized methods for measurement of epiphyte loads is a crucial next step in making an indicator based on epiphyte load operationally useful in monitoring of coastal eutrophication.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements and Disclaimer:

We thank Dr. Tim Nelson for initial technical review of this paper, and two anonymous reviewers for their helpful comments to improve the manuscript. The members of the Office of Research and Development Nutrient Indicators Workgroup (Cheryl Brown, Jim Kaldy, Jim Hagy, Lauri Ruiz-Green, Caitlin White, Nathan Smucker, Catherine Gross, Brad Blackwell) provided fruitful discussion during the formulation of this paper. The analysis of information in this document has been funded wholly by the U.S. Environmental Protection Agency. It has been subjected to review by the National Health and Environmental Effects Research Laboratory's Western Ecology Division and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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Figure 1.
Relationship of mean summer season (May – August) epiphyte load on *Zostera marina* versus mean summer water column TN at four sites along the Roskilde Fjord, Denmark (Data Borum, 1985; Figs. 4, 5).

Figure 2.

Regression relationship of average epiphyte accumulation rate on artificial substrates at stations along a salinity gradient within the Patuxent River, Maryland (Data Stankelis et al., 2001; Fig. 4–14).

Figure 3.

Regression relationships of epiphyte load on *Thalassia testudinum* for winter samples from field sites in the Florida Keys, USA versus A) Total Nitrogen and B) Total Phosphorus (Data Lapointe et al., 1994; Tables 1, 2).

Figure 4. Relationship of epiphytes to nutrients for A) mean annual epiphyte load on seagrass (*Halodule wrightii* and *Thalassia testudinum*) versus annual N load to regions of Charlotte Harbor, FL (Epiphyte data Dixon and Kirkpatrick, 1999; Fig. 19; N loads Squires et al., 1998; Fig. 4.), and B) epiphyte load on mixed seagrass species versus TP in the Indian River Lagoon, Florida, USA (Data Hanisak, 2001; TP, Fig. 3.13; epiphyte data, Fig. 8.8).

Figure 5. Relationship of epiphyte load on *Potamogeton perfoliatus* to nutrients for A) TP concentration and B) N/P ratio, in mesocosm tanks (Data Twilley et al., 1982; epiphyte data, Fig. 13; nutrient data, Tables A-4, A-5).

Figure 6.

Relationship for *Zostera marina* of A) growth rate to epiphyte load per shoot from Padilla Bay, Washington (Data Williams and Ruckelshaus, 1993; field data, Tables 3, 4, laboratory data, Fig. 6), and B) biomass to epiphyte biomass (per unit tank area) for a mesocosm experiment varying nutrient load and light level (Data for all treatments, Kaldy, 1992; Fig. 14).

Figure 7.

Relationship of epiphyte biomass as chl-a on *Zostera marina* to experimental nutrient concentration for a mesocosm experiment varying nutrients and presence of grazers (Data Jaschinski and Sommer, 2008; Fig. 1A and text).

Figure 8.
Relationship of the growth rate of *Halodule wrightii* (as new blade weight) to epiphyte load per unit weight of old blades (Data Virnstein et al., 1989; Figs. 7, 8).

Figure 9.
Relationship of the epiphyte load on *Zostera marina* to isopod density for field experiments from Padilla Bay, Washington (Data Williams and Ruckelshaus, 1993; epiphyte data - Table 3, *Idotea* data - Table 6).

Table 1.

response patterns, qualitative or quantitative, to experimental nutrient additions in micro- or mesocosms for biomass, chlorophyll *a* on metrics for epiphytes on SAV. Studies are also classified by fertilization method and climate zone. Abbreviations: Qualitative QL, Solid - S, Dissolved - D, Temperate - T, Subtropical - ST, Tropical - TR, Yes - Y, N- No, Increase - I, Decrease - D, H, Medium - M, Low - L, Unknown - U.

Location:	Citation:	Data Type	Nutrient Addition	Climate Zone	Measured Nutrients?	Measured Grazers? / Relative Density	Biomass Response	Chl <i>a</i> Response	Taxa changes?	Comments
Adelaide, South Australia	Collings et al. 2006	QU	S	T	Y	N/U	I			Nutrient addition method not clearly stated.
North Carolina USA	Burkholder et al. 1994	QL	D	T	Y	Y/H	NR ¹			¹ Fall 1992 experiment. Cell counts only, epiphytes "negligible"
Florida USA	Lapointe et al. 1994	QU	D	TR	N	N/U	I			"Grazing organisms were added."
Florida USA	Vinstein et al. 1987	QU	D	ST	Y ¹	N/U	I			¹ Nutrient data not provided. Amphipod grazers controlled by fish.
Adelaide, South Australia	Collings et al. 2006	QU	S	T	Y	N/U	I			Nutrient addition method not clearly stated.
Maryland USA	Staver 1984	QU	D	T	N	N/U	I	I		Laboratory microcosms.
Maryland USA	Staver 1984	QU	D	T	Y	N/U	I	I		Field mesocosm ponds.
Maryland USA	Twilley et al. 1985 (& 1982)	QU	D	T	Y ¹	N/U	I	I		¹ Reported in Twilley et al. 1982
Maryland USA	Severn 1998, pt 1	QU	D	T	Y	N/L	I			No grazers included.
Maryland USA	Severn 1998, pt 2	QU	D	T	Y	Y/M	I			Periodic fish exposure still allowed grazer increase 20–100× over 6 weeks.
Maryland USA	Sturgis & Murray 1997, Murray et al. 2000	QU	D	T	Y	N/L		I		"Nutrient Delivery Experiments". Summer-Fall experiment. No grazers.
Maryland USA	Sturgis & Murray 1997, Murray et al. 2000	QU	D	T	Y	N/L		I		"Nutrient Delivery Experiments". Spring-Summer experiment. No grazers.
Maryland USA	Murray et al. 2000	QU	D	T	N	N/L	I			"Exchange Rate Experiment"

Location:	Citation:	Data Type	Nutrient Addition	Climate Zone	Measured Nutrients?	Measured Grazers? Relative Density	Biomass Response	Chl a Response	Taxa changes?	Comments
Maryland USA	Murray et al. 2000	QU	D	T	N	Y/U	I			"Trophic Complexity Experiment", experimental grazer density not provided in reference.
Maryland USA	Twilley et al. 1985 (& 1982)	QU	D	T	Y ¹	N/U	I	I		¹ Reported in Twilley et al. 1982
North Carolina USA	Burkholder et al. 1994	QL	D	T	Y	Y/H	I			Fall 1992 experiment, <i>Polysiphonia</i> "noticeably thicker" on <i>Ruppia</i> than other species
Florida USA	Lapointe et. al 1994	QU	D	TR	N	N/U	I			"Grazing organisms were added."
Florida USA	Tomasko and Lapointe 1991	QU	D	TR	N	N/U	I			"Grazing organisms were added."
Florida USA	Hays 2005	QU	S	ST	Y	Y/M	NR			Nutrient addition levels achieved were very low (<0.015 µM)
Virginia, USA	Murray 1983	QU	S	T	Y	N/L	I			Sig. interaction of nutrient and shading treatments, higher epiphytes in shade treatment.
North Carolina USA	Burkholder et al. 1992	QU	D	T	Y	Y/H	I ¹			¹ Cell counts only, Spring experiment, May samples only
North Carolina USA	Burkholder et al. 1992, Coleman & Burkholder 1994	QU	D	T	Y	Y/M	NR ¹		Y	¹ Cell counts only, Fall experiment, Coleman & Burkholder (1994), some taxa shifts.
New Hampshire USA	Kaldy 1992; Short et al. 1995	QU	S	T	Y	N/L	I			Epiphyte increases except at low light levels, 1990 experiment results.
New Hampshire, USA	Kaldy 1992	QU	S	T	Y	N/L	NR			1991 experiment results.
Virginia, USA	Neckles et al. 1993	QU	D	T	Y	Y/H	I			Early Summer experiment. 4,800 m ⁻² grazers.
Virginia, USA	Neckles et al. 1993	QU	D	T	Y	Y/H	I			Late Summer experiment. 11,400 m ⁻² grazers.
Virginia, USA	Neckles et al. 1993	QU	D	T	Y	Y/H	I			Fall experiment. 3,600 m ⁻² grazers.
Virginia, USA	Neckles et al. 1993	QU	D	T	Y	Y/M	I			Spring experiment. 900 m ⁻² grazers.

Location:	Citation:	Data Type	Nutrient Addition	Climate Zone	Measured Nutrients?	Measured Grazers? / Relative Density	Biomass Response	Chl a Response	Taxa changes?	Comments
Washington, USA	Williams & Ruckelshaus 1993	QU	D	T	N ¹	N/L	I			¹ Water column not measured, sediment porewater nutrients measured.
North Carolina, USA	Burkholder et al. 1994	QL	D	T	Y	Y/H	NR ¹			¹ Cell counts only, epiphytes “negligible”
Virginia, USA	Neckles et al. 1994	QU	D	T	N	N/M	I ¹		Y	¹ Cell counts only, taxon dependent responses. Grazers = 900 m ⁻² .
Rhode Island, USA	Taylor et al. 1995	QU	D	T	N	N/L	NR			Approx. 220 grazing snails m ⁻² added at start.
Rhode Island, USA	Lin et al. 1996	QU	D	T	Y	N/L	NR	NR	Y	Approx. 220 grazing snails m ⁻² added at start.
Rhode Island, USA	Kopp 1999	QU	D	T	Y	N/L	I			Grazers excluded by 1 µm filter bag on input water.
Virginia, USA	Moore & Wetzel 2000	QU	D	T	Y	Y/H	NR			Summer experiment
Virginia, USA	Moore & Wetzel 2000	QU	D	T	Y	Y/H	NR			Fall experiment
Virginia, USA	Moore & Wetzel 2000	QU	D	T	Y	Y/H	I ¹			¹ Spring experiment, high light treatment only
Rhode Island, USA	Bintz et al. 2003	QU	D	T	N	N/L	I			Increase greatest in elevated temperatures
Kiel, Germany	Jaschinski & Sommer 2008	QL	D	T	Y	N/M		I	Y	Average abundance of mesograzers.
Virginia, USA	Spivak et al. 2009	QU	S	T	N	Y/L		I		Grazers as AFDW. Epiphytes ↑ with nutrient ↑ without grazers.
Kiel, Germany	Jaschinski & Sommer 2011	QU	D	T	N	Y/L, M, H		I		Three different grazers; two nutrient levels as separate experiments
Virginia, USA	Blake & Duffy 2012	QU	S	T	Y	Y/M		I	Y	Epiphytes collected from artificial seagrass

Table 2.

response patterns, qualitative or quantitative, to field experimental nutrient additions for biomass, chlorophyll *a* and taxonomic for epiphytes on SAV. Studies are also classified by nutrient addition location and by climate zone. Abbreviations: Quantitative -QU, Qualitative -Q, Water Column -WC, Temperate -T, Subtropical -ST, Tropical -TR, Yes -Y, N- No, Increase - I, Decrease - D, No - H, Medium - M, Low - L, Unknown – U.

Location:	Citation:	Data Type	Nutrient Addition	Climate Zone	Measured Nutrients?	Measured Grazers?	Biomass Response	Chl <i>a</i> Response	Taxa changes?	Comments
Gulf St. Vincent, South Australia	Bryars et al. 2011	QU	WC	T	Y	N	I		Y	Composition altered after 8 months.
Derawan Island, Kalimantan, Indonesia	Christianen et al. 2012	QU	WC	TR	Y	N	I			No effect of experimental nutrient addition, but strong relation to natural P.
Pertido Key, Florida, USA	Wear et al. 1999	QU	WC	ST	N	N	I	I	Y	Biomass, chl <i>a</i> elevated for first 8 months. Pigment and taxon shifts.
Big Lagoon, Florida USA	Heck et al. 2006	QU	WC	ST	Y	Y		I		
Big Lagoon, Florida USA	Myers & Heck 2013	QU	WC	ST	N	Y	NR			Significant interaction of grazer removal and +nutrient at protected site only.
Western Port Bay, Victoria, Australia	Bulthuis & Woelkerling 1981	QL	S	T	Y	N	NR			
Port Phillip Bay, Victoria, Australia	Bulthuis et al. 1992	QU	S	T	Y	N	NR			
Lady Bay, South Australia	McSkimming et al. 2015	QU	WC	ST	Y	Y	I			Epiphyte ↑ > when grazers reduced.
Revellata Bay, Corsica, France	Leoni et al. 2006	QU	WC	ST	Y	N	I			
Fenals Cove, NE Spain	Prado et al. 2008	QU	WC	ST	N	N	I		Y	Biomass and composition changes during 2 summer months only.
Palma Bay, Majorca, Spain	Castejón-Silvo et al. 2012a	QU	WC	ST	N	Y	I			

Species:	Location:	Citation:	Data Type	Nutrient Addition	Climate Zone	Measured Nutrients?	Measured Grazers?	Biomass Response	Chl <i>a</i> Response	Taxa changes?	Comments
	Palma Bay, Majorca, Spain	Castejón-Silvo et al. 2012b	QU	WC	ST	N	N	I			
	Gulf St. Vincent, South Australia	Bryars et al. 2011	QU	WC	T	Y	N	I		Y	Composition altered after 8 months.
	Ninigret Pond, Rhode Island, USA	Harlin & Thorne-Miller 1981	QL	WC	T	Y	N			N	
	Pertido Key, Florida, USA	Wear et al. 1999	QU	WC	ST	N	N	I	I	Y	Biomass, chl <i>a</i> elevated for first 8 months. Pigment and taxon shifts.
	St. John's Island, Singapore	Ali et al. 2012	QU	WC	TR	Y	N	NR			
	Tague Bay, St. Croix, US Virgin Islands	Williams 1987	QL	S	TR	Y	N	I			<i>Thalassia testudinum</i> mixed with <i>Syringodium filiforme</i> or <i>Halodule wrightii</i> .
	Tague Bay, St. Croix, US Virgin Islands	Williams 1990	QL	S	TR	Y	N	NR			<i>Thalassia testudinum</i> mixed with <i>Syringodium filiforme</i> or <i>Halodule wrightii</i> .
	Bailey's Bay, Barbuda	McGlathery 1995	QL	WC	ST	Y	N	I		Y	Calcareous reds to filamentous epiphytes.
	Pertido Key, Florida, USA	Wear et al. 1999	QU	WC	ST	N	N	I	I	Y	Biomass elevated for first 10, chl <i>a</i> for 13 months. Pigment and taxon shifts.
	St. Joseph Bay, Florida, USA	Heck et al. 2000	QU	WC	ST	Y	Y	NR		Y	↑ in % green algae.
	Florida Bay, USA:	Ferdie & Fourqurean 2004	QU	S	TR	N	N	I	I		Nearshore sites
	Florida Bay, USA:	Ferdie & Fourqurean 2004	QU	S	TR	N	N	D	I		Offshore sites, load ↓ with +N, chl <i>a</i> autotrophic index ↑.

Species	Location:	Citation:	Data Type	Nutrient Addition	Climate Zone	Measured Nutrients?	Measured Grazers?	Biomass Response	Chl <i>a</i> Response	Taxa changes?	Comments
	Perdido Bay, Florida, USA	Ibarra-Obando et al. 2004	QU	WC	ST	Y	N	D			Values = standardized differences in treatment versus initial value.
	Florida Bay, USA	Armitage et al. 2005	QU	S	TR	N	N		NR		Duck Key (=site C2).
	Florida Bay, USA	Armitage et al. 2005	QU	S	TR	N	N		D		Bob Allen Key (=site B2).
	Florida Bay, USA	Armitage et al. 2005	QU	S	TR	N	N		NR		Nine Mile Key (=site A2)
	Florida Bay, USA	Armitage et al. 2005	QU	S	TR	N	N		I		Sprigger Bank (=site A1). Significant site x P x N interaction; treatments > controls.
	Florida Bay, USA	Armitage et al. 2005	QU	S	TR	N	N		I		South Nest (=site B1). Significant site x P x N interaction; treatments > controls.
	Florida Bay, USA	Armitage et al. 2005	QU	S	TR	N	N		I		Rabbit (=site C1). Significant site x P x N interaction; treatments > controls.
	Florida Bay, USA	Armitage et al. 2006	QU	S	TR	N	N		I	Y	Duck Key (=site C2). Significant ↑ in chl b and zeaxanthins, chl a = NR.
	Florida Bay, USA	Armitage et al. 2006	QU	S	TR	N	N		I	Y	Bob Allen Key (=site B2). Chl a & fucoxanthins ↑ with +N, ↓ with +P & +NP.
	Florida Bay, USA	Armitage et al. 2006	QU	S	TR	N	N		I	Y	Nine Mile Key (=site A2).

Location:	Citation:	Data Type	Nutrient Addition	Climate Zone	Measured Nutrients?	Measured Grazers?	Biomass Response	Chl <i>a</i> Response	Taxa changes?	Comments
Florida Bay, USA	Armitage et al. 2006	QU	S	TR	N	N		I	Y	Fucoxanthins ↑ with +N. Sprigger Bank (=site A1). Chl <i>a</i> & fucoxanthins ↑ with +P & +NP.
Florida Bay, USA	Armitage et al. 2011	QU	S	TR	N	N		NR		Duck Key (=site C2), @ 44 months.
Florida Bay, USA	Armitage et al. 2011	QU	S	TR	N	N		NR		Bob Allen Key (=site B2), @ 44 months.
Florida Bay, USA	Armitage et al. 2011	QU	S	TR	N	N		I		Nine Mile Key (=site A2), @ 44 months. Significant site x P interaction. Chl <i>a</i> ↑ for +NP.
Florida Bay, USA	Armitage et al. 2011	QU	S	TR	N	N		I		Sprigger Bank (=site A1), @ 44 months. Significant site x P interaction. Chl <i>a</i> ↑ for +P, +NP.
Florida Bay, USA	Armitage et al. 2011	QU	S	TR	N	N		NR		South Nest (=site B1), @ 44 months.
Florida Bay, USA	Armitage et al. 2011	QU	S	TR	N	N		I		Rabbit (=site C1), @ 44 months. Significant site x P interaction. Chl <i>a</i> ↑ for +NP.
Florida Bay, USA	Gil et al. 2006	QU	S	TR	N	Y	NR		Y	Significant ↑ in chl <i>b</i> & zeaxanthin at P limited site.
Florida Bay, USA	Peterson et al. 2007	QU	WC	TR	N	N	I	I	N	Only target gastropods measured.
Florida Bay, USA:	Baggett et al. 2010	QU	WC	TR	N	Y	NR	I	N	Significant ↑ in chl <i>b</i> & zeaxanthin, but

Location:	Citation:	Data Type	Nutrient Addition	Climate Zone	Measured Nutrients?	Measured Grazers?	Biomass Response	Chl <i>a</i> Response	Taxa changes?	Comments
Abaco Island, Bahamas	Stoner et al. 2014	QU	S	TR	N	Y	NR			no relative % changes in pigments.
Mullet Creek, NSW, Australia	York 2010 (Chapter 5)	QU	WC	ST	Y	Y	D			Nutrient effect confounded in experimental design.
Mullet Creek, NSW, Australia	York 2010 (Chapter 6)	QU	WC	ST	Y	Y	NR			Algal loads in all treatments relatively low, non-significant nutrient elevation measured.
Ninigret Pond, Rhode Island, USA	Harlin & Thorne-Miller 1981	QL	WC	T	Y	N			N	↓ biomass with sediment addition in April but not August. Possible grazer effect.
Padilla Bay, Washington, USA;	Williams & Ruckelshaus 1993	QU	S	T	Y	N	D			↓ biomass with water column addition in August but not April. Possible grazer effect.
Padilla Bay, Washington, USA;	Williams & Ruckelshaus 1993	QU	WC	T	Y	N	D			↓ biomass with water column addition in August but not April. Possible grazer effect.
Back Sound, NC, USA	Coleman & Burkholder 1995	QU	WC	T	Y	N			Y	Significant ↑ in all treatments @ 9d, no nutrient effect @ 23d.
York River, VA, USA	Douglass et al. 2007	QU	WC	T	Y	Y		I		Significant nutrient effect at 3 weeks, no effect at 6 weeks.
Gullmar Fjord, Sweden	Moksnes et al. 2008	QU	WC	T	Y	Y		I		Algal response included epiphytes plus
Gullmar Fjord, Sweden	Baden et al. 2010	QU	WC	T	Y	Y	I			

Location:	Citation:	Data Type	Nutrient Addition	Climate Zone	Measured Nutrients?	Measured Grazers?	Biomass Response	Chl <i>a</i> Response	Taxa changes?	Comments
Kalmar Sound, Sweden	Baden et al. 2010	QU	WC	T	Y	Y	NR			loose macroalgae. Algal response included epiphytes plus loose macroalgae.
Gulf of Finland	Baden et al. 2010	QU	WC	T	Y	Y	NR			Algal response included epiphytes plus loose macroalgae.
York River, VA, USA	Whalen et al. 2013.	QU	WC	T	N	Y		I		↑ observed in fall but not in summer experiments with grazer reduction.
York River, VA, USA	Reynolds et al. 2014.	QU	WC	T	N	Y		NR		Short term experiment, 4 weeks.
York River, VA, USA	Reynolds et al. 2014.	QU	WC	T	N	Y		NR		Short term experiment, 3 weeks.
Bodega Bay, CA, USA	Duffy et al. 2015	QU	WC	T	N	Y		I		Duffy et al. Fig. S2
Akkeshi-ko, Hokkaido, Japan	Duffy et al. 2015	QU	WC	T	N	Y		D		Duffy et al. Fig. S2
12 locations, northern hemisphere	Duffy et al. 2015	QU	WC	T	N	Y		NR		NR at 12 sites, Ruesink 2016 results given separately.
Willapa Bay, WA	Ruesink 2016	QU	WC	T	N	Y	I	NR		Nutrients significantly ↑ biomass; chl <i>a</i> ↑ in 2012 but not 2011.
Mullet Creek, NSW, Australia	Kelaher 2013	QU	WC	ST	N	Y	I			<i>Halophila ovalis</i> also present. Only epiphytes in medium + N treatment significantly ↑.