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Epiphyte load and seagrass performance are decoupled in an estuary with low eutrophication risk

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ABSTRACT

Seagrass can decline due to blooms of algae following either loss of top-down control or increase in bottom-up nutrient delivery. Macroalgae negatively affect seagrass especially at high ratios of algal biomass to seagrass size. In contrast, for microalgae, epiphyte loads that affect seagrass performance are not well synthesized. Competition has been demonstrated primarily following nutrient or food web manipulations, which increase epiphyte load from background levels, rather than through experimental removal of epiphytes. Although the Willapa Bay (Washington, USA) is an estuary with low eutrophication risk, microalgae reach large biomass in summer, up to 60% of the mass of their intertidal seagrass host (eelgrass, *Zostera marina*). Based on a novel field technique, experimental reduction of epiphytes did not accelerate growth ($g\ g^{-1}\ d^{-1}$) of *Z. marina*. Neither did two other manipulations that were expected to improve resource access: removing nearby shoots or older, epiphytized leaves. Instead, removing older leaves slowed growth from 2.3% d^{-1} to 1.8% d^{-1}, and removing lightly-epiphytized younger leaves slowed growth to 1.7% d^{-1}. To evaluate top-down and bottom-up causes of epiphyte load, two widespread methods were used. Observationally over time, epiphyte load was positively correlated with eelgrass leaf emergence rate and unrelated to mesograzer density; however, a crossed experiment reducing crustacean mesograzers and adding nutrients indicated top-down control, since chlorophyll-a in epiphytes increased with grazer reduction and no added nutrients (deterrent × nutrient interaction). At current epiphyte loads in the Willapa Bay, intertidal *Z. marina* tolerates seasonally-abundant epiphytes coinciding with rapid leaf emergence, thus producing fresh leaves where microalgal competition cannot be detected.

1. Introduction

Both micro- and macro-algae can form blooms that competitively exclude seagrasses (Duarte, 1995), but this straightforward ecological interaction has been complicated by evidence that blooms result from multiple factors and exert context-dependent effects on seagrasses. Consequently, both the causes and consequences of algal blooms in seagrass beds require case-specific evaluation. The causes of algal blooms have been variously attributed to both top-down and bottom-up controls. With top-down forcing, a trophic cascade from overfishing of top predators allows mesopredators to become abundant and to limit mesograzers (gastropods and crustaceans) that would keep algae in check, and thus overfishing links to both algal blooms and seagrass declines (Heck et al., 2000; Hughes et al., 2013). With bottom-up forcing, competitors gain an advantage over seagrass because of release from nutrient limitation in the water column (Duarte, 1995; Hughes et al., 2004). The relative contribution of bottom-up and top-down pathways in particular seagrass systems has been tested in several cases involving both slow-release nutrient addition and reduction of mesograzers, with grazing of epiphytes a critical component (Heck et al., 2000; Verhoeven et al., 2012; Whalen et al., 2013; Reynolds et al., 2014; Duffy et al., 2015). In contrast to this experimental evidence, epiphyte distribution and abundance can change spatiotemporally in concordance with factors other than grazers, including seasonal changes in light or temperature, eutrophication, and variation in seagrass leaf turnover (Bulthuis and Woelkerling, 1983; Borum, 1987; Nelson and Waaland, 1997). In fact, in observational studies over seasons, epiphytes and grazers can be positively correlated due to similar seasonal dynamics (Nelson and Waaland, 1997; Fong et al., 2000), and mesograzers may release epiphytes from light or nutrient-limitation (Jaschinski and Sommer, 2010).

Competition experiments between macroalgae and seagrass were synthesized recently via meta-analysis (Thomsen et al., 2012), without reference to microalgal epiphytes. Impacts scaled with macroalgal density and were inversely related to seagrass size, but were overall rather weak (Thomsen et al., 2012). In some cases, macroalgae may even benefit seagrass by harboring mesograzers that clean seagrass leaves (Whalen et al., 2013). Epiphytes growing on seagrass leaves have long been implicated to restrict seagrass access to light and carbon, with negative effects more obvious when resources are below saturating levels (Sand-Jensen, 1977). Unlike macroalgae, however, microalgae have not been directly manipulated in seagrass field experiments to compare

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responses with and without competitors. Rather, manipulations of grazers or nutrients tend to increase epiphytes relative to background levels (Hughes et al., 2004, Duffy et al., 2015).

In this study, the causes and consequences of epiphyte loading on intertidal eelgrass (*Zostera marina*) were examined in a context of robust eelgrass populations and low eutrophication risk. The study paired two approaches that are widespread in seagrass-epiphyte studies: 1) an observational study of environmental correlates of epiphytes, and 2) a crossed experimental manipulation of nutrients and grazers. The observational approach cannot be used alone to derive mechanistic conclusions but does define natural variability in epiphyte load. If bottom-up factors control seasonal variability, then positive correlations are expected between epiphytes, light, and nutrients. Alternatively, epiphyte load would be negatively correlated with leaf turnover under a scenario of host control. Top-down control could result in an inverse relationship between epiphytes and grazers. The companion experiment, involving mesograzer reduction and addition of slow-release nutrients in the field, examined two mechanisms that could limit epiphyte load in Willapa Bay during its summer season of peak biomass. In addition to these methods, a novel direct removal of microalgae in the field was employed to determine the consequences of standing levels of epiphytes. Effects of epiphyte load were tested in comparison to other manipulations selected to explore additional mechanisms that could modify resources available for seagrass growth. Specifically, shoots were thinned and outer, epiphytized leaves removed in treatments that were expected to increase resource availability, and young, relatively clean leaves were removed to evaluate their contribution to whole-shoot performance.

2. Material and methods

2.1. Study site

The study took place in an eelgrass (*Z. marina*) bed near the center of its estuarine distribution in Willapa Bay (Washington, USA; N46.475°, W124.022°). *Z. marina* occupies 10% of the area of the bay (>3000 of 34,904 ha; Borde et al., 2003; Ruesink et al., 2006), primarily between +0.5 and −1 m relative to mean lower low water (MLLW; Ruesink et al., 2010). The bay receives tidally-advected nutrients from upwelled ocean water but has low human population density in the watershed and rapid flushing, therefore a “low overall eutrophic conditions rating” (Bricker et al., 2007 p. A153). Within *Z. marina* tissue, nitrogen below 1.8% indicates nutrient limitation (Duarte, 1990). In contrast, tissue nitrogen at the study site greatly exceeds this level (>3%, Duffy et al., 2015), although varying spatially in Willapa Bay from 1.5% to 3.2% in summer (Ruesink et al., 2015). Consistent with this assessment of available nutrients, *Z. marina* growth did not respond to sediment nutrient addition (Wagner et al., 2012). The health of eelgrass populations in the bay is also suggested by resilience to pulse disturbances (Wisehart et al., 2007; Ruesink et al., 2012), including shellfish aquaculture that can overlap with eelgrass but was not present within the target eelgrass bed.

2.2. Seasonal surveys of epiphytes and mesograzers

Epiphytes and mesograzers were surveyed at one- to three-month intervals for a total of 10 times between June 2011 and September 2012 at an elevation near MLLW. Epiphytes primarily consist of microalgae at this tidal elevation and polyhaline conditions (salinity of 18–29 depending on river flow). Macroalgae (*Enteromorpha* spp., *Polysiphonia* spp.) are generally not attached to eelgrass blades and overall are uncommon in Willapa Bay relative to other US west coast estuaries (Hessing-Lewis and Hacker, 2013). At each sampling time, 4–5 *Z. marina* shoots were collected haphazardly along 50 m to evaluate epiphyte load. Epiphytes were collected from leaves extending above the outer sheath by pulling a glass slide along both sides of each leaf and drying the slurry from each leaf separately (60 °C for 1 week). Epiphytes were weighed, along with the corresponding dried leaves and sheath, and epiphyte dry mass standardized by shoot dry mass. To account for variation in number of leaves among shoots across seasons, this metric of epiphyte load was based on the five most recent leaves for each shoot, including the leaf sheaths.

To quantify the mesograzer assemblage, 4–5 eelgrass samples were collected during each sampling time, with each sample consisting of the shoots caught within a mesh bag lowered over the shoots in shallow water (bag diameter of 20 cm, 80 μm mesh; Reynolds et al., 2014). Eelgrass material was agitated and gently scraped in freshwater to remove mesograzers, then dried and weighed to provide a means of standardizing mesograzers by the amount of host material. The water was sieved (0.5 mm), and the trapped mesograzers were identified and counted by size class, using a series of nested sieves to estimate epifaunal biomass (Edgar, 1990; Reynolds et al., 2014).

Seasonal patterns of *Z. marina* growth throughout Willapa Bay were determined from intensive sampling in 2004 (Ruesink et al., 2010). For each sample at each of seven sites, 30–50 shoots were marked with holes near the top of the leaf sheath, and 2–5 days later the shoots were examined for new, unmarked leaves extending beyond the original mark. The fraction of plants with newly-emerged leaves, standardized by the time between marking and collection, represents leaf emergence rate and is also the inverse of the leaf plastochron interval (Herbert and Fourqurean 2009).

Epiphyte load was examined with respect to five total variables. Two of these predictors were abiotic: daylength, and the average water temperature recorded by a nearby logging sensor (iButton, Dallas Semiconductor) on each day when the eelgrass bed was sampled. Two predictors represented the substratum availability (shoot dry mass) and turnover (leaf emergence rate) of *Z. marina*. Leaf emergence rate was interpolated from a sine wave fit to 2004 data (New leaves $d^{-1} = 0.057\cdot(\sin(2\pi\cdot(\text{day of year} - 53)/365)) + 0.098$, $n = 39$). Finally, crustacean mesograzer density was included as a predictor for top-down control, either as the total assemblage or as one of three major taxa (isopods, caprellid amphipods, or gammarid amphipods); thus, mesograzer density was only included as a single predictor variable in any particular model but required four distinct sets of models to accommodate the four ways of defining this value. The best predictors of epiphyte load were identified through a model selection approach,

Table 1
Importance of predictors of seasonal epiphyte load in Willapa Bay, Washington. Parameter importance is the sum of Akaike's weights across containing models in the 95% confidence set. In parentheses is shown the number of models in the 95% confidence set that contain the parameter. Mesograzers were incorporated in four different ways.

Mesograzer parameter as:	Predictors				
	Leaf emergence rate	Daylength	Mesograzers	Shoot dry mass	Water temperature
Total density	0.77 (3)	0.18 (1)	0.04 (1)	0.06 (1)	0.01 (1)
Caprellid amphipod density	0.71 (3)	0.22 (2)	0.13 (4)	0.06 (1)	0.02 (1)
Gammarid amphipod density	0.77 (3)	0.19 (2)	0.05 (2)	0.06 (1)	
Idotea isopod density	0.75 (3)	0.17 (1)	0.07 (2)	0.06 (1)	