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SHORT COMMUNICATION

ESTIMATION AND COMPARISON OF EPIPHYTE LOADING ON HOLOPELAGIC *SARGASSUM FLUITANS* COLLECTED IN THE NORTH ATLANTIC OCEAN AND THE GULF OF MEXICO

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INTRODUCTION

Sargassum natans and *S. fluitans* (collectively referred to as *Sargassum*) are holopelagic brown macroalgae found throughout the Atlantic Ocean and Gulf of Mexico (Coston—Clements et al. 1991). The structural complexity of the *Sargassum* thallus, which includes blades and pneumatocysts, provides surface area for sessile epibiota, including hydroids, bryozoans, polychaetes, diatoms, and other microalgae (Weis 1968, Maples 1984, Stoner and Greening 1984, Calder 1995, Rooker et al. 2006). Combined, *Sargassum* and its associated epibiota create a productive base "community" relative to the surrounding and often oligotrophic open ocean environment (Dooley 1972). As a result, diverse assemblages of fishes (>100 species), sea turtles (4 species), seabirds (>20 species), and invertebrates (>145 species) are found in association with *Sargassum* (SAFMC 2002, Wells and Rooker 2004, Wang and Hu 2016), which provides refuge from predators, enhanced feeding opportunities, and serves to concentrate organisms with flotsam—seeking behaviors (Dooley 1972). *Sargassum* is often referred to as a nursery habitat because many of the associated fishes and sea turtles are juveniles, including commercially and recreationally important fish species such as Gray Triggerfish (*Balistes capriscus*), Dolphinfish (*Coryphaena hippurus*), Tripletail (*Lobotes surinamensis*), and Greater Amberjack (*Seriola dumerili*), among others (Wells and Rooker 2004, Rooker et al. 2006, Casazza and Ross 2008).

Epiphytes are conspicuous members of the *Sargassum* community, and although the dominant taxa have been previously reported (see reviews by Butler et al. 1983, Coston—Clements et al. 1991), relatively little is known about their ecological role within *Sargassum* habitats. There is evidence to suggest that the epiphyte community contributes to nutrient cycling, and makes up an important component in *Sargassum* food webs. For example, epiphytic, nitrogen—fixing cyanobacteria may provide a significant amount of new nitrogen to *Sargassum* communities, which are often found in nutrient—depleted waters (Carpenter and Cox 1974). *Sargassum*—associated hydroids, bryozoans and diatoms have been reported in the diets of invertebrates feeding within *Sargassum*, such as polychaete worms, gastropods, amphipods, and decapod crustaceans (Geiselman 1983). Similarly, hydroids, encrusting bryozoans, and barnacles were observed in the diets of *Sargassum*—associated monacanthids (*Stephanolepis hispidus, S. setifer*) and balistids (*B. capriscus*; Dooley 1972). The degree of epiphytic loading also influences the capacity of *Sargassum* to remain buoyant. For example, Pestana (1985) estimated that between 4.3—21.4% of *Sargassum* wet weight off Bermuda was calcium carbonate, primarily from encrusting barnacles, bryozoans and tube—building polychaetes. Ultimately the level of biofouling can reach a point where *Sargassum* cannot remain afloat (Johnson and Richardson 1977), thus effectively removing the habitat from the near surface and diminishing its nursery function.

The extent of epiphyte loading on *Sargassum* could "potentially" be used as a proxy for relative measures of *Sargassum* "age." *Sargassum* reproduces vegetatively, and newly bloomed *Sargassum* releases anti—fouling tannins that prevent epiphytic organismal growth (Conover and Sieburth 1964, Ryland 1974). These tannins eventually fade and allow for the growth of microorganisms and in succession other epiphytic organisms (Coston—Clements et al. 1991), thus lightly biofouled *Sargassum* is likely to be relatively young compared to heavily biofouled *Sargassum*. Using qualitative measures of epiphyte coverage (e.g., low, intermediate, high epiphyte coverage) and gradients of *Sargassum* color, Stoner and Greening (1984) classified *Sargassum* age and determined that age was a determinant in structuring the associated macrofaunal community. Combined with other methods such as remote sensing observations, estimating the relative age of *Sargassum* may also be useful in determining the source of *Sargassum*, its transport within and between ocean basins, and the dynamics of its life cycle.

As part of a larger study examining the nursery role function of *Sargassum* habitat, we wanted to develop more quantitative methods than those outlined in Stoner and Greening (1984) to estimate epiphyte loading on *Sargassum*, and use these estimates as factors in assessing variability in associated juvenile fish assemblages. Specifically, the objectives of this pilot study were to develop and compare 2 methods of estimating biofouling on *S. fluitans* blades (epiphyte dry weight and epiphyte percent cover), and then apply these methods to compare the relative epiphyte loading on *S. fluitans* samples collected in the Gulf of Mexico

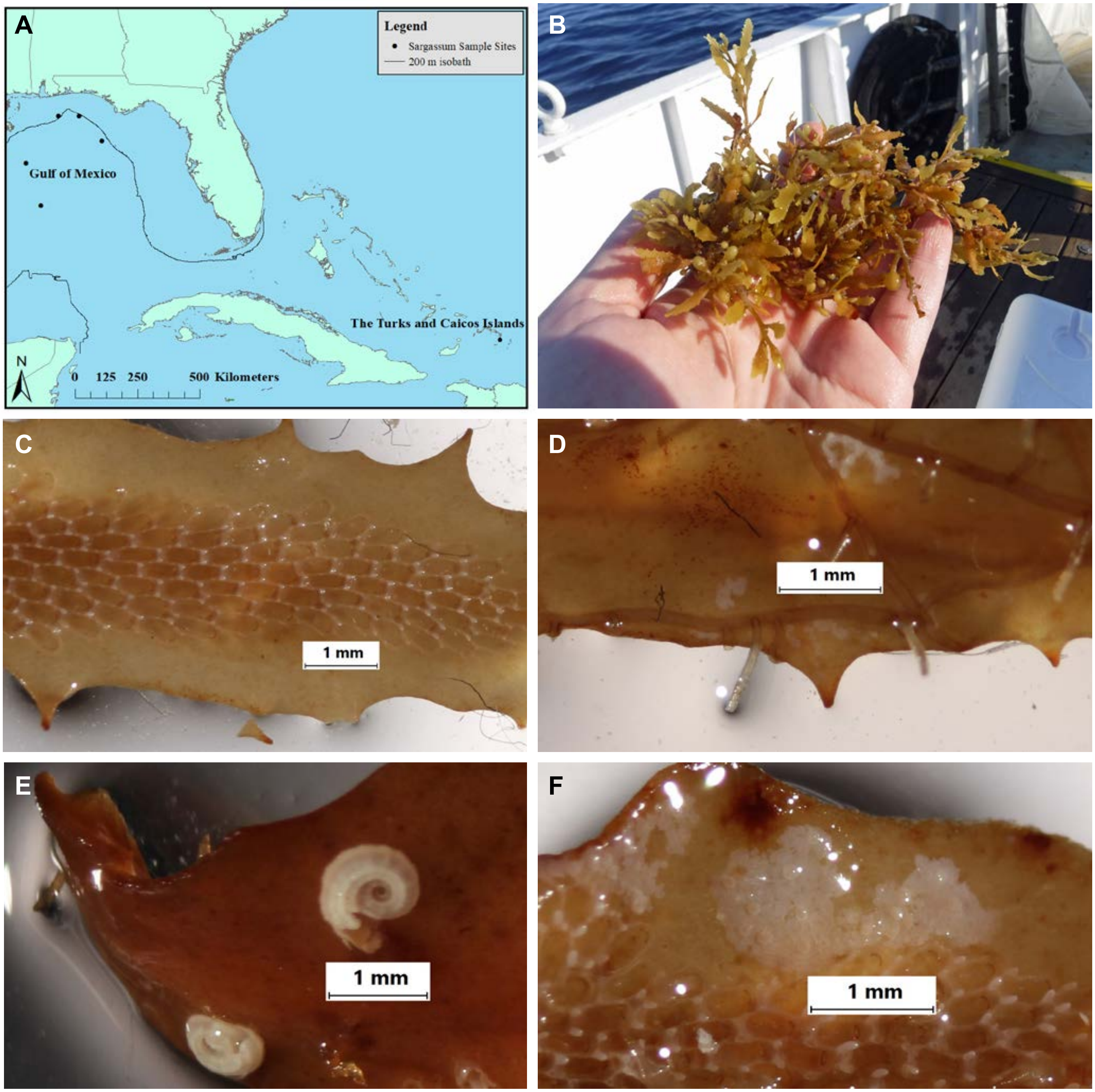

FIGURE 1. Sargassum fluitans *collected for this study. A. Sampling locations in the northern Gulf of Mexico and the Turks and Caicos Islands. B. A clump of* Sargassum fluitans. *Representative epiphytes observed on* S. fluitans *samples included: C. Bryozoans, D. Hydroids, E.* Spirorbis spp., *and F. Unidentified encrusting algae.*

and North Atlantic Ocean (Turks and Caicos Islands).

Materials and Methods

Floating clumps of *S. fluitans* were collected at 5 locations in the northern Gulf of Mexico (between 28 May and 3 June 2019) and at a single location off the coast of South Caicos, Turks and Caicos Islands (16 June 2019; Figure 1) using a variety of net samplers (e.g. dipnet, neuston net). A single clump (Figure 1B) was haphazardly selected from each of the 5 Gulf of Mexico stations (n=5 samples) and 5 clumps were selected from the Turks and Caicos station (n=5 samples). All samples were rinsed of debris and mobile epifauna with seawater and preserved in buffered 95% ethanol.

Five thalli were randomly removed from each *Sargassum* sample, and then one blade was removed from each thalli, resulting in 25 blades from each region (Gulf of Mexico and Turks and Caicos). To determine the percent cover of epiphytes on the blades, digital images were taken of the front and back of each blade using a Zeiss dissecting microscope fitted with a Canon digital camera. Magnification ranged between 6.5—10.0x. The total blade area (mm^2) and the basal area covered by each type of encrusting epiphyte (bryozoans, hydroids, *Spirorbis* spp., and unidentified algae) was measured using iSolution Lite software using a polygon function to trace outlines. These measures were then used to calculate the total epiphyte cover (%) for each blade. To determine the total biomass of epiphytes, each blade was then dried in an oven overnight at 60°C and weighed with a microbalance (mg) with the encrusting invertebrates still intact. Then, all encrusting epiphytes were scraped off using forceps and a scalpel under a dissecting microscope and the cleaned blades were reweighed. The difference in the 2 dry weights (before and after epiphyte removal) was calculated as the total epiphyte dry weight (mg). To account for measurement errors in weight due to rehydration of the blades between weightings, a dry weight correction factor was calculated using mean difference in dry weight for blades (n=5) with zero epiphyte coverage. This correction factor (0.083 mg) was then applied to the dry weights prior to statistical analyses. To examine the influence of blade size on our dry weight estimates, we used a Spearman correlation to examine the relationship between dry weight (mg) and dry weight standardized by blade area (mg/mm^2).

The distribution of the data were skewed towards low dry weight and low percent cover, and did not meet the assumption of normality for parametric statistical analysis (Shapiro—Wilk tests: $W = 0.887$; $p < 0.001$ and $W = 0.855$; $p < 0.0001$, respectively). Therefore, a Spearman correlation using the estimates of epiphyte percent cover and dry weight was used to compare the 2 measurement methods. An independent 2—group Mann—Whitney U test was used to compare taxon—specific percent cover, total epiphyte percent cover, and total epiphyte dry weight between samples collected in the Gulf of Mexico and Turks and Caicos Islands. To examine the influence of blade size on our analyses, the above comparisons between measurement methods (Spearman correlation) and collection location (Mann—Whitney U test) were repeated using dry weight estimates standardized by blade area.

Results and Discussion

Epiphytes associated with the *Sargassum* included bryozoans (Figure 1C), hydroids (Figure 1D), *Spirorbis spp.* (Figure 1E), and unidentified encrusting algae (Figure 1F). We found a significant and positive correlation between total epiphyte percent cover and total epiphyte dry weight (Spearman correlation: $\rho = 0.902$, $p < 0.0001$; Figure 2), which indicates that the methods are comparable in their estimates of epiphyte loading. Overall there was no relationship between epiphyte dry weight and blade surface area (Spearman correlation: $\rho = 0.118$, $p = 0.414$), but a significant and positive relationship was found between total epiphyte percent cover and total epiphyte dry weight when standardized by blade surface area (Spearman correlation: $\rho = 0.969$, $p < 0.0001$). Although both methods were time consuming, the dry weight method was faster. However, due to the relatively low biomass being measured, we encountered issues with rehydration between the initial weighing (with epiphytes) and the second weighing (after epiphyte removal), which required a correction factor. We therefore recommend a second drying of the blades in the oven (after epiphyte removal) to reduce this source of error, although this extra step will extend the time needed for this approach. In our assessment, we found several advantages to the epiphyte percent cover method. First, the digital imaging method allows for taxon—specific analyses, which may be useful in studies related to epiphyte succession or biogeography (Butler et al. 1983). For example, in our study, we found significantly higher bryozoan coverage in the Gulf of Mexico samples, and significantly higher *Spirorbis* spp. coverage in the Turks and Caicos (see data below). These findings are not discernable using the dry weight method. Second, our observations suggest that the dry weight estimates were subject to greater sources of error. In addition to rehydration (mentioned above), some of the encrusting organisms (e.g., bryozoans) were deeply embedded in the algal tissue, and therefore the efficacy of scraping the *Sargassum* blades was variable. Indeed, it was often not possible to scrape off the epiphytes in their entirety. More broadly, scientific digital imaging capabilities are less costly than in years past, as there are numerous free, open—source digital imaging packages available online (e.g., ImageJ). Lastly, digital capture of images and the associated measurements allows for archiving and reanalysis, if required.

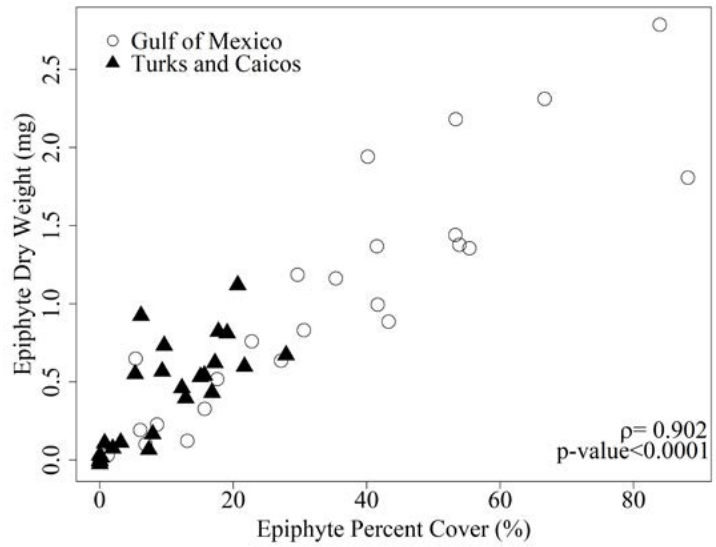

FIGURE 2. *Scatter plot of total epiphyte percent cover (%) and total epiphyte dry weight (in mg) derived from Sargassum fluitan samples collected in the Turks and Caicos (filled triangles) and Gulf of Mexico (open circles). Variables were significantly and positively correlated based on Spearman correlation (ρ) analysis.*

There was a significant difference in the total epiphyte percent cover (Mann—Whitney U test: $W = 500$, $p < 0.001$) and the total epiphyte dry weight (Mann—Whitney U test: $W = 471$, $p < 0.01$) between samples collected from the Turks and Caicos Islands and the Gulf of Mexico (Figure 3). There was also a significant difference in total epiphyte dry weight standardized by blade surface area between the 2 locations (Mann—Whitney U test: $W = 472$, $p < 0.001$). The reasons for variation in epiphyte cover in this study are unknown. Numerous controls on epiphytization have been proposed, including spatial variability in antimicrobial activity (Conover and Sieburth 1964), nutrient

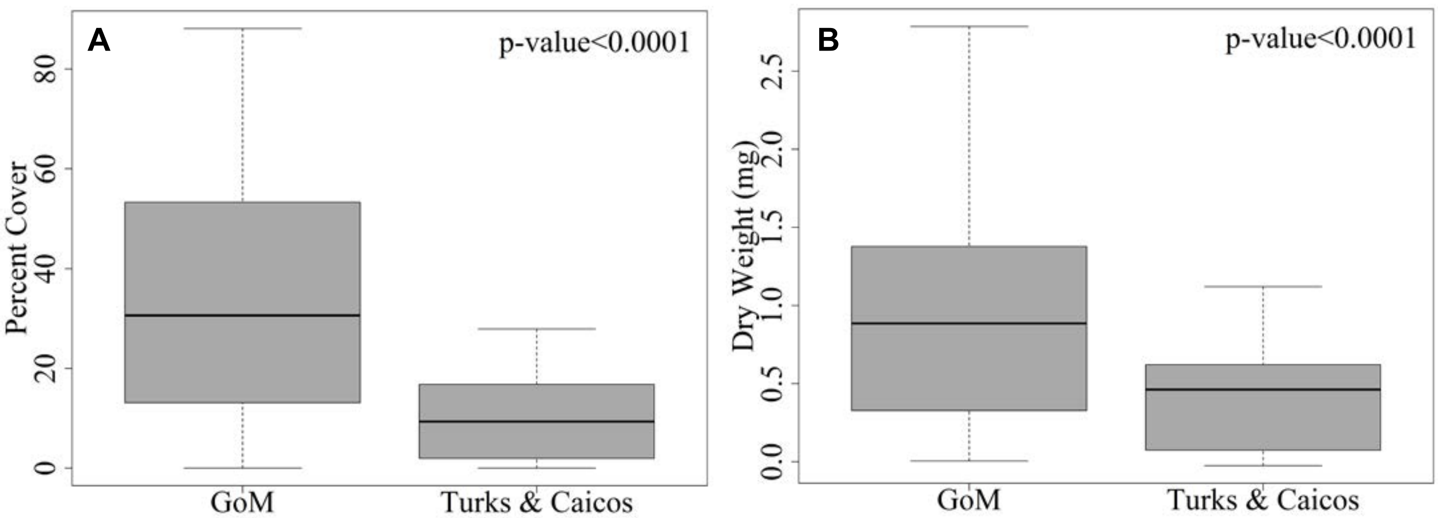

FIGURE 3. *Box and whisker plots comparing Sargassum epiphytes collected in the Gulf of Mexico (GoM) and the Turks and Caicos Islands. A. Total epiphyte percent cover. B. Total epiphyte dry weight. The black bar denotes the sample median, the boundaries of the box denote the 25th and 75th percentiles, and the whiskers denote the 10th and 90th percentiles. The p-values denote significance based on non-parametric Mann-Whitney U tests.*

availability (Carpenter and Cox 1974), epiphyte grazing (Duffy 1990), and seasonal cycles of growth and epiphyte colonization (Butler et al. 1983). Further, the longer *Sargassum* floats in the surface waters, the greater its chance of epiphyte colonization, which is why the degree of biofouling may also serve as a proxy for *Sargassum* age (Stoner and Greening 1984). The associated motile faunal assemblages have been found to be significantly related to both the degree of biofouling and age of *Sargassum* (Stoner and Greening 1984), which are therefore critical drivers of *Sargassum* community dynamics. In recent years, large blooms of *Sargassum* have been documented in the Caribbean and Central Atlantic, and a portion of this biomass extends into the Gulf of Mexico via the Loop Current (Wang et al. 2019). Remote sensing imagery and HYCOM—derived (Global Hybrid Coordinate Ocean Model) estimates of surface current velocities suggest that the Gulf of Mexico samples in our study were collected in features associated with either the Loop Current or associated eddies (e.g., https://optics.marine.usf.edu/cgi—bin/optics_data?roi=SECOORA&Date=6/2/2019; original data available from http://hycom.org, with the methodology used to estimate *Sargassum* biomass and distribution found in Wang and Hu (2016)). The higher epiphyte coverage and biomass observed in our samples from the Gulf of Mexico may suggest that these *Sargassum* clumps were relatively mature and originated from the bloom event that was occurring at the time in the Caribbean and Central Atlantic.

With respect to individual taxa, the bryozoan percent cover of Gulf of Mexico samples was significantly higher than that of Turks and Caicos samples (Mann—Whitney U test: $W = 492$, $p < 0.001$), which indicates that the difference in total percent cover and dry weight between locations may be driven by bryozoans. The percent cover of *Spirorbis* spp. was significantly higher in Turks and Caicos samples than in Gulf of Mexico samples (Mann—Whitney U test: $W = 218$, $p<0.05$). There was no difference in percent cover of hydroids or unidentified algae between locations (Mann—Whitney U tests: $W = 266$, $p = 0.2$ and $W = 364$, $p= 0.12$, respectively). In his description of the succession of epiphytes on *S. natans*, Ryland (1974) observed an abundance of hydroids on new growth, whereas species of bryozoan (*Membranipora tuberculata*) and *Spirorbis* (*S. corrugatus*) were primarily found on older regions of the thalli. These results indicate that the *Sargassum* clumps collected from both regions were relatively mature.

In summary, this pilot project established protocols useful for the assessment of *Sargassum* biofouling, an important habitat attribute with relevance to the structure of associated faunal communities. The results of the dry weight method and percent coverage method were highly correlated, suggesting either would provide an acceptable measure or proxy for epiphyte loading, although the percent coverage (digital imaging) method offers several advantages. Going forward, our future efforts will include similar analyses to examine: 1) finer—scale spatial variability in *Sargassum* epiphyte loadings, e.g., among stations within the northern Gulf of Mexico; 2) potential differences in epiphyte loading between *S. fluitans* and co—occurring *S. natans*; and 3) potential differences in epiphyte loading between *Sargassum* structures (blades, stems, air bladders).

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