



## Review

# 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.

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## Contents

1. Introduction .....	344
2. Methods .....	344
2.1. General methods .....	344
2.2. Estimation of light attenuation .....	344
2.3. Estimation of effects on macrophytes .....	345
3. Results and discussion .....	347
3.1. Metrics of epiphyte response .....	347
3.2. Establishment of epiphyte threshold-response values .....	348
3.2.1. Modeling approaches .....	348
3.2.2. Stressor-response approaches .....	348
4. Conclusions and recommendations .....	352
Acknowledgements and disclaimer .....	353
Appendix A. Supplementary data .....	353
References .....	353

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## 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 and Woelkerling, 1983; Borum, 1985; Twilley et al., 1985; Cambridge et al., 1986; Silberstein et al., 1986; Jensen and Gibson, 1986; Neverauskas, 1987a,b; 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 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 and Van Montfrans, 1984; Brandt and Koch, 2003), negative impacts predominate (Borowitzka and Lethbridge, 1989). Negative effects include: 1) reduction in light available for photosynthesis, 2) reduction in the rate of diffusion of materials such as CO<sub>2</sub> 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 and 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 and Koch, 2003), and as a factor potentially limiting desiccation damage for plants in the upper intertidal zone (Penhale and 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 and Lavery, 2000; U.S. EPA, 2010; Sutula, 2011, and references in Table 1 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; Giovannetti et al., 2010; Castejón-Silvo and 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 et al., 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 in 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 and 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, 1997; Hasegawa et al., 2007). Searching for epiphyte studies relied on previous reviews of seagrass epiphytes (e.g. 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

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