EPA Public Access

Author manuscript

. Author manuscript; available in PMC 2019 August 01.

About author manuscripts

Submit a manuscript

Published in final edited form as:

. 2018 August 1; 148: 53–63. doi:10.1016/j.aquabot.2018.04.010.

An evaluation of factors controlling the abundance of epiphytes on *Zostera marina* along an estuarine gradient in Yaquina Bay, Oregon, USA

Walter G. Nelson

United States Environmental Protection Agency, Western Ecology Division, Pacific Coastal Ecology Branch, 2111 SE Marine Science Drive, Newport, OR, 97365, USA

Abstract

Epiphytes on seagrass (*Zostera marina*) growing in the lower intertidal were examined along an estuarine gradient within Yaquina Bay, Oregon over a period of 4 years. The Yaquina Estuary receives high levels of nutrients from the watershed during the wet season and from the ocean during the dry season. Mean epiphyte biomass per unit seagrass leaf surface area (epiphyte load) peaked during the summer, and thus epiphyte load was higher during dry season than wet season in both marine and riverine dominated regions. Epiphyte load was greater in marine than in riverine dominated areas in both wet and dry seasons, although only dry season differences were significant. There was no evidence that grazers controlled epiphyte load differences. Annual DIN concentration was inversely related to epiphyte load, principally because of elevated wet season dissolved inorganic nitrogen from river inputs. While there was a positive annual relation of epiphyte load to PO_4 concentration, it is not clear that phosphorus becomes a limiting nutrient for epiphyte growth. Water column light attenuation tends to increase linearly with distance from the estuary mouth, while both epiphyte load and *Z. marina* biomass tend to decrease. Both seagrass and seagrass epiphytes may be increasingly light limited in the upper estuary, and thus, epiphyte loads may have proportionally more impact on seagrass occurrence in this estuarine region.

Keywords

Epiphyte load; Nutrients; Yaquina Bay; Dissolved Inorganic Nitrogen; Phosphorus; Mesograzers

1.0 Introduction

Epiphytic growth on the surface of seagrass leaves is a natural part of the seagrass community that may have high biodiversity (e.g. Saunders et al., 2003; Piazzini et al., 2004; Uku et al. 2007) and may contribute significantly to the overall seagrass community productivity (Penhale, 1977; Moncreiff et al., 1992; Wear et al., 1999). Additionally, epiphytes provide support to higher trophic levels (Jernakoff and Neilsen, 1997, 1998; Spivak et al., 2009; Whalen et al., 2013; Reynolds et al., 2014). However, dense epiphytic cover leads to decreased seagrass growth and can reduce survival because of light limitation (reviewed by Nelson, 2017b). High levels of anthropogenic nutrients have been associated with increases in seagrass epiphytes and decreased health of seagrass beds (reviewed by Nelson 2017a, 2017b). In some systems, light reduction to seagrasses from epiphyte load

may reach 60 – 80%, at least seasonally (Harden, 1994; Dixon and Kirkpatrick, 1999). Mesograzers are believed to be an important factor that may influence the degree to which epiphyte load impacts seagrasses (Reynolds et al., 2014). Epiphyte load appears to be a major variable determining the ability of seagrasses to grow and survive under elevated nutrient conditions. Effects of epiphyte load on light available to seagrasses is thus an important factor which must be quantified to be able to develop accurate seagrass stressor response models for evaluating overall impacts of nutrients to seagrass systems (Kemp et al., 2000, 2004).

In contrast to seagrass systems such as Chesapeake Bay, (Stankelis et al., 2001; Kemp et al., 2004), Florida (Frankovich and Fourqurean, 1997; Fourqurean et al., 2010), and the Mediterranean (Piazzi et al., 2004; Balata et al., 2007), where many studies of effects of seagrass epiphytes have been conducted, estuaries on the west coast of the U.S. have important differences in fundamental ecological drivers. The estuaries have high natural loadings of nutrients, which occur both from the watershed in winter and from the coastal ocean in summer (Brown et al., 2007; Brown and Ozretich, 2009). Water temperatures are also strongly influenced by the coastal ocean and are typically low all year (flood tide, 8–16°C) in the portion of the estuaries supporting seagrass/epiphyte systems (Brown and Folger, 2009). Tidal amplitudes (~3 m) are macrotidal (Monbet, 1992), and the majority of seagrass habitat is intertidal rather than subtidal. For example, less than 10% of the population coverage of *Z. marina* in three Oregon estuaries is found in subtidal areas 0.6 m below mean lower low water (MLLW) (Young et al., 2009). Thus, application of seagrass nutrient, stressor-response models requires calibration of the epiphyte load element to correctly capture the role of epiphytes in influencing seagrass growth and survival.

Quantitative knowledge of the dynamics of epiphytes on seagrasses remains relatively limited for the Pacific Northwest region of the USA (Thom, 1990; Thom et al., 1991; Williams and Ruckelshaus, 1993; Nelson and Waaland, 1997), and particularly for systems located outside of Puget Sound and adjacent waters (Kentula, 1982; Ruesink, 2016). Previous studies have not quantified seagrass epiphytes along the estuarine transition from marine dominated to river dominated regions of the system, nor has there been a spatial and temporal assessment of the impacts of epiphyte loads on light availability to seagrasses. Therefore, a study was conducted to assess the following hypotheses: 1) epiphyte load on *Zostera marina* varies along the estuarine gradient in Yaquina Bay, 2) spatial and temporal patterns of epiphytes on *Z. marina* correlate with spatial and temporal patterns of nutrient distribution, 3) epiphyte loads result in levels of light reduction to *Z. marina* that may impact the seagrass, and 4) mesograzers may ameliorate impacts of epiphyte load to *Z. marina*.

2.0 Methods

2.1 Study Area

Yaquina Bay is a small (19 km²) estuary located on the central coast of Oregon, USA (Fig. 1). The watershed (660 km²) is 85% forested, 6.5% grasslands, and is <1% developed land, with the remaining area as wetlands and estuary. Population density is relatively low (12 km⁻²). Within Yaquina Bay and in the Pacific Northwest coastal region in general, there are two

distinct hydrological seasons. In the winter wet season, river flows are high and can be five times greater than during the summer dry season, and the dominant (74%) source of dissolved inorganic nitrogen (DIN) into the estuary is the watershed. During the summer period of low river flow, coastal upwelling is a major (82%) source of nitrogen and phosphorus into Yaquina Bay (Brown et al., 2007). Relative to river flow and upwelling, atmospheric deposition, wastewater input, and benthic recycling are minor (1–2 orders of magnitude lower) sources of nitrogen to both the estuary and the watershed (Brown and Ozretich, 2009). Through analysis of new cruise data, historical water quality data, hydrodynamic modeling and stable isotope analyses of nitrogen sources, Yaquina Bay has been divided into Marine and Riverine zones which tend to be dominated by differing sources of nutrients (Brown et al., 2007). The division is approximately associated with a median salinity of 26 (Fig. 1).

2.2 Epiphyte Abundance

Epiphytes growing on *Z. marina* leaves were collected at six stations, located within the lower intertidal zone between 3.5 and 17 km upriver from the estuary mouth, over the period from August 2000 through November 2004 (Fig. 1). Stations WN1-WN4 were all within the elevation range +0.2 to -0.2 m MLLW, while due to bathymetric variation up-estuary, WN5 was at +0.7 and WN6 was at -1.6 m MLLW. Over the course of the study, sampling intensity was reduced and sampling was discontinued at Stations WN2 and WN5 in February 2002, and at Station WN6 in March of 2003. As resources varied, sampling occurred at approximately monthly intervals from 2000 – 2001 and again in 2003, and at approximately bimonthly intervals in 2002 and 2004. Sampling dates and locations are provided in Supplementary Table 1.

At each station, 6 eelgrass shoots were collected into plastic bags. Because epiphyte load varies with blade age within a shoot, to obtain an average estimate for load within a shoot, one exterior leaf (older) and one internal leaf (younger) were selected from each shoot in the laboratory. Variation among shoots within a station was not a prime focus of the current study, and the replicate observations for the 6 shoots were pooled to calculate mean values of all parameters per site and date, which were used for subsequent analyses. Epiphytes were scraped from both sides of each leaf collected, and both epiphytes and seagrass leaves were separately placed in a drying oven for 24–36 hours at 60–70°C and dry weights of the material removed were determined for each leaf. While the term epiphyte biomass is used to refer to dry weight (g) of the material collected, the measurements included inorganic material since this material also reduces light available to the leaf. Leaf length and width were recorded and used to calculate leaf surface area for epiphyte attachment. The term epiphyte load is used here to refer to mg epiphytes cm⁻² of seagrass leaf surface area (area of both sides of leaf). Laboratory observations included notes on relative cover and broadly described type of epiphytes present, and served as a quality check for consistency of the weights obtained. Epiphyte biomass was compared with *Zostera* biomass using Box and Whiskers plots, and mean biomass was compared between external and internal leaves for both variables using the non-parametric Mann-Whitney U-test. Relationships between epiphyte load and seagrass biomass were examined with linear regressions. All plots and

statistical analyses were conducted using Sigmaplot 13[®] software. In all statistical tests, α 0.05, was used to infer significant differences.

For analysis, epiphyte load data for both external and internal leaves were divided by collection date into wet (November-April) and dry seasons (May – October) and into estuarine salinity zones (Marine vs. Riverine) that represent a difference in the predominant source of nitrogen and phosphorus (Brown et al., 2007). Mean epiphyte load was compared between the main factors Season and Zone with Two -way ANOVA. Assumptions of normality (Shapiro-Wilk test) and homoscedasticity (Brown-Forsythe) were examined. Seasonal pattern in epiphyte load was examined by plotting epiphyte load versus Julian day of samples and by fitting a polynomial (quadratic) regression to the data which gave slightly improved fit versus a sinusoidal function.

2.3 Light reduction

To estimate the extent of photosynthetically active radiation (PAR) attenuation to the surface of eelgrass leaves due to epiphyte cover, samples collected at stations WN1, WN3, WN4 from July 2002 through November 2004, and from station WN6 from July 2002 through January 2003 were additionally processed following the method developed by Dixon (2000). Light was measured with a Li-Cor[®] data logger and PAR sensors (2 π , air, LI-190SA). In the laboratory, freshly removed epiphytes from a single seagrass leaf were washed into a plastic cylinder with distilled water (60 ml). A light source was placed above the cylinder which was covered with a plastic diffuser plate, while the PAR sensor was located below the chamber, and the amount of irradiance reaching the sensor was measured. This value was then compared to a similarly measured irradiance value obtained using a second identical cylinder containing 60 ml of distilled water without epiphytes to control for temporal variations in strength of the light source. PAR sensors received factory calibration annually. Biomass values for epiphytes and seagrass were obtained as described above. The regression relationship of percent light attenuation to epiphyte load was fit with an exponential rise to maximum curve.

2.4. Nutrient sampling

Samples of DIN and dissolved inorganic phosphorus (DIP) were obtained over the period 2003–2004 from water quality monitoring stations along the length of the Yaquina Estuary that were a part of a separate study (Brown and Ozretich, 2009). While the nutrient stations were in the channel rather than on the tide flats where epiphytes were sampled, potential input of DIN via benthic regeneration is estimated to be an order of magnitude less than either river or ocean inputs (Brown & Ozretich, 2009). The nutrient sample sites should therefore be a reasonable representation of nutrient levels experienced at the epiphyte stations. Details of sampling methods for nutrient and other water quality data collected are provided in Brown et al. (2007) and Brown and Ozretich (2009). Nutrient data were selected from the subset of stations nearest to the epiphyte sampling stations (Fig. 1). Data were collected on 31 dates over the entire year in 2003 at stations 4, 5, 6, 8, and 10. Data were collected at stations 3, 7, 9, and 11 on the same dates in 2003, as well as on 21 dates between April and September in 2004.

2.5 Mesograzer Abundance

Potential invertebrate mesograzers, typically small (<2.5 cm) crustacean or gastropod herbivores, associated with the eelgrass shoots collected for epiphyte measurements were recorded and counted. Because sites were intertidal and samples were generally collected at lower tide stages, grazers recorded may represent the low end of the range of potential grazer abundances, but should be adequate for within system comparisons. More rigorous mesograzer sampling (Motley, 2017) found virtually identical composition of mesograzers, supporting the limited use of these data. While taxonomic composition of the mesograzers collected was similar across all years of the collection, only samples collected during 2001 were used for mesograzer analyses, since this was the period with the most complete collection of stations and most frequent sampling interval (Supplementary Table 1). Mesograzer abundance values were expressed on a mean abundance shoot⁻¹ basis, where the number of mesograzers collected on external and internal leaves were combined for n=6 shoots per station. Two-way ANOVA was used to compare mesograzer abundance among estuarine salinity zones (Marine - WN1, WN2, WN3: Riverine - WN4, WN5, WN6) among 11 sample dates over the one-year period. Data were transformed with a 4th root transformation to meet assumptions of normality and homogeneity of variances.

3.0 Results

3.1 Spatial and Temporal Variability of Epiphyte Load

Epiphyte loads have been reported on both a weight/surface area basis (biomass per unit seagrass leaf surface area, mg cm⁻²), and on a weight/weight basis (epiphyte biomass per unit biomass of seagrass (mg mg⁻¹ seagrass, or g g⁻¹ seagrass, usually as DW) (Nelson 2017a). Therefore, the regression relationships between the two metrics were computed to allow ready comparison of the present results to other studies. Regression equations between the two metrics for external and internal leaves were highly similar, and therefore both leaf types were pooled to a single regression. For the relationship passing through (x, y) = (0, 0), $y \text{ (mg cm}^{-2}\text{)} = 2.19x \text{ (mg mg}^{-1}\text{ seagrass)}$ ($r^2 = 0.87$, $p < 0.0001$, $n = 328$). The unconstrained regression was very similar: $y \text{ (mg cm}^{-2}\text{)} = 2.1x + 0.24 \text{ (mg mg}^{-1}\text{ seagrass)}$ ($r^2 = 0.87$; $p < 0.0001$; $n = 328$).

There were significant, positive linear relationships between epiphyte biomass (y) and *Z. marina* biomass (x) (both variables as g DW based on measurements of individual leaves), both for external and internal leaves, as well as for all data pooled, although the coefficient of determination was highest for internal leaves. The regression equations were: internal leaves ($y = 0.98x - 0.02$; $r^2 = 0.48$; $p < 0.001$, $n = 166$); external leaves ($y = 1.45x + 0.05$; $r^2 = 0.32$; $p < 0.001$; $n = 166$); all leaves ($y = 1.41x - 0.002$; $r^2 = 0.35$; $p < 0.001$; $n = 332$).

As expected, both mean epiphyte biomass (g) and mean *Z. marina* biomass (g) per station were significantly greater on external compared to internal leaves (Supplementary Fig. 1, Mann-Whitney Rank Sum test, epiphytes $p < 0.001$, *Zostera* $p < 0.003$). This was also the case for epiphyte load expressed either as mg cm⁻² seagrass or as g g⁻¹ seagrass (Supplementary Table 2). Median values for epiphyte biomass on external leaves, however expressed, were approximately 4x those on internal leaves, while the median value for *Z. marina* biomass

was only approximately 15% greater (Supplementary Fig. 1). There was a significant positive relationship between epiphyte load on internal leaves versus external leaves in the shoot, expressed either as mg cm^{-2} ($y = 0.26x + 0.17$; $r^2 = 0.52$; $p < 0.0001$) and as g g^{-1} ($y = 0.26x + 0.016$; $r^2 = 0.39$; $p < 0.0001$). Given the consistent differences in external and internal leaves, additional statistical comparisons analyzed data for these two categories separately.

Averaged over all sample dates, there was little difference in mean epiphyte load among stations during the wet season period (Fig. 2A, B). During the dry season, mean values for stations within the Marine zone (WN1, WN2, WN3) tended to be higher than those in the Riverine zone (WN4, WN5, WN6) (Fig 3). These spatial and temporal trends were confirmed by results of Two-way ANOVAs (Supplementary Table 3) that indicated that epiphyte loads on both external and internal leaves were significantly higher ($p < 0.001$) in the Marine zone than in the Riverine zone. Epiphyte load was also significantly higher in the dry season than during the wet season for both external ($p = 0.004$) and internal ($p < 0.001$) leaves. The interaction term between estuarine Zone and Season was not significant for either external or internal leaves.

Over the period 2000–2003, epiphyte load increased in the spring to a maximum in the summer (near Julian day 200, mid-July) and then decreased towards winter. There was a statistically significant, 2nd order polynomial relationship between load and Julian day ($y = -1.15 + 0.076x - 0.002x^2$; $r^2 = 0.6$; $p < 0.001$) (Fig. 4). While the seasonal relationship was most clearly seen on the older, external seagrass leaves, there was a similar relationship, but with higher variability, for internal blades ($y = -0.08 + 0.008x - 0.0002x^2$; $r^2 = 0.26$; $p < 0.013$). This seasonal pattern was not observed in the 2004 samples, when epiphyte load stayed relatively high throughout the year (Fig. 4). With inclusion of the 2004 data, the regression relationship for epiphyte load versus Julian day was also significant, but with much greater variation ($y = 0.97 + 0.056x - 0.0001x^2$; $r^2 = 0.28$; $p < 0.027$).

3.2 Epiphyte relation to nutrients

Median DIN concentration measured at water quality monitoring stations nearest the epiphyte sampling stations showed little difference along the estuarine gradient from Marine zone to Riverine zone during the dry season (Fig. 5A). Reflecting the large inputs of DIN due to watershed runoff during the winter months, DIN concentrations tended to be higher in the Riverine zone than the Marine zone during the wet season (Fig. 5B).

The composite temporal patterns of both epiphyte load and water column DIN in the Yaquina estuary are shown in Fig. 6A for data organized into consecutive bins of 15 Julian days. There were significant 2nd order polynomial relationships for both metrics versus Julian day, convex for epiphyte load (Fig. 6A; $r^2 = 0.75$; $n = 20$; $P < 0.0001$) and concave for DIN concentration (Fig. 7A; $r^2 = 0.69$; $n = 19$; $P < 0.0001$). These opposing trends resulted in a significant negative correlation between epiphyte load and DIN ($r = -0.77$; $r^2 = 0.59$; $n = 15$; $P < 0.0009$). In contrast, there was a significant positive correlation of epiphyte load with PO_4 concentration (Fig. 6B; $r^2 = 0.4$; $n = 15$; $P < 0.01$). The water column N/P ratio (Supplementary Fig. 2) in the Yaquina estuary was greater than 22 between Julian days 1 – 140 (January – April), < 13.5 between Julian days 165 – 300 (mid-June – late October), and again > 22 in December.

3.3 Light Reduction by Epiphytes

There was a significant, curvilinear relationship (exponential rise to maximum, Fig. 7) between percent light attenuation and epiphyte load, for data from external and internal leaves combined. Maximum light attenuation estimated from the regression was 94% at the highest values of epiphyte load.

There were clear tendencies (Fig. 8) for lower levels of light attenuation by epiphytes in the Riverine zone as compared to the Marine zone, and for internal leaves as compared to external leaves within shoots. Two-way ANOVA (Supplementary Table 4) utilizing station mean values (n=116) found highly significant differences ($p<0.001$) in light attenuation in the Marine (61%) versus Riverine (36.7%) zones, and highly significant differences ($p<0.001$) on external (63.8%) compared to internal (33.9%) leaves. The interaction term was not significant. Light attenuation to the surface of *Z. marina* leaves varied greatly from month to month, ranging from 3 – 99% for external leaves, and 1 – 89 % for internal leaves. The range in mean light attenuation for a shoot, estimated as a simple average of external and internal leaf values, ranged from 3 – 76 %, with an estimated mean light attenuation across space and time of 53% for eelgrass shoots within the Yaquina Estuary.

3.4 Mesograzer abundance patterns

Gastropods (94.2%), primarily *Lacuna* spp., gammaridean amphipods (5.2%), and isopods (0.6%) were the principal mesograzers recorded during the 2001 sampling period.

Abundance was extremely low at Riverine zone sites, and periodically (November-March) higher in the Marine zone due to gastropods (Supplementary Fig. 3). Mean mesograzer abundance (external + internal leaves combined) was greater in the Marine versus Riverine zones ($p<0.001$), varied significantly among months sampled ($p<0.001$), and had different seasonal patterns between zones ($p<0.001$, Two-way ANOVA, Supplementary Table 5).

4.0 Discussion

4.1 Spatial and Temporal Variability of Epiphyte Load

In Yaquina Bay, epiphyte biomass was significantly higher in the ocean dominated portions of the estuary (Fig. 3). Results from mesocosm experiments (Short et al., 1995; Lin et al. 1996; Moore and Wetzel, 2000) have suggested that epiphyte biomass may be strongly regulated by light limitation. Even under experimental nutrient addition, epiphyte response can be limited if water column light levels are reduced (Short et al., 1995). For *Z. marina*, a principle limiting factor is light availability, and there were significant negative relationships of normalized leaf biomass (mg cm^{-2} (one sided leaf surface area)) of *Z. marina* with distance upriver, which were also significantly negatively correlated with light extinction coefficient K_d (Supplementary Fig. 4). Increased biomass in the Marine zone for both *Z. marina* and macroalgae was also found in the Yaquina estuary (Kentula and DeWitt, 2003), as well as a negative correlation with water column turbidity along the estuarine gradient. Thus, a likely explanation for reduced epiphyte biomass is also the reduction in water clarity in the Riverine section of the estuary (Boese et al., 2009).

Greatest biomass for epiphytes occurred during the summer - fall dry season. Low levels of epiphytes on *Z. marina* in winter is typical for the PNW region (Kentula, 1982; Thom, 1990; Thom et al., 1991; Williams and Ruckelshaus, 1993; Nelson and Waaland, 1997; Thom et al., 2008; Ruesink, 2016), to the point of being virtually absent (Kentula, 1982; Thom et al., 2008). The shorter day length coupled with frequent cloud cover typical of the PNW in winter may contribute to the limited winter epiphyte abundance.

The decrease in epiphyte load during the typical peak, annual abundance period in July – September 2004 differed from other study years. Physical variables such as river flow, rainfall, air temperature and cloud cover, showed no obvious qualitative differences for 2004. While, the mean dry season, flood tide water temperature was 2° C higher in the summer of 2004 as compared with the average from 2001–2003 (Ruggerio et al., 2010), mesocosm experiments (Bintz et al., 2003) have found that epiphyte load tends to increase with increased water temperature. Comparison of data on macroalgal abundance from two areas in the Marine zone near the epiphyte stations showed no differences between 2003 and 2004. Thus, the 2004 difference in epiphyte load compared to previous years remains unexplained.

Biomass of epiphytes was positively related to aboveground biomass of *Z. marina* both for external and internal leaves, with coefficients of determination intermediate between those found by Kentula (1982; $r^2=0.6$) and Nelson and Waaland (1997; $r^2=0.21$). This is consistent with the similarity in spatial and temporal patterns described above for both epiphytes and seagrass, and suggests that the limiting factors for both may also be similar.

A quantitative intercomparison of seagrass epiphyte results within the PNW is provided in Table 1. An important caveat in such comparisons is having comparable units. While in all cases, mean epiphyte load expressed as g epiphyte g⁻¹ seagrass could be extracted, in some cases the collection units were “shoots”, implying inclusion of the leaf sheath, and in some cases, were “leaf” only. Precisely which leaves within a shoot were measured was often not specified, and when specified, may vary (e. g. all leave per shoot, Kentula, 1982; five leaves per shoot including leaf sheath, Ruesink, 2016; two leaves per shoot, this study). Jorgensen et al. (2007) found that *Z. marina* sheath weight averaged 28% of leaf weight in summer and 50% of leaf weight in winter, and thus inclusion of sheath weight would tend to reduce the g g⁻¹ relationship relative to leaf vs leaf comparisons. Nelson (1997) showed that approximately 50% of total epiphyte biomass was on the oldest *Z. marina* leaf. In the current study, epiphyte biomass or load was 2.6 – 3x greater on older, external leaves compared to younger internal leaves (Supplementary Table 2). Thus, selection of sample leaves may also have a potentially large effect on the ability to compare results.

Despite these potential issues, site inter-comparisons suggest differences within the PNW region. Studies from the San Juan Islands and Padilla Bay of WA found maximum epiphyte loads which represented from 23 – 71 % of *Z. marina* aboveground biomass (shoots) (Thom, 1990; Thom et al., 1991; Nelson and Waaland, 1997). Maximum epiphyte biomass for the PNW region (Table 1) expressed as a percentage relative to seagrass leaf biomass ranged from 75 to 410% (Kentula, 1982; Williams and Ruckelshaus, 1993; Motley, 2017; this study), and up to 199% related to biomass of leaf + sheath (Ruesink, 2016). Using

conversion factors where feasible to compare regional epiphyte loads on a leaf area basis (mg cm^{-2} , Table 1), the Yaquina estuary stands out as an order of magnitude higher in average epiphyte load. Motley (2017) also found epiphyte loads in Yaquina were significantly higher than in two other Oregon estuaries studied. Nelson (2017a) has recently proposed threshold levels for impacts of seagrass epiphytes based on relationships of epiphyte load to seagrass response. Compared to these globally derived thresholds, the Yaquina epiphyte load would be rated High, whereas most of the remaining values observed across the PNW would be rated Low to Moderate. In comparison, Narragansett Bay, an estuary with known anthropogenic nitrogen problems (Lee et al., 2004), had epiphyte loads spanning the Low to High epiphyte load categories proposed by Nelson (2017a).

4.2 Epiphyte load in relation to nutrients

In the Yaquina estuary, epiphyte load was significantly lower in the Riverine compared to the Marine zone in both wet and dry seasons. This does not correlate with patterns of nitrogen concentration, which tend to be very high in the river dominated segment during the wet season (Brown et al., 2007). Similarly, for comparisons among estuaries (described above, Section 4.1), while wet season median DIN tends to be higher in Yaquina Bay than in some other Oregon estuaries, dry season median DIN concentration falls in the middle range among systems (Brown and Folger, 2009), and thus does not readily explain the higher epiphyte load in the Yaquina.

Seasonally, there was a negative correlation of epiphyte load in the Yaquina estuary with DIN concentration (Fig. 7). Nelson (1997) also found significant negative relationships between both nitrate and total nitrogen concentrations and epiphyte biomass at two locations in the Sand Juan Islands, WA, although there was a very weak, positive relationship of DIN to epiphyte load ($r^2=0.1$) in field samples from Padilla Bay, WA (Williams and Ruckelshaus, 1993). Thus, in contrast to the observations of Borum (1985) for *Z. marina* in Denmark, there is little evidence from the PNW that seasonal variation in water column nitrogen is influencing the seasonal pattern of seagrass epiphyte load. However, in the Yaquina estuary there was an unanticipated, significant, positive temporal correlation of epiphyte biomass to PO_4 concentration. Nelson and Waaland (1997) found no such correlation of either biomass or productivity of epiphytes with water column PO_4 concentrations at a site in Sand Juan Islands, WA. Field experimental addition of either nitrogen (Williams and Ruckelshaus, 1993) or nitrogen and phosphorus (Ruesink, 2016) to the water column in PNW seagrass beds found no significant increases of epiphyte biomass relative to controls. In the former case, the authors suggested that grazing by *Idotea* prevented an increase of epiphytes in response to the nutrient additions, since parallel laboratory experiments resulted in epiphyte increases.

Considering both nitrogen and phosphorus, water column N/P ratio (Supplementary Fig. 2) in the Yaquina would suggest phosphorus limitation ($\text{N/P}>22$) in the period January-April, with either optimal nutrient conditions or slight nitrogen limitation ($\text{N/P}<13$) for the rest of the year, based on the nutrient ratios determined for periphyton by Hillebrand and Sommer (1999). While there is evidence that phosphorus may become limiting for phytoplankton at times within the Riverine zone of the estuary, Brown et al. (2007) found that the Yaquina

estuary is not generally limited by either nitrogen or phosphorus. It is presently unclear whether the observed relationship of epiphyte biomass and PO_4 represents nutrient limitation for phosphorus under persistent high nitrogen conditions.

Besides seasonally varying nutrient ratios, both residence time and water temperature may have interactive effects influencing nutrient effects on epiphytes. Hessing-Lewis et al. (2015) found little response of macroalgae to in-situ nutrient elevation experiments in the Yaquina estuary, while mesocosm experiments showed strong responses. In contrast to Williams and Ruckelshaus (1993), they attributed the differences to the high rates of water movement in the field (Hessing-Lewis et al., 2015). This is consistent with residence times in the lower Yaquina estuary that were estimated as 1 d (2 tidal cycles) under high river discharge conditions, and from 1–10 days under low river discharge (Lemagie and Lerczak, 2015). More recent mesocosm experiments (Kaldy et al., 2017), which varied both temperature and nutrient loads under high water turnover rates to mimic conditions in PNW estuaries, found limited nutrient impacts on macrophytes except at higher water temperatures. In the face of the many possible ecosystem characteristics that affect the expression of vegetation response to elevated nutrients (Cloern, 2001), Krause-Jensen et al. (2008) reviewed the empirical relationships of marine vegetation to eutrophication and concluded that to generalize from cross system comparisons remains extremely difficult.

The ultimate susceptibility of seagrass within the estuaries of the PNW region to elevation of anthropogenic nutrients remains an open question. While seagrass may experience major blooms of green macroalgae in addition to epiphyte loading, there do not currently appear to be major impacts (Hessing-Lewis et al. 2015). Shelton et al. (2016) recently concluded that total *Z. marina* coverage in the Puget Sound region has remained relatively stable over the past 40 years despite major urbanization of adjacent coastline over the same period, although significant site-specific changes have occurred. Ruesink (2016) suggested that there may be a tipping point at which seagrass would be unable to cope with epiphyte accumulation if the current balance between mitigating factors and seagrass persistence should be disturbed. In some estuaries in the region (Table 1), light reduction from epiphyte loads could already potentially impact seagrass. Mesocosm experiments indicate that *Z. marina* should be particularly susceptible to the multiple effects of nutrients, including epiphyte increases, as environmental temperatures rise (Bintz et al., 2003; Kaldy and Shafer, 2013; Kaldy, 2014; Kaldy et al., 2017). Ruggerio et al. (2010) suggested that the river dominated portions of Oregon estuaries were most likely to experience increased water temperatures with climate change. *Z. marina* beds located in the Riverine zone would be particularly susceptible, due both to the decrease in light availability from increased epiphyte loads and to the higher water temperatures in this region.

4.3 Light Reduction by Epiphytes

At intertidal sites in the Yaquina estuary, the mean light attenuation at seagrass leaf surfaces due to epiphytes is about 61% in the Marine zone and 37% in the Riverine zone, similar to the range (31% – 63%) for two eelgrass systems in Waquoit Bay, MA (Hauxwell et al., 1998). The Yaquina light attenuation values per shoot are also comparable to those from

other seagrass species (Dixon and Kirkpatrick, 1995, 1999; Dixon and Leverone, 1995; Dixon, 2000; Harden, 1994).

While epiphytic algae may have some beneficial effects on seagrasses (Orth and van Montfrans, 1984), heavy epiphytic cover on seagrasses primarily results in decreased growth and survival caused by reduced light availability (reviewed by Nelson 2017a, 2017b, 2017c), especially if ambient light levels are low (Morgan and Kitting, 1984; Twilley et al., 1985; Wetzel and Neckles, 1986). Short term, in situ, epiphyte removal experiments (4–6 d, Bohrer et al., 1995; 5–9 d, Ruesink, 2016) found no significant effects on the growth rate of *Z. marina*. Meta-analysis (McMahon et al., 2013) has found that for seagrass leaf extension, the response timescale is weeks, suggesting that the in-situ experiments above may have been too short to generate measurable effects. Nelson (2017c) reviewed experimental light reduction impacts on seagrasses and found that manifestation of impacts to biomass and shoot density required on the order of 2 months. Light attenuation from epiphytes on external leaves at station WN1, the most seaward station, ranged from 77 – 99 % from May 2003 through March 2004, which is long enough for evident impacts on seagrass. Comparison of *Zostera* biomass during this period with that obtained in the remainder of the study found no significant difference in either biomass (g) or leaf surface area normalized biomass (mg cm^{-2}), suggesting that the seagrass plants were not being affected by the epiphytes. The intertidal site location may allow the seagrass to still receive the daily compensation light intensity (Thom et al., 2008) needed to maintain itself in the face of heavy epiphyte load.

4.4 Mesograzers

Recent quantitative sampling of mesograzers during summer in intertidal *Zostera* beds of Yaquina bay (Motley 2017) confirmed the composition of dominants found in the current study, i.e. >99% of abundance was *Lacuna* spp, gammaridean amphipods and *Idotea resicata*. These taxa also tend to be the dominants in intertidal seagrass beds in the PNW (Nelson, 1997; Thom et al., 1991; Williams and Ruckelshaus, 1993), with caprellid amphipods (Thom et al., 1991), and the sea hare *Phyllaplysia taylori* (Motley 2017) also important in some locations. Mesograzers in the PNW appear to be extremely patchy in space and time. In the present study, mesograzers were rarely recorded during summer months, consistent with studies from some other locations (Thom et al., 1991; Ruesink 2016), while other studies in the region recorded some mesograzers as abundant in summer or fall (Kentula, 1982; Ruesink, 2016; Motley, 2017). Timing and peak density of mesograzers can vary within a site across tidal elevations (Thom et al., 1991), among sites along salinity gradients (this study), and among estuaries sampled contemporaneously (Motley, 2017).

Experiments have shown that *Lacuna* and *Idotea* (Thom et al., 1991; Williams and Ruckelshaus, 1993; Nelson, 1997), as well as caprellids amphipods at high densities (Caine, 1980; Thom et al., 1991), can potentially reduce epiphyte load on *Z. marina*. However, experimental reduction of crustacean mesograzers in situ (Ruesink, 2016) had no significant effect on epiphyte biomass, nor was seasonal variation in epiphyte load explained by grazer abundances. Based on a cross system assessment of epiphyte load and grazer abundance,

Motley (2017) found that mesograzers at only two of nine locations (not including Yaquina) appeared to impact epiphytes, and concluded that, in general, bottom-up processes were primarily controlling epiphytes at most locations in the Oregon eelgrass beds studied.

4.5 Integration of controlling factors

Williams and Ruckelshaus (1993) suggested that epiphytes may control seagrass growth in the Puget Sound region when; a) herbivory by mesograzers is low and b) nitrogen in the water column is abundant ($>15 \mu\text{M}$) and c) water temperatures are below 10°C , which tends to reduce *Z. marina* growth. In the Yaquina estuary, median DIN concentrations in both the dry ($25 \mu\text{M}$) and wet ($95 \mu\text{M}$) seasons (C.A. Brown, U.S. EPA unpublished data) are well above the proposed threshold, and mesograzers during the study period were predominantly very low. Epiphyte loads, particularly in the Marine zone of the Yaquina estuary, can be temporally persistent and represent levels known to significantly impact *Z. marina* (Nelson 2017a). In comparison, epiphyte levels (as a mean of old and new leaves) recorded by Ruesink within Willapa Bay, WA (Fig. 1A, 2016) ranged from a 1.04 to 1.45 g g^{-1} , which are approximately equivalent to $2.2\text{--}3.2 \text{ mg cm}^{-2}$ (conversion from Nelson, 2017a). These would also be considered very high levels for epiphyte load (Nelson, 2017a), with a high potential for negative impacts to seagrass. However, there is no evidence for loss of seagrass within the Yaquina estuary over a period of 30 years (Kaldy, 2014; Young et al., 2015). Ruesink (2016) suggested *Z. marina* can tolerate seasonally abundant epiphytes because at intertidal locations, the seagrass receives high light levels during summer low tides, which may largely compensate for the high levels of epiphyte light attenuation that also occur during the summer. This is consistent with observations from the Yaquina estuary.

While flood tide water temperatures in the Yaquina during summer months were often near 10°C in the Marine zone because of advection of upwelled water (Brown and Folger, 2009; Kaldy, 2014), temperatures at the sediment surface in *Z. marina* beds during low tides are often well above this value (Specht, US EPA, unpublished sediment temperature database, available from author on request). This would be an additional, potentially ameliorating factor for epiphyte impacts on seagrass growth. Areal leaf productivity for *Z. marina* tends to be highest during summer (Kaldy and Lee, 2007), which tends to confirm that low water temperature is not a primary influence in the seagrass-epiphyte interaction in the Yaquina system.

Ruesink (2016) also suggested that the process of sloughing of old leaves with replacement by new leaves that have lower epiphyte loads may provide one mechanism by which *Z. marina* can tolerate high loads. Kentula (1982) found that leaf life was 34 d in late spring and increased to 46 days in summer, and suggested leaf sloughing was associated with periodic decreases in mean epiphyte biomass. On an annual basis, a very high level of leaf loss occurs for *Z. marina* in the Yaquina estuary during the early fall, which often coincides with annual “King” tides, increased storm activity, and decreased ambient light levels both from shorter day length and increased cloud cover. Kaldy and Lee (2007) found that light is the principal limiting factor in winter for *Z. marina* populations in Yaquina estuary, and is most probably similarly limiting for epiphytes. Light limitation together with loss of fouled

leaves in the fall in most years appears to reset the intertidal seagrass meadows to low epiphyte loads over the winter in most years.

4.6 Conclusions

Standardization of the methods and metrics for measurement of seagrass epiphyte load would greatly improve the ability to synthesize and compare epiphyte results among studies. In terms of the hypotheses examined, epiphyte load was lower in the river dominated portion of the estuary than the marine dominated region. Spatial and temporal patterns of nutrient concentration did not clearly relate to patterns of epiphyte load. There was a negative relationship of epiphyte load to nitrogen concentration and a positive relationship to phosphorus concentration, while N/P ratios were consistent with nitrogen limitation in the dry season. However, relatively high absolute concentrations of nitrogen and rapid replacement from upwelling make it unlikely that nutrients are a limiting factor for epiphyte load. Rather than nutrient patterns, the seasonal differences in epiphyte load observed in the Yaquina estuary are most likely a function of light limitation on epiphyte growth during the winter, wet season. Observed epiphyte loads could result in negative impacts to seagrass, while observed mesograzers did not appear to be high enough to reduce epiphyte loads at the intertidal sites sampled. Currently, seagrasses appear to be able to cope with epiphyte loading. However, seagrass beds within the Riverine zone are potentially vulnerable, since this region may experience increased water temperatures in the future, which may increase epiphyte loads and impacts. Whether seagrass can continue to be resilient to combined effects of environmental and anthropogenic perturbations should be a question of considerable concern.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Acknowledgements and Disclaimer:

This project was made possible by field and laboratory support provided by Dynamac Inc. through contract to EPA, and included the assistance of David Adamson, Casey Wendling, Susan Garlow, Dana Scarborough, Daren Swisher, Allen Taylor, Gonzalo Castillo, Stacia Kimbell, Susan Gerlach, and Greg Wendling. Pat Clinton of U.S. EPA produced the station map. Dr. Robert Ozretich (US EPA, retired) was responsible for the water quality data collection, and the data were compiled and provided by Dr. Cheryl A. Brown (US EPA). Dr. Jud Kenworthy and Jim Markwiese provided extremely helpful initial technical reviews of the manuscript. The manuscript greatly benefited from frequent conversations with Drs. Jim Kaldy and Cheryl Brown. 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.

References

- Balata D, Nesti U, Piazzzi L, Cinelli F. Patterns of spatial variability of seagrass epiphytes in the north-west Mediterranean Sea. *Marine Biology*. 2007; 151:2025–2035.
- Bintz JC, Nixon SW, Buckley BA, Granger SL. Impacts of temperature and nutrients on coastal lagoon plant communities. *Estuaries*. 2003; 26:765–776.

- Boese, BL., Nelson, WG., Brown, CA., Ozretich, RJ., Lee, HL., II, Clinton, PJ., Folger, CL., Mochon-Collura, TC., DeWitt, TH. Lower depth limits of *Zostera marina* in seven target estuaries. In: Lee, H., II, Brown, C., editors. Classification of Regional Patterns of Environmental Drivers and Benthic Habitats in Pacific Northwest Estuaries. Office of Research and Development, U.S. Environmental Protection Agency; 2009. p. 219-241. Technical Rept. EPA/600/R-09/140
- Bohrer T, Wright A, Hauxwell J, Valiela I. Effect of epiphyte biomass on growth rate of *Zostera marina* in estuaries subject to different nutrient loading. The Biological Bulletin. 1995; 189:260–260. [PubMed: 27768472]
- Borum J. Development of epiphytic communities on eelgrass (*Zostera marina*) along a nutrient gradient in a Danish estuary. Marine Biology. 1985; 87:211–218.
- Brown, C., Nelson, W., Boese, B., DeWitt, T., Eldridge, P., Kaldy, J., Lee, H., II, Power, J., Young, D. An Approach to Developing Nutrient Criteria for Pacific Northwest Estuaries: A Case Study of Yaquina Estuary, Oregon. USEPA Office of Research and Development, National Health and Environmental Effects Laboratory, Western Ecology Division; 2007. EPA/600/R-07/046
- Brown, CA., Folger, CL. Water quality surveys in seven target estuaries. In: Lee, H., II, Brown, C., editors. Classification of Regional Patterns of Environmental Drivers and Benthic Habitats in Pacific Northwest Estuaries. Office of Research and Development, U.S. Environmental Protection Agency; 2009. p. 96-114. Technical Rept. EPA/600/R-09/140
- Brown CA, Ozretich RJ. Coupling between the coastal ocean and Yaquina Bay, Oregon: Importance of oceanic inputs relative to other nitrogen sources. Estuaries and Coasts. 2009; 32:219–237.
- Caine E. Ecology of two littoral species of caprellid amphipods (Crustacea) from Washington, USA. Marine Biology. 1980; 56:327–335.
- Cloern JE. Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology Progress Series. 2001; 210:223–253.
- Dixon LK, Kirkpatrick GJ. Light attenuation with respect to seagrasses in Sarasota Bay, Florida, Sarasota Bay National Estuary Program. Mote Marine Laboratory Technical Report 407. 1995:59. plus appendices.
- Dixon, LK., Leverone, JR. Mote Marine Laboratory Technical Report no 425. 1995. Light requirements of *Thalassia testudinum* in Tampa Bay, Florida. South Florida Water Management District; p. 77 plus appendices
- Dixon, LK., Kirkpatrick, G. Final Report to Sarasota Bay National Estuary Program Mote Marine Laboratory Technical Report. Sarasota, FL: 1999. Causes of light attenuation with respect to seagrasses in upper and lower Charlotte Harbor; p. 101
- Dixon, LK. Establishing light requirements for the seagrass *Thalassia testudinum*: An example for Tampa Bay. In: Bortone, SA., editor. Seagrasses: Monitoring, Ecology, Physiology and Management. CRC Press; Boca Raton, FL: 2000. p. 9-31.
- Fourqurean JW, Muth MF, Boyer JN. Epiphyte loads on seagrasses and microphytobenthos abundance are not reliable indicators of nutrient availability in oligotrophic coastal ecosystems. Marine Pollution Bulletin. 2010; 60:971–983. [PubMed: 20381091]
- Frankovich TA, Fourqurean JW. Seagrass epiphyte loads along a nutrient availability gradient, Florida Bay, USA. Marine Ecology Progress Series. 1997; 159:37–50.
- Harden, SW. MS thesis. Florida Institute of Technology; Melbourne FL: 1994. Light requirements, epiphyte load and light reduction for three seagrass species in the Indian River Lagoon, FL; p. 130
- Hauxwell J, McClelland J, Behr PJ, Valiela I. Relative importance of grazing and nutrient controls of macroalgal biomass in three temperate shallow estuaries. Estuaries. 1998; 21:347–360.
- Hessing-Lewis ML, Hacker SD, Menge BA, McConville S-o, Henderson J. Are large macroalgal blooms necessarily bad? nutrient impacts on seagrass in upwelling-influenced estuaries. Ecological Applications. 2015; 25:1330–1347. [PubMed: 26485959]
- Hillebrand H, Sommer U. The nutrient stoichiometry of benthic microalgal growth: Redfield proportions are optimal. Limnology and Oceanography. 1999; 44:440–446.
- Jernakoff P, Nielsen J. The relative importance of amphipod and gastropod grazers in *Posidonia sinuosa* meadows. Aquatic Botany. 1997; 56:183–202.
- Jernakoff P, Nielsen J. Plant–animal associations in two species of seagrasses in Western Australia. Aquatic Botany. 1998; 60:359–376.

- Jorgensen P, Ibarra-Obando SE, Carriquiry JD. Top-down and bottom-up stabilizing mechanisms in eelgrass meadows differentially affected by coastal upwelling. *Marine Ecology Progress Series*. 2007; 333:81–93.
- Kaldy JE, Lee KS. Factors controlling *Zostera marina* L. growth in the eastern and western Pacific Ocean: comparisons between Korea and Oregon, USA. *Aquatic Botany*. 2007; 87:116–126.
- Kaldy JE, Shafer DJ. Effects of salinity on survival of the exotic seagrass *Zostera japonica* subjected to extreme high temperature stress. *Botanica Marina*. 2013; 56:75–82.
- Kaldy JE. Effect of temperature and nutrient manipulations on eelgrass *Zostera marina* L. from the Pacific Northwest, USA. *Journal of Experimental Marine Biology and Ecology*. 2014; 453:108–115.
- Kaldy JE, Brown CA, Nelson WG, Frazier M. Macrophyte community response to nitrogen loading and thermal stressors in rapidly flushed mesocosm systems. *Journal of Experimental Marine Biology and Ecology*. 2017; 497:107–119. [PubMed: 29225370]
- Kemp WM, Bartleson R, Murray L. Epiphyte contributions to light attenuation at the leaf surface. Chesapeake Bay Submerged Aquatic Vegetation Water Quality and Habitat-Based Requirements and Restoration Targets: A Second Technical Synthesis. CBP/TRS. 2000; 245(00):55–70.
- Kemp WM, Bartleson R, Bergstrom P, Carter V, Gallegos CL, Hunley W, Wilcox DJ. Habitat requirements for submerged aquatic vegetation in Chesapeake Bay: Water quality, light regime, and physical-chemical factors. *Estuaries*. 2004; 27:363–377.
- Kentula, ME. PhD Dissertation. Oregon State University; Corvallis, OR: 1982. Production dynamics of a *Zostera marina* L. bed in Netarts Bay, Oregon; p. 158
- Kentula ME, DeWitt TH. Abundance of seagrass (*Zostera marina* L.) and macroalgae in relation to the salinity-temperature gradient in Yaquina Bay, Oregon, USA. *Estuaries*. 2003; 26:1130–1141.
- Krause-Jensen D, Sagert S, Schubert H, Boström C. Empirical relationships linking distribution and abundance of marine vegetation to eutrophication. *Ecological Indicators*. 2008; 8:515–529.
- Lee KS, Short FT, Burdick DM. Development of a nutrient pollution indicator using the seagrass, *Zostera marina*, along nutrient gradients in three New England estuaries. *Aquatic Botany*. 2004; 78:197–216.
- Lemagie EP, Lerczak JA. A comparison of bulk estuarine turnover timescales to particle tracking timescales using a model of the Yaquina Bay estuary. *Estuaries and Coasts*. 2015; 38:1797–1814.
- Lin HJ, Nixon S, Taylor D, Granger S, Buckley B. Responses of epiphytes on eelgrass, *Zostera marina* L., to separate and combined nitrogen and phosphorus enrichment. *Aquatic Botany*. 1996; 52:243–258.
- McMahon K, Collier C, Lavery PS. Identifying robust bioindicators of light stress in seagrasses: a meta-analysis. *Ecological Indicators*. 2013; 30:7–15.
- Monbet Y. Control of phytoplankton biomass in estuaries: a comparative analysis of microtidal and macrotidal estuaries. *Estuaries*. 1992; 15:563–571.
- Moncreiff CA, Sullivan MJ, Daehnick AE. Primary production dynamics in seagrass beds of Mississippi Sound: the contributions of seagrass, epiphytic algae, sand microflora, and phytoplankton. *Marine Ecology-Progress Series*. 1992; 87:161–161.
- Moore KA, Wetzel RL. Seasonal variations in eelgrass (*Zostera marina* L.) responses to nutrient enrichment and reduced light availability in experimental ecosystems. *Journal of Experimental Marine Biology and Ecology*. 2000; 244:1–28.
- Morgan MD, Kitting CL. Productivity and utilization of the seagrass *Halodule wrightii* and its attached epiphytes. *Limnology and Oceanography*. 1984; 29:1066–1076.
- Motley, JM. MS thesis. Marine Resources Management. Oregon State University; Corvallis, Oregon: 2017. Local and regional patterns in eelgrass (*Zostera marina* L.) communities along an upwelling-productivity gradient in Oregon estuaries, USA; p. 97
- Nelson TA. Interannual variance in a subtidal eelgrass community. *Aquatic Botany*. 1997; 56:245–252.
- Nelson TA, Waaland JR. Seasonality of eelgrass, epiphyte, and grazer biomass and productivity in subtidal eelgrass meadows subjected to moderate tidal amplitude. *Aquatic Botany*. 1997; 56:51–74.

- Nelson WG. Development of an epiphyte indicator of nutrient enrichment: threshold values for seagrass epiphyte load. *Ecological Indicators*. 2017a; 74(2017):343–356. DOI: 10.1016/j.ecolind.2016.11.035
- Nelson WG. Development of an epiphyte indicator of nutrient enrichment: a critical evaluation of observational and experimental studies. *Ecological Indicators*. 2017b; 79:207–227.
- Nelson WG. Patterns of shading tolerance determined from experimental light reduction studies of seagrasses. *Aquatic Botany*. 2017c; 141:39–46.
- Orth RJ, Van Montfrans J. Epiphyte-seagrass relationships with an emphasis on the role of micrograzing: a review. *Aquatic Botany*. 1984; 18:43–69.
- Penhale PA. Macrophyte-epiphyte biomass and productivity in an eelgrass (*Zostera marina* L.) community. *Journal of Experimental Marine Biology and Ecology*. 1977; 26:211–224.
- Piazzi L, Balata D, Cinelli F, Benedetti-Cecchi L. Patterns of spatial variability in epiphytes of *Posidonia oceanica*: differences between a disturbed and two reference locations. *Aquatic Botany*. 2004; 79:345–356.
- Reynolds PL, Richardson JP, Duffy JE. Field experimental evidence that grazers mediate transition between microalgal and seagrass dominance. *Limnology and Oceanography*. 2014; 59:1053–1064.
- Ruggiero, P., Brown, CA., Komar, PD., Allan, JC., Reusser, DA., Lee, H, II. Oregon Climate Assessment Report (2010). Dello, KD., Mote, PW., editors. Oregon Climate Change Research Institute, College of Oceanic and Atmospheric Sciences, Oregon State University; Corvallis, OR: 2010.
- Ruesink JL. Epiphyte load and seagrass performance are decoupled in an estuary with low eutrophication risk. *Journal of Experimental Marine Biology and Ecology*. 2016; 481:1–8.
- Saunders JE, Attrill MJ, Shaw SM, Rowden AA. Spatial variability in the epiphytic algal assemblages of *Zostera marina* seagrass beds. *Marine Ecology Progress Series*. 2003; 249:107–115.
- Shelton AO, Francis TB, Feist BE, Williams GD, Lindquist A, Levin PS. Forty years of seagrass population stability and resilience in an urbanizing estuary. *Journal of Ecology*. 2016; doi: 10.1111/1365-2745.12682
- Short FT, Burdick DM, Kaldy JE. Mesocosm experiments quantify the effects of eutrophication on eelgrass, *Zostera marina*. *Limnology and Oceanography*. 1995; 40:740–749.
- Spivak AC, Canuel EA, Duffy JE, Richardson JP. Nutrient enrichment and food web composition affect ecosystem metabolism in an experimental seagrass habitat. *PLoS ONE*. 2009; 4(10):e7473.doi: 10.1371/journal.pone.0007473 [PubMed: 19829713]
- Stankelis, RM., Frank, JM., Lawrence, JM., Boynton, WR. Technical Report TS 297-01 CBL. Maryland: US Army Research Laboratory, Chesapeake Biological Laboratory; 2001. An assessment of seasonal submerged aquatic vegetation (SAV) epiphyte loading at Blossom Point.
- Thom R. Spatial and temporal patterns in plant standing stock and primary production in a temperate seagrass system. *Botanica Marina*. 1990; 33:497–510.
- Thom, RM., Miller, B., Kennedy, M. Temporal patterns of grazers and vegetation in a temperate seagrass system. University of Washington, Fisheries Research Institute; Seattle, WA: 1991. p. 28Report FRI-UW-9122
- Thom RM, Borde AB, Rumrill S, Woodruff DL, Williams GD, Southard JA, Sargeant SL. Factors influencing spatial and annual variability in eelgrass (*Zostera marina* L.) meadows in Willapa Bay, Washington, and Coos Bay, Oregon, estuaries. *Estuaries*. 2003; 26:1117–1129.
- Thom RM, Southard SL, Borde AB, Stoltz P. Light requirements for growth and survival of eelgrass (*Zostera marina* L.) in Pacific Northwest (USA) estuaries. *Estuaries and Coasts*. 2008; 31:969–980.
- Twilley R, Kemp W, Staver K, Stevenson JC, Boynton W. Nutrient enrichment of estuarine submersed vascular plant communities. 1. Algal growth and effects on production of plants and associated communities. *Marine Ecology Progress Series*. 1985; 23:179–191.
- Uku J, Björk M, Bergman B, Díez B. Characterization and comparison of prokaryotic epiphytes associated with three East African seagrasses. *Journal of Phycology*. 2007; 43:768–779.
- Wear DJ, Sullivan MJ, Moore AD, Millie DF. Effects of water-column enrichment on the production dynamics of three seagrass species and their epiphytic algae. *Marine Ecology Progress Series*. 1999; 179:201–213.

- Wetzel RL, Neckles HA. A model of *Zostera marina* L. photosynthesis and growth: simulated effects of selected physical-chemical variables and biological interactions. *Aquatic Botany*. 1986; 26:307–323.
- Whalen MA, Duffy JE, Grace JB. Temporal shifts in top-down vs. bottom-up control of epiphytic algae in a seagrass ecosystem. *Ecology*. 2013; 94:510–520. [PubMed: 23691669]
- Williams SL, Ruckelshaus MH. Effects of nitrogen availability and herbivory on eelgrass (*Zostera marina*) and epiphytes. *Ecology*. 1993; 74(3):904–918.
- Young, DK., Clinton, PJ., Lee, H., II, Specht, DT., Mochon-Collura, TC. Aerial measures of estuarine intertidal and shallow subtidal *Zostera marina* coverage. In: Lee, H., II, Brown, C., editors. *Classification of Regional Patterns of Environmental Drivers and Benthic Habitats in Pacific Northwest Estuaries*. Office of Research and Development, U.S. Environmental Protection Agency; 2009. p. 158-172. Technical Rept. EPA/600/R-09/140
- Young D, Clinton P, Specht D, Collura T. Comparison of non-native dwarf eelgrass (*Zostera japonica*) and native eelgrass (*Zostera marina*) distributions in a northeast Pacific estuary: 1997–2014. *Botanica Marina*. 2015; 58:239–250. DOI: 10.1515/bot-2014-0088

Highlights

Nelson - An evaluation of factors controlling the abundance of epiphytes on *Zostera marina* along an estuarine gradient in Yaquina Bay, Oregon, USA.

Epiphyte load on intertidal *Zostera marina* was greater in the dry season and in marine-dominated estuarine areas.

Epiphyte load correlated inversely to DIN and positively to PO₄ concentration, but P may not limit growth.

Grazers did not appear to control epiphyte load differences.

Increased up-estuary water-column light attenuation may limit both epiphyte load and *Z. marina* biomass.

Figure 1.
Sample locations for seagrass epiphytes and nutrients along the length of the Yaquina estuary. Three epiphyte stations were located within both the Marine and Riverine zones. Gray bar indicates the approximate boundary of the two estuarine zones.

Figure 2. Mean (+1 s.d.) epiphyte load (mg cm⁻²) during wet and dry seasons on A) external leaves, and B) internal leaves from shoots of *Zostera marina* at six sites in the Yaquina estuary over the 4-year period of study.

Figure 3. Mean epiphyte load (+1 s.d.) on external and internal *Zostera marina* leaves by season (wet vs. dry) and salinity zone (Marine vs. Riverine) in the Yaquina estuary. Sample size ranged from 36–51.

Figure 4. Temporal relationship of epiphytic load on *Zostera marina* external leaves in the Yaquina estuary, 2000 – 2003. Gray diamonds indicate data from 2004, which were not included in the seasonality regression equation shown.

Figure 5.

Box and Whiskers plots of nitrogen (DIN) concentration at the water quality monitoring stations nearest to the epiphyte sampling stations in the Yaquina estuary during 2003–2004 for A) the dry season, and B) the wet season. Gray boxes present the 25th and 75th percentiles with median. Whiskers are the 10th and 90th percentiles; individual values are plotted outside these limits.

Figure 6.

Comparison of the seasonal relationship of mean epiphyte load on external leaves and A) mean water column DIN concentration, or B) mean water column PO₄ concentration versus Julian Day for data from 2000–2003. Data are means for all stations, with data binned and further averaged within intervals of 15 Julian days.

Figure 7.

Regression relationship (exponential rise to maximum) between the percent of light attenuation relative to epiphyte load, based on mean monthly values at each station from July 2002 through November 2004 (n=116).

Figure 8.

Box and Whiskers plot for percent light attenuation by epiphytes at 4 stations for both external (E) and internal (I) leaves within *Zostera marina* shoots. Distributions are for means of $n=6$ leaves on each sample date, with 18 sample dates for Stations WN1, WN3, and WN4, and 4 dates for Station WN6. Percentiles as described for Fig. 5. WN1, WN3 (Marine zone), WN4, WN6 – (Riverine zone).

Table 1

Comparison of studies with data on seagrass epiphyte biomass and load in the Pacific Northwest region of the USA. NA – not available

Location (Latitude, Longitude)	Observation period (# sample dates)	Month of maximum epiphyte biomass	Maximum epiphyte biomass value	Mean epiphyte biomass value	Units for max value	Mean epiphyte load (g g ⁻¹)	Mean epiphyte load (mg cm ⁻²)	Reference	Sampling Design
Friday Harbor, WA (48.54, -122.99)	Jul. 1990 – Jul. 1992 (20)	Sep.	202.3	31	g DW m ⁻²	0.14	0.28	Nelson and Waaland 1997	1 site, 8 subtidal depths
Padilla Bay, WA (48.51, -122.496)	Jun. 1986 – May 1987 (9)	Jun.	95.9	30.2	g DW m ⁻²	0.22	0.45 ¹	Thom 1990	1 site, 3 intertidal elevations
Padilla Bay, WA (48.51, -122.496)	Jun. 1987 – Aug. 1989 (13)	Aug.	0.07	0.03	g DW shoot ⁻¹	0.021		Williams and Ruckelshaus 1993	1 ² intertidal site
Padilla Bay, WA (48.51, -122.496)	Oct. 1989 – Nov. 1990 (7)	Jul.	24.1	11.9	g DW m ⁻²	0.09	0.18 ¹	Thom et al. 1991	1 site, 2 intertidal elevations
Willapa Bay, WA (48.54, -122.99)	Jul. – Aug. 1998–2001 (4)	NA	NA	0.025	g DW shoot ⁻¹	0.013		Thom et al. 2003	6 intertidal sites on salinity gradient
Willapa Bay, WA (46.475, -124.022)	Jul. 2011 – Sep. 2012 (10)	Aug.	1.99 ²	1.3 ²	g g ⁻¹ (leaf)	0.031 ³	0.52 ¹	Ruesink 2016	1 intertidal site
Netarts Bay, OR (45.4, -123.953)	Apr. 1980 – May 1981 (11)	May	87	24.4	g DW m ⁻²	0.52 ⁴	0.54	Kentula 1982	1 site, 3 intertidal elevations
Netarts Bay, OR (45.4, -123.953)	May–Sept. 2015 (6)	Jun.	194.5	132.6	g DW m ⁻²	0.57 ⁴		Motley 2017	3 intertidal sites on salinity gradient
Yaquina Bay, OR (44.596, -124.014)	Aug. 2000 – Nov. 2004 (38)	Jul.	4.1	1.4	g g ⁻¹ (leaf)	1.42 ⁴	1.43	this study **	6 intertidal sites on salinity gradient
Yaquina Bay, OR (44.596, -124.014)	May–Sept. 2015 (6)	Jun.	190.5	132.2	g DW m ⁻²	1.12 ⁴		Motley 2017	3 intertidal sites on salinity gradient
Coos Bay, OR (43.4, -124.25)	Jul. Aug. 1998–2001 (4)	NA	NA	0.022	g DW shoot ⁻¹	0.025		Thom et al. 2003	6 intertidal sites on salinity gradient
Coos Bay, OR (43.4, -124.25)	May–Sept. 2015 (6)	Jun.	175.6	104.8	g DW m ⁻²	0.9 ⁴		Motley 2017	3 intertidal sites on salinity gradient

¹ used conversion formula shoot wt (mg) = -59.1 + 2 leaf area cm² from Table 1, Thom (1990)

² From Fig. 3A, Ruesink (2016), t=0 for experiment

³ g epiphyte g⁻¹ (leaf + sheath)

⁴ g epiphyte g⁻¹ (leaf)