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Development of an epiphyte indicator of nutrient enrichment: threshold values for seagrass epiphyte load

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Abstract

Metrics of epiphyte load on macrophytes were evaluated for use as quantitative biological indicators for nutrient impacts in estuarine waters, based on review and analysis of the literature on epiphytes and macrophytes, primarily seagrasses, but including some brackish and freshwater rooted macrophyte species. An approach is presented that empirically derives threshold epiphyte loads which are likely to cause specified levels of decrease in macrophyte response metrics such as biomass, shoot density, percent cover, production and growth. Data from 36 studies of 10 macrophyte species were pooled to derive relationships between epiphyte load and –25 and –50% seagrass response levels, which are proposed as the primary basis for establishment of critical threshold values. Given multiple sources of variability in the response data, threshold ranges based on the range of values falling between the median and the 75th quantiles of observations at a given seagrass response level are proposed rather than single, critical point values. Four epiphyte load threshold categories - low, moderate, high, very high, are proposed. Comparison of values of epiphyte loads associated with 25 and 50% reductions in light to macrophytes suggest that the threshold ranges are realistic both in terms of the principle mechanism of impact to macrophytes and in terms of the magnitude of resultant impacts expressed by the macrophytes. Some variability in response levels was observed among climate regions, and additional data collected with a standardized approach could help in the development of regionalized threshold ranges for the epiphyte load indicator.

Keywords

Epiphyte; nutrient indicator; water quality indicator; indicator development; seagrass

1. Introduction

Field and laboratory observations and experiments obtained from varied aquatic systems have repeatedly demonstrated that elevated levels of water column nutrients can result in increased levels of epiphytic algal material on submerged aquatic vegetation and other relatively stable substrates within relatively short time periods (Phillips et al. 1978, Bulthuis & Woelkerling 1983, Borum 1985, Twilley et al. 1985, Cambridge et al. 1986, Silberstein et al. 1986, Jensen & Gibson 1986, Neverauskas 1987a, b, Dunton 1990, Tomasko & LaPointe 1991, Frankovich & Fourquean 1997, Neckles et al. 1993, Williams & Ruckelshaus 1993, Lapointe et al. 1994, Murray et al. 2000, and numerous others). Assessment of epiphyte

responses on attached macrophytes is particularly of interest since the substrate itself (e.g. seagrass) is often an endpoint of interest for evaluating the impacts of elevated nutrients. Macrophytes tend to remain in place long enough to integrate local nutrient loads. Response patterns in freshwater lakes and streams appear generally similar to those in estuarine and marine systems. Based on a review of field observations, and laboratory and field mesocosm experiments Nelson (2016 submitted) concluded that the balance of evidence suggests that epiphyte load on SAV can be a useful indicator of persistent nutrient enhancement in many situations.

While epiphytic algae may have some beneficial effects on seagrasses (Orth & van Montfrans 1984, Brandt & Koch 2003), negative impacts predominate (Borowitzka & Lethbridge 1989). Negative effects include: 1) reduction in light available for photosynthesis, 2) reduction in the rate of diffusion of materials such as CO₂ across the seagrass blade surface, and 3) increase in physical drag, resulting in increased loss of leaves or plants. Seagrass leaves with heavy epiphyte cover may become more brittle and break off (Borowitzka & Lethbridge 1989, Heijs 1985), an effect that may be aggravated by nitrate enrichment (Kopp 1999, Nafie et al. 2012). Harlin (1975) suggested that epiphytes may compete with seagrass for water column nutrients, but the magnitude of any effect should be minor relative the main effects listed above. Suggested positive benefits of epiphytes include serving as a UV-B filter, which might be most important in tropical, oligotrophic waters (Trocine et al. 1981; Brandt & Koch 2003), and as a factor potentially limiting desiccation damage for plants in the upper intertidal zone (Penhale & Smith 1977, Bendell 2006).

Epiphyte metrics as indicators of system response to nutrient loadings have been commonly considered (e.g. Bricker et al 2003, Wood & Lavery 2000, US EPA 2010, Sutula 2011, and references in Table 2 herein), and there has been recent increased interest in epiphyte metrics within the European Water Framework Directive (e.g. Gobert et al. 2009, Balata et al. 2010, Giovanetti et al. 2010, Castejón-Silvo & Terrados 2012, Marbà et al 2013). While ideally it would be desirable to tie an epiphyte indicator directly to water column nutrient loads, conceptual models of eutrophication effects on macrophytes (e.g. Phillips et al. 1978, Dennison 1992, Krause-Jensen et al. 2008) make clear that there are multiple interacting processes which make development of such relationships extremely difficult. Additionally, there are relatively few studies which include data on nutrient loads at ecologically relevant scales in relation to seagrass response metrics (Table 2, Krause-Jensen et al., 2008), and even fewer that also include data on epiphyte loads. Epiphyte load was one indicator in the initial development of the ASSETS method for estuarine trophic status assessment (Bricker et al 2003; Scavia & Bricker 2006), but the indicator was dropped from subsequent assessments due to lack of available data (Whitall et al. 2007). In light of such limitations, the present study accepts, based on a parallel literature review (Nelson 2016 submitted), as well as previous reviews (Harlin 1980, 1995, Hughes et al. 2004, Burkholder et al. 2007, Leoni et al. 2008, Krause-Jensen et al. 2008, Nelson 2009, Thomsen et al. 2012), that excessive nutrients will cause increases in epiphyte load on macrophyte hosts in the absence of mitigating factors. It then empirically determines threshold levels of epiphyte loads which are likely to cause given levels of decreases in macrophyte response metrics such as biomass, shoot density, percent cover, production and growth. The thesis of this approach is

that if measured epiphyte loads fall within the higher ranges of the proposed threshold, excess nutrients should be investigated as a causal agent.

2. Methods

2.1. General Methods

An extensive review of the literature on epiphytes and macrophytes, primarily seagrasses and some brackish and freshwater species (e.g. *Potamogeton*, *Ruppia*), was conducted in order to evaluate whether epiphyte metrics can be used as quantitative biological indicators for nutrient impacts in estuarine waters. The research literature examined focused on field studies which included data on seagrass epiphyte responses to nutrient inputs (e.g. waste water, fish farms, bird guano), and both field and laboratory experimental studies which manipulated nutrient levels and recorded epiphyte responses. Studies that assessed light reduction in relation to quantity of epiphytes were also reviewed as being an important mechanism in the stress-response relationship between nutrients, epiphytes and macrophytes (Nelson, submitted). Additionally, some autecological studies of seagrass systems had data on epiphyte load and seagrass response metrics which allowed examination of relationships between the two factors (e.g. Nelson 1987, Hasegawa et al. 2007). Searching for epiphyte studies relied on previous reviews of seagrass epiphytes (e.g. Smith et al. 1994, Hughes et al. 2004, Burkholder et al. 2007, Leoni et al. 2008, 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 scientific journal web sites. In excess of 400 publications were examined, including peer reviewed literature, theses and dissertations, and “gray” literature technical reports.

To acquire data from published 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. Comparison of repeated measurements of the same data points with the software gave a measurement precision within <0.1%. Comparison of the values extracted via software to values for the same data points which were given in the publication gave a measurement accuracy of <3%.

The capture of data from the original papers followed by independent analysis provided a QA check for analyses in the original papers. Data issues noted included regression equations in original sources that were clearly in error, and either ambiguous or erroneous units for data were presented. For the former, recalculated equations were used. For the latter issue, data sets were excluded.

2.2. Estimation of Light Attenuation

The literature review found 29 studies (Supplemental Table 1) that determined the relationship between light attenuation and various epiphyte load metrics, for nine submerged aquatic vegetation (SAV) species including several freshwater species, and multiple types of artificial substrates, spanning a range of latitudes. These studies form one basis for translation to potential thresholds for different regions and macrophyte species. A variety of

response variables, measurement methods, and curve fitting methods were used to describe the relationships between epiphyte load and light reduction. Various aspects of measurement approaches to assess epiphyte light attenuation have been previously reviewed (Vermatt & Hootsmans 1994, Brush & Nixon 2002, Frankovich & Zieman 2005).

In a subset of studies, limited data were collected ($n < 10$), and the authors reported linear relationships of epiphyte load to light reduction (Bulthuis & Woekerling 1983, Neundorfer & Kemp 1993, Carter et al. 1995, Glazer 1999, Brandt & Koch 2003), which are at variance with the large majority of studies showing best fit relationships of the type “exponential increase to a maximum”. Since the small data sets could be fit with exponential curves and comparable r^2 to the original equations, they were included in the final analysis. Only studies which expressed epiphyte biomass as mg DW cm^{-2} (the most frequently used metric) were selected, resulting in a subset of 28 independent cases where data could be reanalyzed to provide consistent curve fitting approaches for the purposes of comparison (Table 2). Data were classified as to substrate type (artificial, natural), climate region (Temperate, Subtropic, Tropic), and a very general morphological type: Broad blade (*Posidonia australis*, *Posidonia sinuosa*, *Posidonia oceanica*, *Thalassia testudinum*, *Zostera marina*), Leafy (*Amphibolis antarctica*, *Potamogeton perfoliatus*), and Narrow blade (*Halodule uninervis*, *Halodule wrightii*, *Zostera japonica*). Broad versus Narrow blade refers to blade width.

Equations of the form $Y = a * (1 - e^{(-b * x)})$, exponential rise to a maximum with two parameters, were fit to each data set using Sigmaplot 13®. Coefficient “a” represents the maximum value for light attenuation, and coefficient “b” represents the exponential rate of increase. Mean values of the two equation coefficients were compared among subsets of the data using one-way ANOVA, t-tests or non-parametric equivalents where assumptions of the parametric test were not met. Comparisons included substrate type, climate region, and species (with sufficient data – *Z. marina*, *T. testudinum*). For comparison of climate region, three studies conducted in freshwater (Supplemental Table 1) were pooled with macrophyte studies from the Temperate climate region. There were insufficient data to compare equation coefficients among seagrass morphotypes.

2.3. Estimation of Effects on Macrophytes

The compiled seagrass-epiphyte literature was examined to find data sets containing both epiphyte load and seagrass response metrics such as biomass, shoot density, productivity, or growth. A total of 36 publications provided data for 10 macrophyte species (Table 2). Regression analyses were conducted in Sigmaplot 13 ® software on seagrass metrics as a function of epiphytic load. Based on inspection of the data, either linear or non-linear regression was applied. Data sets with either no relationship or a positive relationship of epiphytic load and seagrass biomass were not further considered. Data sets from oligotrophic areas where epiphytic loads tended to be very low often fell in this category. A particular challenge was that both epiphytic load (Table 1) and seagrass response metrics were reported in a wide range of metric categories and measurement units within a category. In a few cases, disparate units could be directly converted to a common basis (e.g. mg g^{-1} DW to g g^{-1} DW), or an appropriate conversion factor could be developed and applied to a

metric (AFDW to DW). In all cases, biomass ratio metrics (e.g. g g^{-1} DW) refer to DW of epiphytes compared to DW of aboveground biomass of SAV.

For each data set for which there was a negative regression relationship, the regression equation was used to develop estimated values of epiphytic load (the dependent variable) associated with fixed reduction levels (25 and 50%) in seagrass response metrics from their maximum estimated values (y intercepts). The goal was to provide a reasonable quantitative basis for establishing threshold levels of concern when measuring epiphytic loads on seagrasses. While 25 and 50% reduction levels are somewhat arbitrary, they are commonly computed percentile levels and would represent moderate to severe impacts to seagrass beds, and are likely to be detectable in field surveys without prohibitive sample sizes (Heidelbaugh & Nelson 1996). Other reduction levels could be selected if lower or higher reduction levels are desired.

Epiphyte loads associated with the 25 and 50% reductions were compiled together with any additional data points identifying quantitative levels of epiphyte load associated with seagrass response (Table 3). Data were additionally categorized to assess the importance of factors such as morphological type (defined as above) and seagrass response metric (Biomass, Shoot Density, and Productivity or Growth) on the epiphytic load values. To confirm that 25 and 50 % yielded significantly different median values of epiphytic load, data were parsed into these two categories. Medians of grouped data were compared with Kruskal-Wallis One-Way ANOVA on Ranks where sufficient data were available to analyze three groups, and the Mann Whitney Rank Sum test was used for comparison of two groups. These analyses, together with production of Box and Whisker plots, were conducted in Sigmaplot 13 ®.

3. Results and Discussion

3.1. Metrics of Epiphyte Response.

An extremely wide variety of metrics for characterization of epiphytes have been published, and many of these are summarized in Table 1. The metrics may be broadly divided into measures of amount of epiphytes present, epiphyte community composition, and epiphyte production functions.

A number of publications have explored the response of epiphytic community composition to anthropogenic stressors including nutrients (Wear et al. 1999, Lavery & Vanderklift 2002, Cambridge et al. 2007, Balata et al. 2007, 2008, Giovannetti et al. 2010). However, other types of metrics such as biomass or chlorophyll *a* are most directly relevant to impacts on macrophytes, such as seagrasses, and protection of seagrasses may be incorporated as a success metric for water quality criteria. Also, the effort and cost per data point required to characterize community composition may limit use of such indicators in water quality monitoring, especially across larger scales. As costs decrease and capabilities increase for genomic approaches to organismal identification (Uku et al. 2007), utilization of epiphyte community composition for protection of water quality may become more feasible. However, Cambridge et al. (2007) found that while changes in epiphyte species composition

correlated with nutrient loading and seagrass decline, composition shifts did not predict future seagrass loss and thus were of limited utility as an indicator.

A current problem with a number of common epiphyte metrics is the plethora of units used for their expression (Table 1). Biomass of epiphytes has been expressed in the literature in more than a dozen different units, some of which may be easily interconverted, while for others there may not be a ready conversion factor. For example, given the major morphological differences among macrophyte species, biomass expressed as weight per shoot is not comparable among species. Conversion to a standard measure such as weight per cm^{-2} of macrophyte from weight per shoot requires data on surface area, which may or may not be provided. Imprecision or omission of details in Methods sections may also preclude intercomparison of potentially useful data. For example, it was sometimes difficult to determine whether units expressed in terms of m^{-2} refer to area of the benthic environment, or to surface area of the macrophyte substrate from which the epiphyte samples were collected. Authors should be cognizant of these issues, and it would be beneficial if conversion factors for epiphyte load per substrate surface area and substrate biomass were routinely provided.

In spite of the above limitations, sufficient data exist such that some assessment metrics may be intercalibrated through the establishment of empirical relationships, e.g. chlorophyll *a* versus epiphyte DW, or DW versus AFDW, at least at local to regional scales. Such interconversions, allow for increasing sample sizes in the review of data, allowing greater flexibility in measurement and monitoring (Heijs 1987).

For the purpose of selecting epiphyte metrics most useful to a goal of quantifying factors influencing the health of the macrophyte substrate, both biomass as weight per unit area of substrate (e.g. mg DW cm^{-2}) and chlorophyll *a* per unit area of substrate (e.g. $\mu\text{g chl-a cm}^{-2}$) have been directly related to the reduction of light available to macrophytes (see below). An additional advantage is that the methods for these metrics are well established. Chlorophyll *a* is the most direct measurement of epiphyte plant response to nutrient enrichment. However, the epiphyte complex generally includes both inorganic and non-photosynthetic organic components, which also may attenuate light to macrophyte substrates. Thus, biomass may more accurately reflect the aggregate light attenuation of all components. Relatively few studies ($n=5$) have computed both biomass and chl-*a* measurements on the same samples, but a comparison of the mean r^2 values indicates a slight trend for biomass to explain more of the variance in light reduction versus epiphytes than chl-*a* (one tailed paired comparisons t-test, $p=0.049$).

Epiphyte “load” is usually expressed as biomass of epiphyte per unit biomass of plant substrate. This measure is both efficient and may be quite useful in comparing levels at which impacts are observed across either space or species. It does not require measurement of leaf area, which may be a more problematic metric than biomass for some species. However, effective intercomparison requires a standardized definition particularly for the denominator, where units have included “per leaf” or “per shoot”. Various authors have used different selection criteria for which leaves to measure, and whether to include or exclude senescent leaf tissue from consideration. Removal of possible ambiguity by agreement on a

standard definition of the measurement approach for epiphyte load within a given species would also be beneficial for intercomparison and data synthesis. Kendrick & Lavery (2001) provide detailed guidance on measurement of epiphyte biomass, but do not address the issue of a standardized measurement approach for load. Measurement standardization issues could not be fully resolved in the current data review, and represent one potential source of variation in the data analyses that follow.

3.2. Establishment of Epiphyte Threshold-Response Values

3.2.1. Modeling Approaches—Mechanistic modeling systems have been developed that incorporate the impacts of epiphytes on macrophyte growth and survival for various macrophyte substrates, including *Ruppia maritima* (da Silva & Asmus 2001), *T. testudinum*, *H. wrightii*, and *Syringodium filiforme* (Fong & Harwell 1994), *Z. marina* (Wetzel & Neckles 1986) and multiple submerged aquatic vegetation species in Chesapeake Bay (Madden & Kemp 1996, Kemp et al. 2000, Best et al. 2001, Cerco & Moore 2001, Kemp et al. 2004). Kemp et al. (2000) provided a detailed modeling framework to link water column DIN and DIP values to resultant epiphyte biomass, and then to determine an epiphyte biomass-specific attenuation coefficient for photosynthetically active radiation (PAR). By combining the results of both numerical models and regression models of empirical data, the model can calculate PAR at the macrophyte leaf surface, and then compare this value to minimum light requirements for the macrophyte. This model uses common water quality monitoring parameters (DIN, DIP, TSS, K_d) to estimate suitability of a location with given depth Z for macrophyte growth. In theory, this approach should be generally applicable, although there do not appear to be examples of its application in other systems beyond Chesapeake Bay. Unless transferability of the model terms can be demonstrated, use of this approach would require extensive site specific data collection and calibration of the multiple formulas leading to the final estimate of light reduction.

3.2.2. Stressor-Response Approaches

3.2.2.1. Light Attenuation Estimation: An alternative to the modeling approach is direct measurement of the contribution of epiphytic material to total light attenuation, which Kemp et al. (2004) suggest will be prohibitively expensive. However, a number of assessments have been conducted at estuarine scale, which suggests that an empirical approach may be feasible in some cases (Harden 1994; Dixon 2000, Dixon & Leverone 1995, Dixon & Kirkpatrick 1995, Yarbro et al. 2004) especially if rapid assessment techniques for epiphyte biomass (Miller-Myers & Virnstein 2000) can be regionally calibrated. As developed below, the use of existing attenuation-biomass relationships (Supplemental Table 1) may be adequate in many cases. However, adoption of a standardized approach to the determination of light attenuation by epiphytes is another area where agreement on standard methods would be greatly advantageous. Methods for the collection of the data on light attenuation by epiphytes (Supplementary Table 1) involved use of a wide variety of methods (reviewed by Brush & Nixon 2002, Nelson, 2009, Chapter 7), which was a probable major source of variation in the current analyses.

Studies of light attenuation by epiphytes accumulating on seagrasses or on seagrass mimics (glass slides or plastic strips) indicate that the presence of epiphytes can lead to nearly 100%

attenuation of incident light to the surface of individual seagrass blades (Brush & Nixon 2002, Nelson 2009). Over an annual period, Dixon and Kirkpatrick (1995) found a range of from 13–98 % in epiphytic light attenuation in Sarasota Bay, with an annual average of about 50%. When averaged over entire shoots to account for heterogeneity of epiphyte distribution on blades of different ages, average light reduction to a seagrass shoot is more typically 50–60% (Harden 1994). At the deep edge of its distribution in Tampa Bay, Florida where epiphyte growth can be light limited, annual averages of epiphyte-caused light reduction were in the range of 32–36% for *T. testudinum* (Dixon and Kirkpatrick 1995; Dixon 2000), while shallower sites where light was not limiting tended to have a higher level of light attenuation by epiphytes (Dixon 2000). Data for salinity zones of Chesapeake Bay indicated that epiphytes tended to reduce light at the leaf surface by 20–60 % in the lower salinity regions, and 10–50% in the mesohaline and polyhaline regions (Appendix J, U.S. EPA 2003).

Based on the data compiled from the literature review, the equation coefficients (a and b) for curve fits of light attenuation versus epiphytic biomass were compared for artificial (n=18) versus natural (n=10) substrates. There was no significant difference between these groups for either coefficient (for a, t-test, $P=0.83$, n.s.; for b, Mann-Whitney Rank Sum test, $P=0.38$ n.s.). Deployment of artificial substrates can help to remove effects of substrate variability (Silberstein et al 1986, van Dijk 1993, Vermatt & Hootsmans 1994, Burt et al 1995; Glazer 1999, Stankelis et al 2001, Frankovich & Zieman 2005, Köhler et al 2010). Comparisons between epiphyte assemblages on natural and artificial substrates find that there tend to be relatively minor differences in species composition and biomass (Silberstein et al., 1986, Neverauskas, 1987a, Horner, 1987, Lethbridge et al., 1988, Burt 1995). Therefore, use of artificial substrates appears to be a reasonable reflection of the response pattern of light attenuation versus epiphyte biomass from natural substrates, and may be a useful approach in standardized monitoring programs.

Comparison of the curve fit equation coefficients (a and b) among the Temperate (n=14), Subtropic (n=9), and Tropic (n=5) climate zones (Table 2) found that the median values of coefficient a (the maximum light attenuation) were significantly lower within the Subtropic zone as compared to the Temperate zone (Kruskall-Wallis One Way ANOVA with Dunn's test, $p=0.003$), but other differences were not significant. Coefficient b was significantly higher in the Subtropics compared to the Tropics, but other differences were not significant (Kruskall-Wallis One Way ANOVA with Dunn's test, $p=0.032$). While these analysis results are inconclusive, they suggest the possibility of regional differences in the response patterns of light attenuation to epiphyte biomass. Temperate sites may have a higher maximum percent light attenuation due to epiphyte biomass, which is also achieved more rapidly per unit biomass. However, sample size for Tropic zone locations was limited (n=5) and additional observations would be particularly helpful.

Comparisons of coefficients between *Z. marina* (n=7) and *T. testudinum* (n=4) reflected the pattern for the climate zones (Table 2), since the *Zostera* data were exclusively from Temperate sites and *Thalassia* data were solely from Subtropical sites. Coefficient a for *Zostera* was higher, but not significantly so (t-test, two tailed, $P=0.068$), while values of coefficient b were very similar between the two species (t-test, two tailed, $P=0.63$).

To generate a first order estimation of critical thresholds of epiphyte loads, the mean values of the regression coefficients (a, b) from 28 quantitative studies (Table 2) were computed. The resultant equation, $y=79.1*(1-e^{-.59x})$ (Fig. 1), represents a composite, generalized relationship of percent light reduction relative to epiphyte biomass, averaged across multiple substrate types and geographic areas. Epiphyte loads associated with selected levels of light attenuation, e.g. 25 and 50% can then be calculated from the regression equation (Fig. 1).

Recognizing that there appear to be some significant regional differences in the light attenuation and epiphyte load relationships, regionalized estimates could be developed in a similar fashion. As examples, the composite light reduction curves for Temperate (Fig. 2), Subtropic (Fig. 3) and Tropic (Fig. 4) studies are shown together with the projections for epiphyte load associated with the 25th and 50th percentiles of light reduction. Load values are presented in Table 3

3.2.2.2. Estimation of Effects on Macrophytes: Based on the data compiled from the literature review, there were three epiphytic load metrics for which there were potentially sufficient data to conduct exploratory data analysis, $g\ g^{-1}\ DW$ (n=30), $mg\ DW\ cm^{-2}$ (n=11), chl a $\mu g\ cm^{-2}$ (n=8). However, only data expressed as $g\ g^{-1}\ DW$ were adequate to assess potential sources of variation such as seagrass response type, seagrass morphotype, and climate zone.

Light reduction most immediately affects the physiological responses of aquatic plants (Longstaff & Dennison 1999, Leoni et al. 2008). As suggested by McMahon et al. (2013), it might therefore be assumed that responses in primary production or growth rate may be more sensitive to epiphytic loads than biomass or density responses. Only Biomass and Production/Growth had sufficient sample size for statistical comparison, but there was no significant difference in the epiphyte load associated with a 25% reduction in these two parameters. Data for all three categories of seagrass response metrics were therefore pooled for additional analyses.

Comparing the epiphyte load ($g\ g^{-1}\ DW$) data among Broad (n=20), Leafy (n=5) and Narrow (n=3) blade morphotypes indicated that Leafy species had a significantly higher median value of load than Broad blade species (Kruskall-Wallis One Way ANOVA with Dunn's test, $p < 0.022$), while other pairwise comparisons were not significant. Although sample sizes were inadequate for a statistical comparison of load expressed as $mg\ DW\ cm^{-2}$, the magnitude of difference between Leafy and Broad blade medians was similar ($\sim 3\times$).

There was no significant difference in epiphyte load generating a 25% reduction in seagrass parameters among the three climate zones (Temperate (n=13), Subtropic (n=12), Tropic (n=3); Kruskal-Wallis One Way ANOVA with Dunn's test, $p < 0.483$),

In using linear regression analysis to estimate epiphyte loads associated with 25 and 50 % reductions in seagrass parameters, there is a constant proportionality for relationships such that estimated load for a 50% reduction is always $2\times$ that for a 25% load. Any small variation in proportionality in Table 2 in the values shown is due to rounding. However, it was of interest to examine whether values of epiphyte load for 50% reductions were

statistically greater than for 25% reductions in seagrass response metrics. Values expressed as g g^{-1} DW ($n=28$) (Fig. 5) were significantly different (Mann Whitney Rank Sum Test, $P=0.015$), whereas those expressed as mg DW cm^{-2} ($n=9$), chl $\text{a } \mu\text{g cm}^{-2}$ ($n=8$), and mg DW shoot^{-1} ($n=4$) were not, presumably because of low power due to much more limited sample sizes. Based on the statistical comparisons and available sample size, g g^{-1} DW would be the preferred epiphytic load metric for threshold purposes.

3.2.2.4. Derivation of Epiphyte Threshold-Response Ranges: There are a wide variety of factors that may potentially influence the temporal and spatial expression of the response of epiphyte load on macrophytes to nutrients in the environment, (reviewed by Nelson, 2016 submitted). These may include variations in leaf turnover rate and morphology among macrophyte species, the temporal period of sampling during an annual growth cycle, physical site characteristics such as temperature and intensity of wave action, and biological interactions such as grazing, as well as the interactions among these factors (Nelson 2016 submitted). Accounting for every potential source of variation influencing epiphyte load during routine sampling to monitor epiphyte load would be prohibitively complex. Rather, the approach suggested is to acknowledge unmeasured sources of variation and to deal with statistical ranges in the metric value, rather than individual, which may be associated with increasingly serious potential impacts to macrophytes.

The proposed approach is to use the empirically derived relationships between epiphyte load and seagrass response (Fig. 5) at 25 and 50% response levels as the primary basis for establishment of critical response ranges. To provide weight of evidence and validation for the selected ranges, the empirical relationships of epiphyte load and light reduction (Fig. 1) are evaluated for equivalent percentile reductions (25, 50%) in available light. Estimated values for these parameters are provided (Table 3) based on all available data combined, as well as for data grouped by three climate regions.

Recognizing that there are multiple sources of variability in the seagrass response data (reviewed by Nelson 2016), the suggested approach is to define threshold ranges based on the quantiles of the data distributions shown in Fig. 5. Specifically, ranges of values falling between the median and the 75th percentile of observations at a given seagrass response level, e.g. -25%, are used. For the example of a 25% reduction in seagrass response metrics, the median and 75th percentiles of epiphyte load associated with such a response were 0.38 and 0.74 g g^{-1} DW. For a 50% reduction response level, the median and 75th percentiles of epiphyte load were 0.76 and 1.47 g g^{-1} DW.

The proposed epiphyte load threshold ranges (g g^{-1} DW) are visually summarized in Fig. 6. Epiphyte loads associated with seagrass responses are compared to loads associated with light reductions of -25 and -50% (from Fig. 1). In both cases, the independently estimated values relative to light reduction fall within the range of the equivalent impact level in terms of seagrass response. Thus, there is reasonable congruence between the magnitude of epiphyte loads associated with levels of light reduction (the principle mechanism of impact to macrophytes), and epiphyte load associated with the same percentage negative response by the macrophytes.

Suggested threshold ranges for epiphyte loads on macrophytes are also indicated on Fig. 6, and correspond to the following:

“Low” epiphyte load	$< 0.4 \text{ g g}^{-1} \text{ DW}$
“Moderate” epiphyte load	$0.4 - 0.7 \text{ g g}^{-1} \text{ DW}$
“High” epiphyte load	$0.8 - 1.5 \text{ g g}^{-1} \text{ DW}$
“Very High” epiphyte load	$< 1.5 \text{ g g}^{-1} \text{ DW}$

Although epiphyte-macrophyte interactions have been studied for over 30 years, there do not appear to be quantitatively established thresholds for epiphyte load impacts for any macrophyte substrate species. In terms of qualitative comparisons, Neverauskas (1987c, cited in Hillman et al. 1991, p. 29) suggested that for *Posidonia* spp. in Australia, an epiphyte load of $> 0.5 \text{ g g}^{-1} \text{ DW}$ was indicative of “excessive” epiphyte growth. Bricker et al. (2003) and Scavia and Bricker (2006), citing Dennison et al. (1992), stated that epiphyte biomass equal to SAV biomass (i.e. 1 g g^{-1}) results in SAV mortality, but the source data for this statement have not been found. These two estimates would fall in the “Moderate” and “High” epiphyte threshold categories derived in the present analysis. Based on transplant survival rates for *Z. marina*, Glazer (1999) estimated that epiphytes at 20 mg DW cm^{-2} caused “some” mortality, while loads at $200 \text{ mg DW cm}^{-2}$ resulted in near 100% mortality. For the Australian seagrass species *Halophila ovalis*, Bendell (2006) suggested that a negative impact of epiphyte load occurred at $2 \text{ mg cm}^{-2} \text{ AFDW}$, or roughly $4.7 \text{ mg cm}^{-2} \text{ DW}$. Using the conversion equation given in Table 3 to convert these literature values to g g^{-1} , all three estimates would be in the “Very High” epiphyte load category. Thus, the epiphyte threshold levels developed appear to reasonably agree with the limited literature observations of impacts.

A comprehensive review of epiphytes on algal substrates was not conducted, but Worm & Sommer (2000) projected a regression of epiphyte loads versus algal growth rate to suggest that loads $> 0.15 \text{ g g}^{-1} \text{ DW}$ on the alga *Fucus vesiculosus* resulted in a growth rate of 0, a load value falling in the “Low” range for rooted macrophytes. This suggests that the epiphyte threshold ranges developed in the present study may not be appropriate for macroalgal substrates.

With additional data, the approach presented here could be used to develop regionalized epiphyte load thresholds. Although differences were not significantly different, the mean epiphyte load (g g^{-1}) associated with a 25% response in seagrass parameters for Tropical sites was only about 40% of that observed for Temperate and Subtropic sites (Table 3). However, this trend is highly tentative due to small sample size ($n=3$), and the fact that all studies were from the relatively limited geographic region of Florida Bay, USA. Similarly, the benefit of regionalized values is suggested by the significant differences observed in the curve fit equation coefficients for light attenuation versus epiphyte load. Additional data would also help to resolve whether accounting for varying morphotypes among macrophyte species would narrow the ranges of epiphyte loads associated with the various levels of impact.

In any analysis based on a composite of existing data it is important to recognize constraints and limitations. Difference in measurement techniques for light attenuation (Section 3.2.2.1) may introduce variability in estimation of responses. Macrophytes with different morphologies (Section 3.2.2.2) may exhibit different response relationships of epiphyte load to light attenuation. Macrophyte morphology only partially represents different evolutionary lineages in seagrasses, and to assess the importance of differences in responses among different life history strategies (Kilminster et al. 2016) will require additional data.

4. Conclusions and Recommendations

Studies assessing light attenuation in relation to epiphytes have tended to measure either epiphyte weight or chlorophyll a per unit area of substrate. Although comparable data are limited, weight per unit area of substrate appears to explain a larger percentage of the variance.

Selection of a standard metric for assessing epiphyte impacts would be very helpful. Biomass of epiphyte per unit biomass of plant substrate appears optimal for comparing epiphyte levels at which impacts are observed across space or among species. Removal of possible ambiguity by agreement on a standard definition of the basis for calculation of seagrass biomass both within and among species would be extremely beneficial for intercomparison. At a minimum, inclusion of conversion factors to allow data expression as epiphyte load (DW) per unit substrate weight (DW) is highly recommended.

The literature review confirms that increased epiphytic material has been demonstrated to reduce light availability to macrophyte hosts, and to negatively affect a range of macrophyte response measures, including host biomass, shoot density or percent cover, photosynthetic rate, and host growth rate. Responses have been demonstrated for multiple macrophyte species in many geographic areas.

There was no significant difference in the light reduction by epiphytes recorded from natural as compared to artificial substrates used in both field and laboratory exposure experiments. Thus artificial substrates may be an extremely useful tool for assessing epiphyte status in comparison to proposed threshold levels, and would mitigate the need for standardized definitions for measurement of seagrass biomass.

While there were some significant differences among climate zones in the relationships between light attenuation and epiphyte load, analysis was limited by small sample sizes, and additional data are needed to determine whether regional calibration of epiphyte light attenuation relationships may be valuable.

Although samples sizes limited complete analyses, there were no significant differences in the epiphyte load associated with a 25% reduction among the seagrass response metrics tested. There were some significant differences in the epiphyte loads associated with three categories of macrophyte host morphotype, but no significant difference among climate zones in the epiphyte load generating a 25% reduction in the seagrass response metrics.

Empirically derived relationships from the pooled global data set between epiphyte load and 25 and 50% seagrass response levels are proposed as the primary basis for establishment of threshold response ranges. Threshold ranges (low, moderate, high, very high) are proposed to account for multiple sources of variability in the seagrass response data. Comparison of values of epiphyte loads associated with 25 and 50% reductions in light to macrophytes suggest the threshold ranges are realistic both in terms of the principle mechanism of impact to macrophytes and the magnitude of resultant macrophyte impacts. If regional differences are confirmed by additional data, regional thresholds would be feasible.

Epiphyte load on SAV appears to have significant potential for use as a biological indicator of water quality conditions with respect to nutrients. The present analysis provides estimates of the range of loads associated with selected levels of negative impacts to seagrasses. Due to the multiple trophic pathways by which aquatic systems may respond to elevation of nutrients, epiphyte load cannot serve as a sole means of biological assessment for elevated nutrients in coastal systems. However, application of the proposed statistical threshold approach can be extremely useful in signaling to resource managers situations which require careful assessment of natural versus anthropogenic nutrient sources. An epiphyte load indicator, with additional local to regional calibration, could also be useful in providing quantitative support for narrative type water quality criteria.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Composite light attenuation relationship to epiphyte biomass for all data combined (n=28). Horizontal and vertical lines indicate predicted epiphyte biomass associated with the 25th and 50th percentiles of light attenuation. Other points plotted are intervals of 10% between 10–70%.

Figure 2.

Composite light attenuation relationship to epiphyte biomass for Temperate measurements (n=14). Horizontal and vertical lines indicate predicted epiphyte biomass associated with the 25th and 50th percentiles of light attenuation. Other points plotted are intervals of 10% between 10–80%.

Figure 3.

Composite light attenuation relationship to epiphyte biomass for Subtropical measurements (n=9). Horizontal and vertical lines indicate predicted epiphyte biomass associated with the 25th and 50th percentiles of light attenuation. Other points plotted are intervals of 10% between 10–60%.

Figure 4.

Composite light attenuation relationship to epiphyte biomass for Tropic measurements (n=5). Horizontal and vertical lines indicate predicted epiphyte biomass associated with the 25th and 50th percentiles of light attenuation. Other points plotted are intervals of 10% between 10–60%.

Figure 5. Box and Whiskers plot comparing the distribution of epiphyte loads associated with 25% and 50% reduction in seagrass response metrics. Gray boxes present the 25th and 75th percentiles and median. Whiskers are the 10th and 90th percentiles; individual values are plotted outside these limits.

Figure 6.

Graphical representation of the statistical ranges of epiphyte loads associated with –25% and –50% Seagrass Responses. Also shown are the means and quantiles (50%=median, 75%) of the response data from Fig. 5. Values for Light Responses represent the mean epiphyte loads associated with –25 and –50% reduction in light, as computed from the regression relationship in Fig. 1. Threshold Ranges (Low, Moderate, etc.) are defined in the text.

Table 1.

Summary of metrics used to characterize epiphytes associated with Submerged Aquatic Vegetation (SAV).
DW – Dry Weight, AFDW – Ash Free Dry Weight, AW – Ash Weight, WW – wet weight, C - carbon.

Metric	Units	Example References
Biomass	mg DW shoot ⁻¹ ; g DW shoot ⁻¹	Williams & Ruckelshaus 1993; Stoner et al. 2014
Biomass (Load)	g (DW epiphyte) g ⁻¹ (DW SAV leaf); g (DW epiphyte) g ⁻¹ (DW SAV shoot)	Twilley et al. 1985; Castejón-Silvo et al. 2012a
Biomass	g (DW epiphyte) m ⁻² (artificial substrate)	Carter et al. 1985; Van Dijk 1993; Köhler et al. 2010
Biomass	g (fresh weight epiphyte) g ⁻¹ (fresh weight SAV)	Collings et al. 2006
Biomass	mg WW (SAV leaf area)	Dimech et al. 2000
Biomass	mg DW cm ⁻² (SAV leaf area)	Fourqurean et al. 2010
Biomass	mg DW 10 cm ⁻¹ (SAV leaf); mg DW cm ⁻¹ (shoot)	Nelson 1997; Hughes et al. 2013
Biomass	g (DW epiphyte) m ⁻² (bottom area)	Short et al. 1995; Christianen et al. 2012
Biomass	mg AFDW cm ⁻² (SAV leaf area)	Vermaat & Hootsmans 1994
Biomass	g AFDW (epiphyte) g ⁻¹ DW (SAV), g AFDW (epiphyte) shoot ⁻¹	Strand & Weisner 1996; Gacia et al. 1999
Biomass	mg C (epiphyte) mg C ⁻¹ (SAV)	Kemp et al. 2004
Biomass	mg C (epiphyte) m ⁻² (SAV leaf area)	Lebreton et al. 2009
Biomass (inorganic)	g AW (epiphyte) m ⁻² (artificial substrate); or mg AW cm ⁻² , or %DW	Van Dijk 1993; Burt et. al. 1995; Terrados & Pons 2008
Biovolume (microalgae)	µm ⁻³	Coleman & Burkholder 1994; Lebreton et al. 2009
Chlorophyll <i>a</i>	µg <i>Chl a</i> cm ⁻² (SAV leaf area)	Neundorfer & Kemp 1993
Chlorophyll <i>a</i>	mg <i>Chl a</i> m ⁻² (SAV leaf area; or artificial substrate area)	Van Dijk 1993; Wear et al., 1999; Heck et al. 2006; Peterson et al. 2007
Chlorophyll <i>a</i>	g <i>Chl a</i> g ⁻¹ DW (SAV)	Hanisak 2001
Chlorophyll <i>a</i> (autotrophic index)	µg <i>Chl a</i> mg ⁻¹ DW (epiphytes)	Wear et al. 1999; Heck et al. 2006; Peterson et al. 2007
Chlorophyll <i>a</i>	mg <i>Chl a</i> g ⁻¹ AFDW (epiphytes)	Van Dijk 1993
Misc. Pigments: Chlorophyll <i>b</i> , Fucoxanthin; Violaxanthin; Lutein, Zeaxanthin	ng cm ⁻² (SAV leaf area); µg cm ⁻² (SAV leaf area)	Wear et al. 1999; Gil et al. 2006; Baggett et al 2010
Epiphyte carbonate	Percent	Wood & Lavery 2000
Epiphyte carbonate	mg DW cm ⁻² (SAV leaf area)	Burt et. al. 1995
Epiphyte abundance	Cells 10 ³ cm ⁻² ; cells mm ⁻²	Coleman & Burkholder 1994; Sultana et al. 2004; Lebreton et al. 2009
Relative percent cover	Percent	Balata et al. 2008; Giovannetti et al. 2010; Lobelle et al. 2013; Green et al. 2015
Relative percent cover	Categorical visual assessment, video transects	Wear et al. 2006
Relative percent cover	Categorical visual assessment, laboratory	Miller-Myers & Virnstein 2000
Epiphyte C:N; C:P; N:P ratios	Dimensionless	McGlathery 1995; Johnson et al. 2005; Baggett et al. 2010

Metric	Units	Example References
Epiphyte nitrogen content	Percent DW	Castejón-Silvo et al. 2012a; Christianen et al. 2012
Epiphyte phosphorus content	Percent DW	Castejón-Silvo et al. 2012b
Epiphyte carbon content	Percent DW	Alcoverro et al. 1997
Epiphyte $\delta^{15}\text{N}$	o/oo	Pérez et al. 2008; Christianen et al. 2012
Epiphyte community composition	Taxonomic composition; visual identification	Wear et al. 1999; Balata et al. 2008; Giovannetti et al. 2010
Epiphyte community composition	Taxonomic composition as functional groups; visual identification	Saunders et al. 2003, Piazzzi et al. 2004
Epiphyte community composition	Taxonomic composition; genomic identification	Uku et al. 2007
Epiphyte primary production	$\text{mg C cm}^{-2} \text{ h}^{-1}$	Wear et al. 1999
Epiphyte relative abundance	Optical density	Alcoverro et al. 1997

Table 2.

Epiphyte loads, recorded as various metrics, associated with decreases of 25% / 50% or other response level (footnote) in seagrass biomass, density, production or growth. Unless noted, epiphyte levels were determined based on linear regression relationships derived from the data sources given under "Reference".

Species	g g ⁻¹ DW	mg DW cm ⁻²	Chl a μ g cm ⁻²	mg DW shoot ⁻¹	AFDW DW ⁻¹	g m ⁻²	mg AFDW cm ⁻²	Production or Growth	Morphotypes: B - broad blade, T - thin blade, L - leafy	Temperature (T), Subtropic (ST), Tropic (TR)	Reference
<i>Amphibolis antarctica</i>	0.43/0.85							B	L	T	Bryars et al. 2009, Fig. 4, Fig. 8
<i>Amphibolis antarctica</i>	0.76/1.15							B	L	T	Bryars et al. 2011, Fig. 3
<i>Halodule uninervis</i>		3.8/7.6					1.1/2.3	D	T	TR	Bendell 2006, Figs. 10, 11
<i>Halodule wrightii</i>			2.7/5.4					B	T	ST	Heck et al. 2006, Fig. 8, 9 (T1, T2 data only)
<i>Halodule wrightii</i>	0.31/0.63							B	T	ST	Howard & Short 1986, Figs. 1, 4
<i>Halodule wrightii</i> (with some <i>T. testudinum</i> , <i>S. filiforme</i> , <i>H. engelmannii</i>)	3.3/6.6							B	T	ST	Hanisak 2001, Fig. 8.8
<i>Halodule wrightii</i>	0.28/0.67 ⁷							B	T	ST	Vimstein et al. 1987, Figs. 4, 7, 8
<i>Potamogeton perfoliatus</i>	1.2/2.4							B	L	T	Staver 1984 Fig. 7
<i>Potamogeton perfoliatus</i>	1.1/2.1							P	L	T	Staver 1984 Fig. 9
<i>Potamogeton perfoliatus</i>		2.4/4.9	1.4/2.8					B	L	T	Sturgis and Murray 1997, Tables 3, 4

Species	g g ⁻¹ DW	mg DW cm ⁻²	Chl a μ g cm ⁻²	mg DW shoot ⁻¹	AFDW DW ⁻¹	g m ⁻²	mg AFDW cm ⁻²	Production or Growth	Morphotypes: B - broad blade, T - thin blade, L - leafy	Temperature (T), Subtropic (ST), Tropic (TR)	Reference
<i>Potamogeton perfoliatus</i>	2.5/5.0	6.3/12.8						P	L	T	Twilley et al. 1985, Fig. 7b
<i>Posidonia australis</i>		1.8/5.4	0.7/2.2					P	B	ST	Silberstein et al. 1986, Fig. 8
<i>Posidonia australis</i> , <i>Posidonia sinuosa</i>	0.5							OTHER ⁴	B	ST	Hillman et al. (1990, p. 17; 1991, p. 29), citing Neveauskas (1987c)
<i>Posidonia australis</i> , <i>Posidonia sinuosa</i>	0.58/1.16							B	B	ST	Neveauskas 1987b, Table 1
<i>Posidonia australis</i> , <i>Posidonia sinuosa</i>	0.68/1.35							D	B	ST	Neveauskas 1987b, Table 1
<i>Posidonia oceanica</i>	0.13/0.25							B	B	ST	Castejón-Silvo and Terrados 2012, Table 1
<i>Posidonia oceanica</i>	0.39/0.77							B	B	ST	Castejón-Silvo et al. 2012a, Figs. 2
<i>Posidonia oceanica</i>	0.35/0.7							B	B	ST	Castejón-Silvo et al. 2012a, Figs. 3
<i>Posidonia oceanica</i>	0.14/0.28							B	B	ST	Castejón-Silvo et al. 2012b, Figs. 2,3
<i>Posidonia oceanica</i>	0.30/0.61							B	B	ST	Delgado et al. 1999, Table III

Species	g g ⁻¹ DW	mg DW cm ⁻²	Chl a μ g cm ⁻²	mg DW shoot ⁻¹	AFDW DW ⁻¹	g m ⁻²	mg AFDW cm ⁻²	Production or Growth	Morphotypes: B - broad blade, T - thin blade, L - leafy	Temperature (T), Subtropic (ST), Tropic (TR)	Reference
<i>Posidonia oceanica</i>		0.23/0.46		75.8/151.6				D	B	ST	Delgado et al. 1999, Table III; Cancemi et al. 2003, Table 6
<i>Posidonia sinuosa</i>	0.35/0.70							B	B	T	Bryars et al. 2011, Fig. 3
<i>Thalassia testudinum</i>			0.45/0.91					D	B	TR	Armitage et al. 2005, Figs. 5a, 5e
<i>Thalassia testudinum</i>			0.59/1.18					P	B	TR	Armitage et al. 2005, Figs. 5b, 5e
<i>Thalassia testudinum</i>	0.39/0.79							B	B	TR	Ferdie & Fourqurean 2004, Figs. 5, 7
<i>Thalassia testudinum</i>	0.29/0.58							P	B	TR	Ferdie & Fourqurean 2004, Figs. 5, 7
<i>Thalassia testudinum</i>				215/431				D	B	TR	Frankovich & Ziemann 1994, Tables 2, 3 (load >0.9 g shoot ⁻¹)
<i>Thalassia testudinum</i>	0.82/1.64							B	B	ST	Heck et al. 2000, Fig. 5, 10
<i>Thalassia testudinum</i>	0.38/0.75						0.13/0.27	B	B	ST	Heck et al. 2000, Fig. 5, 9
<i>Thalassia testudinum</i>							0.09/0.23 ⁷	B	B	TR	Lapointe et al. 1994, Table 2
<i>Thalassia testudinum</i>	0.11/0.23							B	B	TR	Tomasko & Lapointe

Species	g g ⁻¹ DW	mg DW cm ⁻²	Chl a μ g cm ⁻²	mg DW shoot ⁻¹	AFDW DW ⁻¹	g m ⁻²	mg AFDW cm ⁻²	Production or Growth	Morphotypes: B - broad blade, T - thin blade, L - leafy	Temperature (T), Subtropic (ST), Tropic (TR)	Reference
<i>Zostera japonica</i>	4.1							D ¹	T	TR	1991, Table 3 (0.5 m data only)
<i>Zostera marina</i>		20						OTHER ²	B	T	Fong et al. 2000, Fig. 3
<i>Zostera marina</i>		200						OTHER ³	B	T	Glazer 1999, p 55
<i>Zostera marina</i>			1.16/2.79					B	B	T	Glazer 1999, p 55
<i>Zostera marina</i>	0.11/0.22 ⁵							P	B	T	Hasegawa et al. 2007, Figs. 4, 7
<i>Zostera marina</i>			0.6/1.1					P	B	T	Hootsmans & Vermaat 1985, Table 1, end of experiment only
<i>Zostera marina</i>	0.42/0.76 ⁶	0.85/1.7						B	B	T	Jaschinski & Sommer 2008, Figs. 1A, 3
<i>Zostera marina</i>						17.1/34.2		B	B	T	Kaldy 1992, Figs. 11, 13 – 1990 data,
<i>Zostera marina</i>								B	B	T	Kaldy 1992, Figs. 11, 14 – 1991 data,
<i>Zostera marina</i>	1.4/2.8							B	B	T	Moore & Wetzel 2000, Table 5, High light only without outlier
<i>Zostera marina</i>	0.19/0.38							B	B	T	Moore 2004, Fig. 4a, c

Species	$\text{g g}^{-1} \text{ DW}$	mg DW cm^{-2}	$\text{Chl a } \mu\text{g cm}^{-2}$	mg DW shoot^{-1}	AFDW DW^{-1}	g m^{-2}	mg AFDW cm^{-2}	Seagrass response, B =	Biomass, D =	Density, P =	Production or Growth	Morphotypes: B - broad blade, T - thin blade, L - leafy	Temperature (T), Subtropic (ST), Tropic (TR)	Reference
<i>Zostera marina</i>	0.52/1.0							B	B	B	B	B	T	Moore et al. 1996, Tables 3, 4, 5
<i>Zostera marina</i>		7.6/15.3						P	P	P	P	B	T	Nelson 1997
<i>Zostera marina</i>			16.7/33.5					P	P	P	P	B	T	Nelson, 1987, Table 2
<i>Zostera marina</i>	0.12/0.24	1.3/2.61		71/142				P	P	P	P	B	T	Williams & Ruckelshaus 1993, Fig. 6
<i>Zostera marina</i>	0.03/0.06	0.31/0.61		17/33				P	P	P	P	B	T	Williams & Ruckelshaus 1993, Tables 3, 4

¹ 80% reduction in seagrass % cover

² "some" mortality of transplants"

³ 100% mortality of transplants

⁴ "excessive epiphyte growth"

⁵ AFDW basis

⁶ by conversion factor

⁷ negative exponential regression

⁸ negative hyperbolic regression