Hydrological alteration exacerbates the negative impacts of invasive Eurasian milfoil *Myriophyllum spicatum* by creating hypoxic conditions in a northern Gulf of Mexico estuary

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ABSTRACT: Gulf of Mexico estuaries contain an abundance of habitat-forming submerged vegetation that provide various ecosystem services. However, these estuaries now harbor numerous invasive macrophytes, such as Eurasian milfoil *Myriophyllum spicatum*. Previously, we showed that milfoil gained a foothold in the Mobile-Tensaw Delta, Alabama, USA, in protected waters north of a manmade causeway that significantly reduces wave action. Here, we collected associated organisms using a suction sampler and compared the composition and abundances of fauna residing in *M. spicatum* and wild celery *Vallisneria americana*, the most abundant native macrophyte, during day and night. North of the causeway, where water flow is limited, a 5-fold reduction in the abundance of organisms was documented in *M. spicatum* at night, while abundance in *V. americana* varied little. We found that this nocturnal decrease was accompanied by changes in community structure in *M. spicatum* north of the causeway, driven by reductions in invertebrates such as *Gammarus* amphipods, *Neritina* snails, and *Callinectes sapidus* crustaceans. In contrast, communities in *V. americana*, although distinct from *M. spicatum*, exhibited little spatial or temporal difference. Subsequent analyses indicated that reduced dissolved oxygen in *M. spicatum* north of the causeway at night drives assemblage patterns. These results suggest that hydrological alterations exacerbate *M. spicatum*'s negative effects through the creation of hypoxic zones and that daily migrations into these habitats may be necessary for organisms to garner the benefits of this vegetation. Finally, this work highlights that our understanding of the concomitant impacts of altered hydrology and invasive macrophytes is incomplete without considering diel variability.

KEY WORDS: Anoxia · Community · Dissolved oxygen · Eelgrass · Submerged vegetation · *Vallisneria* · Watermilfoil

INTRODUCTION

Understanding the factors that determine the strength of links between productivity and habitat quantity and quality in estuaries is crucial to the successful conservation and management of coastal waters (Beck et al. 2001). In the northern Gulf of Mexico (GOM), a region of extraordinarily rich biological diversity (Moore et al. 1970, Darnell et al. 1983, Lydeard & Mayden 1995), estuaries are frequently exposed to a diverse array of anthropogenic (oil spills, eutrophication, overfishing, pollution, erosion, climate change) and natural disturbances (hurricanes, watershed-driven freshets, tidal fluctua-

tions). Because of the numerous ecosystem services these waters provide, GOM estuaries are frequently sites of intense urbanization and commercial exploitation. Among the many consequences of estuarine urbanization are the increased alterations of the availability of freshwater resources (Havens et al. 2013), landscape alterations (Rozas et al. 2013), and increasingly successful invasions by non-native species. In many cases, establishment of these invaders triggers dramatic alterations of ecological function and reorganization of the structure of these vital ecosystems (Stachowicz et al. 1999, Grosholz et al. 2000, Martin & Valentine 2010).

Invasive species that can modify ecosystems, either by the creation of new habitats or alterations of abiotic conditions in existing habitats, have been hypothesized to have disproportionately negative effects on food webs (Crooks 2002). In some cases, invasive vegetation can be much larger, and structurally more complex, than native species (Posey et al. 1993, Duffy & Baltz 1998, Chaplin & Valentine 2009). The presence of structurally complex invasive plants in estuaries can also reduce the foraging efficiency of higher-order predators (Heck & Thoman 1981, Martin & Valentine 2011, Valinoti et al. 2011). For this reason, it has been hypothesized that structurally complex invasive macrophyte species may limit the flow of energy to higher trophic levels and thus represent a trophic dead end in successfully invaded estuarine food webs (Chaplin & Valentine 2009).

Among the most prominent of all submerged aquatic vegetation (SAV) species occurring in northern GOM estuaries is Eurasian milfoil *Myriophyllum spicatum*. In Mobile Bay and the Mobile-Tensaw Delta (MTD; Fig. 1), Alabama, USA, the location of this study, milfoil consistently dominates the shallow submerged landscape, with up to 80% coverage in sampled locations (Vittor 2003, Chaplin & Valentine 2009, Martin & Valentine 2012). *M. spicatum* is an invasive, structurally complex SAV species commonly encountered in freshwater and coastal ecosystems in North America (Martin & Valentine 2011). This invasive macrophyte can spread rapidly, displacing native species (Sloey et al. 1997, but see Martin & Valentine 2012) and forming dense beds in many areas. Despite this, the biological diversity and density of fauna in *M. spicatum* can be significantly greater than in structurally simpler native habitat, such as beds of the once-dominant wild celery *Vallisneria americana* (Chaplin & Valentine 2009, Goecker et al. 2009; Fig. 2). Martin & Valentine (2011) reported that the composition of fishes and macroinvertebrates in *M. spicatum* was significantly different from that
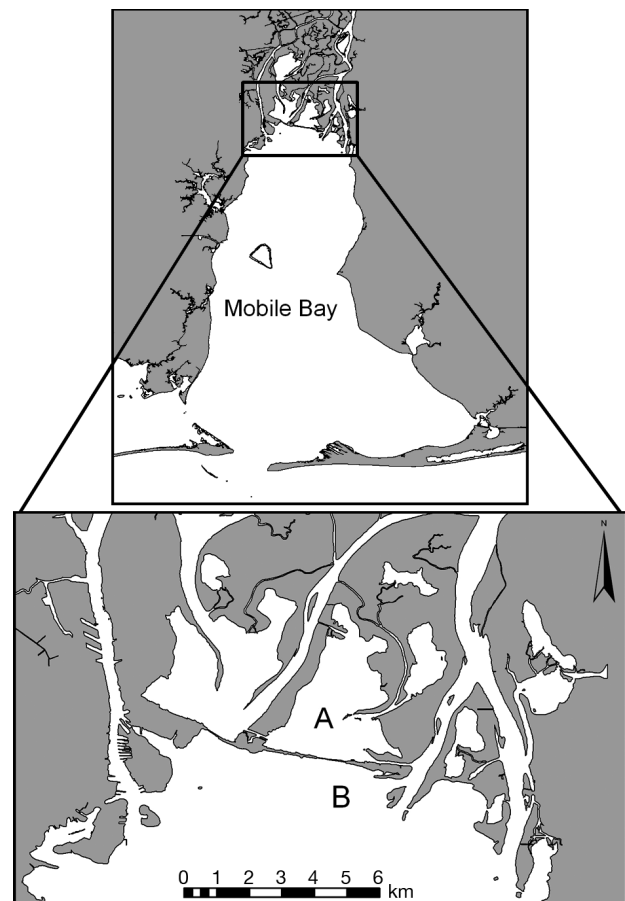

Fig. 1. Mobile Bay, Alabama, USA, with detailed Mobile-Tensaw delta inlay showing general sampling location (A) above causeway and (B) below causeway

found in native *V. americana* beds. While much is known about the aesthetic effects of *M. spicatum* on aquatic ecosystems and its effects on food web structure and function in freshwater systems (Smith & Barko 1990), its ecological effects in coastal systems, especially in the GOM, remain less clear (Duffy & Baltz 1998, Chaplin & Valentine 2009, Martin & Valentine 2014).

Previous research on factors influencing *M. spicatum*'s distribution in the lower MTD (Fig. 1) has indicated that *M. spicatum* gained a foothold in the area via a manmade earthen causeway constructed to facilitate the movement of people and goods across Mobile Bay. This causeway represents an ecosystem-wide alteration of natural hydrography that limits the exchange of water between the watershed and the GOM to a few bridge overpasses (Martin & Valentine 2012). As a result, water turnover rate in semi-enclosed embayments north of the causeway has been reduced (Goecker et al. 2009). Importantly, this
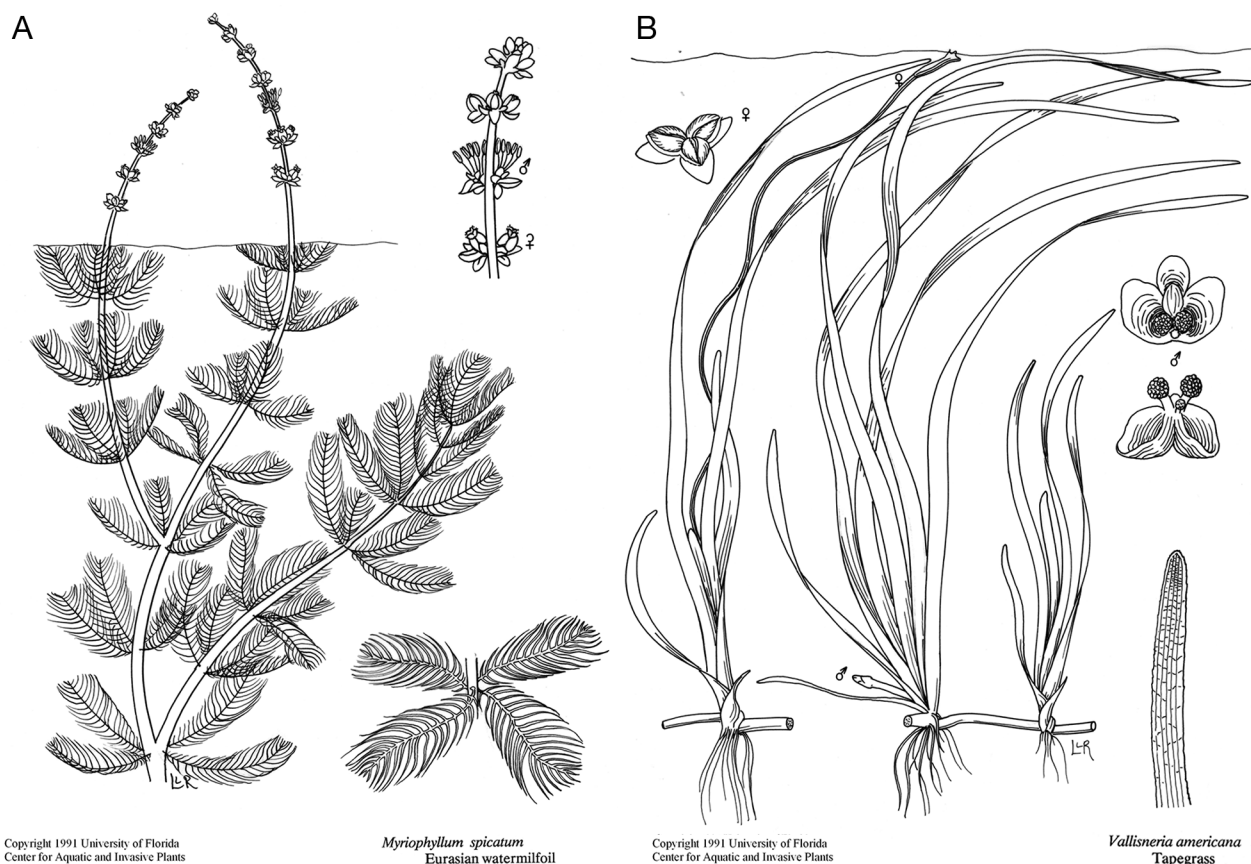
Fig. 2. Submerged aquatic vegetation species compared in this study, (A) the complex invasive species *Myriophyllum spicatum* and (B) the structurally simpler native species *Vallisneria americana*. Line drawings courtesy of UF/IFAS Center for Aquatic and Invasive Plants

causeway also functions as a breakwater that provides a refuge from wave action for large canopy-forming species with shallow root systems, such as invasive *M. spicatum* (Fig. 2), thus allowing these foliose species to proliferate north of the causeway (Martin & Valentine 2012).

Our understanding of the effects of *M. spicatum* as habitat for faunal organisms, however, is limited to studies conducted during daylight hours (Chaplin & Valentine 2009, Martin & Valentine 2011), and very little is known about impacts at night. This gap in our understanding is largely due to the logistical challenges of working in a lightless environment, resulting in a lack of traditional field sampling after dark and an absence of a historical baseline for which to compare results. Despite this paucity of information, it is widely acknowledged that many organisms are nocturnally active, exhibit vastly different behavior, and display altered patterns of community structure (Eggleston et al. 1998, Hammerschlag et al. 2017). Organisms in ecosystems ranging from coral reefs to polar regions and the open ocean are known to have evolved important adaptations to take advantage of the lack of visual cues to both avoid predators and more effectively forage (Helfman 1986, Hammerschlag et al. 2010, Goebel et al. 2017). As such, disregarding potentially important diel patterns in habitat use patterns when conducting impact assessments of invasive species can overlook important negative effects.

Previous studies of *M. spicatum*'s impact as estuarine habitat (Duffy & Baltz 1998, Martin & Valentine 2011) have been conducted during daylight, when these meadows generate large quantities of oxygen. After sunset, respiration in dense beds of SAV may lead to oxygen depletion that could render these meadows uninhabitable, especially in the summer. Dense canopies in *M. spicatum* have been shown to create hypoxic conditions in underlying waters at night in freshwater ecosystems (Frodge et al. 1990, Unmuth et al. 2000), but whether these hypoxic events occur in *M. spicatum* in estuarine conditions,

especially those with limited water exchange such as is the case in parts of the MTD, and how estuarine fauna might respond to these potential perturbations remain unclear.

Here, we assess diel variability in the efficacy of *M. spicatum* as a viable habitat for shelter-seeking organisms by making comparisons with *V. americana* at sites both north (where water exchange is reduced) and south (where water moves freely) of the MTD causeway. Moreover, we explore the extent to which diel fluctuations in environmental characteristics (e.g. dissolved oxygen, DO) might influence these communities across SAV species and locations (cf. Frodge et al. 1990, Unmuth et al. 2000, Brady & Targett 2013). Based on our current understanding of this system (Chaplin & Valentine 2009, Martin & Valentine 2012, 2014), we hypothesized that the reduced wave action and water exchange north of the causeway would promote more intense hypoxic conditions in dense SAV stands than in SAV habitats to the south of the causeway, where water movement is unimpeded. We further hypothesized that hypoxic conditions would be more intense in *M. spicatum* stands, which have much greater aboveground biomass (Martin & Valentine 2012), than in *V. americana* stands and that these predicted differences would have cascading effects on faunal density and composition. This work provides the first diel assessment of the effects of *M. spicatum* on environmental conditions and the ensuing faunal responses to changes in such conditions in an estuarine setting.

MATERIALS AND METHODS

Study sites

The MTD, located in lower Alabama, encompasses an area formed from the convergence of the Tombigbee and Alabama rivers, extending 72 km south and 16 km wide, south to the Mobile Bay causeway (US Highway 90). Ranked as the fourth largest drainage delta in terms of discharge, the MTD drains approximately 15% of the United States' freshwater before entering the GOM (Gastaldo 1985). The landscape gradually shifts from forested wetlands in the north to tidal freshwater and oligohaline marshes as the MTD empties into Mobile Bay to the south. The lower MTD is a hydrologically variable area where salinities can range from <2 PSU during the spring to ≥18 PSU in the fall. Low-frequency, episodic high-intensity events such as storms and hurricanes can also have

profound effects on salinity in this area (Park et al. 2007).

The MTD contains a variety of structurally complex habitats ranging from submerged and emergent vegetation to biogenic reefs composed of *Rangia cuneate* and *Crassostrea virginica.* These habitats support a suite of commercially and recreationally important juvenile organisms such as blue crabs *Callinectes sapidus*, penaeid shrimps, red drum *Sciaenops ocellatus*, and spotted seatrout *Cynoscion nebulosus* (Martin & Valentine 2011). Among the habitats in the MTD supporting the greatest densities of juveniles of these species are SAV beds (Chaplin & Valentine 2009, Martin & Valentine 2011, Rozas et al. 2013).

To test the interactive effects of reduced water exchange and invasive SAV meadows on diel changes of hydrography and community structure, study sites were haphazardly chosen in locations south of the causeway, where there are no impediments to water exchange, and just north of the causeway, where water exchange is limited (Fig. 1). Site selection within each location was also based on the presence of established *Myriophyllum spicatum* and *Vallisneria americana* beds. Chocalatta Bay (30.691° N, 87.983° W), located just north of the causeway, is best described as a broad shallow embayment, approximately 1 m deep, bound by emergent marsh vegetation on 3 sides. The other sites were located just south of the causeway to the west of Meaher Park (30.667° N, 87.975° W). This site is a broad shallow mud flat, approximately 1 m deep, that is bound to the north by the causeway and open to Mobile Bay to the south.

Environmental conditions

Abiotic parameters were monitored during this study to document the extent to which environmental conditions vary within the 2 habitat-forming SAV species at both sites. Measurements were taken continuously using YSI 6600 sondes. The YSIs were deployed in the vicinity (within 0.2 km) of the same established monotypic *M. spicatum* or *V. americana* beds that fish and invertebrate samples were taken from (see below). One YSI was placed at each site and in each SAV species and maintained routinely to download data, service and clean the instrument, and change batteries.

Instruments were mounted horizontally on a PVC frame, with the instrument resting on the benthos just above the sediment–water interface. Deployed instruments were programmed to record

water temperature (°C), specific conductance, turbidity (NTU), depth (m), DO (mg l⁻¹), and salinity, at 15 min intervals. Salinity was calculated from the recorded specific conductance and temperature using an algorithm and recorded at the same interval. Measurements were averaged over time intervals that faunal collections were made (10 August to 5 October 2012). Conditions were typical of the region for this time of year.

Faunal collection

We collected organisms in the SAV species at 2 locations (north and south of the causeway) and at 2 different time periods (day and night) to evaluate the potential effects of diurnal hypoxic conditions on fishes and macroinvertebrates in the MTD. Samples were collected in monotypic stands of *M. spicatum* and *V. americana* (Fig. 2) at each location, randomly selected within each bed and around the YSI instrument described above. We used a Venturi-driven suction sampler, based on the original design used by Orth & van Montfrans (1987), to collect organisms in each habitat. Samples were collected by rapidly placing a plastic cylinder over a 0.28 m² area of SAV (Valentine & Heck 1993). Once the cylinder was positioned, it was driven into the sediment to create a seal, thus preventing escape of organisms. Next, water was evacuated from the cylinder with the pump and passed through a 0.8 mm mesh collecting bag, thus retaining the fauna trapped within the cylinder. Collections were placed on ice and transported back to the Dauphin Island Sea Lab for processing. In the lab, collected organisms were identified to the lowest taxonomic level and counted.

Collections were made at haphazardly selected locations within each species of established SAV during the late summer/fall of 2012, a time of year when SAV densities and coverage is highest (Martin & Valentine 2012). Daytime collections were made in the afternoon, when DO levels were hypothesized to be highest. Nighttime collections were made within 2 h of sunrise, when DO levels were anticipated to be lowest, if in fact the plants exhausted the DO. A total of 48 samples were taken, equally distributed among one of 8 unique treatments (n = 6 for each SAV species in each diel period at each causeway location). Treatments included location (above or below the causeway), SAV species (*M. spicatum* or *V. americana*), and diel period (day or night).

Statistical analyses

Comparisons of the total abundance of collected organisms were made using a 3-way ANOVA with factors including causeway locations (above or below), SAV species (*M. spicatum* or *V. americana*), diel periods (day or night), and all interactions. A categorical subset of organisms with stronger affinities to the benthos (termed benthic organisms), and therefore more responsive and vulnerable to stratified, low DO events, was also analyzed separately using this technique. Assumptions (normality and homogeneity of variance) were tested and transformations conducted when violations occurred.

Treatment effects on environmental conditions were tested using the BIOENV analysis described below but not analyzed using the 3-way ANOVA because of the lack of true replication. We tested for differences in community structure, among treatments, using ANOSIM (square-root transformed, Bray-Curtis similarity), and species contributing to differences were identified using SIMPER tests generated with PRIMER v7 (PRIMER-E) software (Clarke & Warwick 2001). A biological–environmental (BIOENV) procedure, part of the BEST routine in the PRIMER-E package, was used to link environment variables (temperature, salinity, DO, turbidity) to faunal similarity data to identify abiotic variables, or combinations of variables, that covary with assemblage patterns. The normalized environmental similarity matrix was compared to the faunal assemblage similarity matrix based on combinations of environmental variables to maximize the rank correlation with the assemblage matrix. BIOENV calculates a Spearman's rank correlation coefficient (ρ, with high correlation = 1, low = 0) to assign covariance to specific environmental variables and combinations of variables, and variables with the highest correlations are reported (Whitman et al. 2004, Majewski et al. 2013).

RESULTS

Environmental conditions

With the exception of DO, most environmental variables differed little between sites or SAV species. Salinity, for example, was consistently low at all locations during the sampling period (1 to 2.5 PSU). Although the differences were small, temperature was consistently higher during the day in *Vallisneria americana* (average 28.3°C) than in *Myriophyllum spicatum* (25.7°C), likely due to shading by *M. spica-*

tum's canopy and higher structural complexity, leading to reduced sunlight reaching underlying waters.

DO varied greatly between macrophytes, study sites, and diel periods (Fig. 3). DO was consistently low in *M. spicatum*, with daytime measurements north of the causeway and concentrations for both diel periods below the causeway ranging between 1.8 and 4 mg l^{-1}. Above the causeway at night, however, average *M. spicatum* DO concentrations dropped to just 0.88 mg l^{-1}. In contrast, DO in *V. americana* was consistently above the threshold thought to impair nekton, with values consistently above 5 and reaching 10 mg l^{-1} during the day south of the causeway. In general, we found *M. spicatum* above the causeway to have extremely low DO (<2.3 mg l^{-1}) approximately 49.8% of the time and low DO (2.3 to 3.8 mg l^{-1}) 24.3% of the time compared to south of the causeway, which had extremely low DO 35.7% of the time and low DO 26.4% of the time. Conversely, *V. americana* had fewer extremely low DO events north (0.9% of the time) and south (5.9% of the time) of the causeway and fewer low DO events (6.1% north of the causeway and 7.8% south of the causeway).
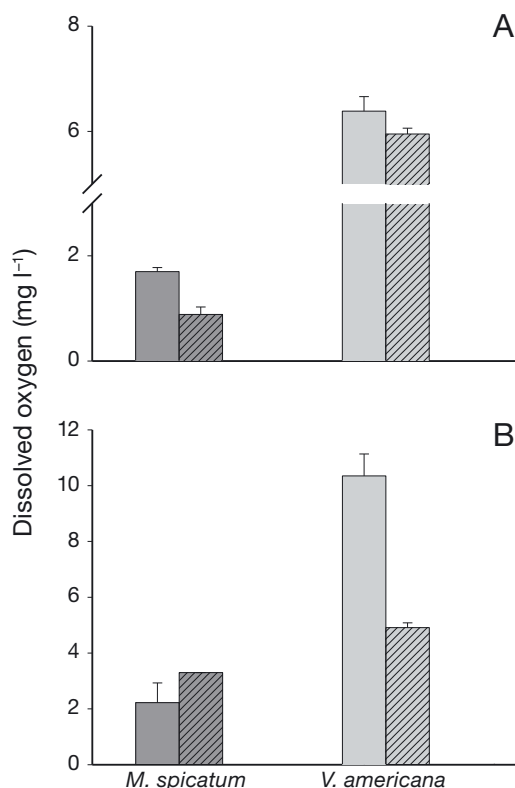

Fig. 3. Dissolved oxygen (mean ± SE) (A) above and (B) below the causeway in each submerged aquatic vegetation species (see Fig. 2) during the day (gray) and night (hatched)

Faunal collection

Total organism abundance (log-transformed) (Fig. 4A,B) varied significantly between SAV species ($F_{1,40} = 27.03$, p < 0.001) and locations ($F_{1,40} = 7.28$, p = 0.010) and varied marginally between diel periods ($F_{1,40} = 3.11$, p = 0.085). Significant interactions were detected for SAV species × location ($F_{1,40} = 7.60$, p = 0.009), and marginally significant interactions were detected for species × time × location ($F_{1,40} = 3.04$, p = 0.089). With one notable exception, total organism density (ind. m^{-2}) was consistently higher in *M. spicatum* (with densities approximately 5000 to 6000 m^{-2}) than in *V. americana*. Nocturnal densities in *M. spicatum* north the causeway, however, dropped to approximately 1000 ind. m^{-2}. Densities recorded in *V. americana* in both locations, and sampling periods, were approximately 1000 m^{-2} (Fig. 4).

Similarly, the density of benthic organisms varied significantly between SAV ($F_{1,37} = 20.11$, p < 0.001) and locations ($F_{1,37} = 21.7$, p < 0.001) and varied marginally between diel periods ($F_{1,37} = 3.28$, p = 0.078). Again, reductions in the densities of organisms in *M. spicatum* occurred at night above the causeway (from >200 m^{-2} during the day to <50 m^{-2} at night, Fig. 4C,D) but not elsewhere. Daytime densities of benthic organisms in *M. spicatum* above the causeway were also lower than in *V. americana* and *M. spicatum* south of the causeway (500 to 750 m^{-2}). Significant interactions were detected for species × location ($F_{1,37} = 33.49$, p < 0.001) and species × time × location ($F_{1,37} = 5.70$, p = 0.008), and marginal interactions were detected for time × location ($F_{1,37} = 3.56$, p = 0.067).

Changes in density among treatments coincided with important shifts in community structure. ANOSIM detected significant differences in the composition of small fish and invertebrate assemblages between causeway locations (R = 0.093, p = 0.016); therefore, separate subsequent comparisons were made in each area using 2-way crossed ANOSIM (Bray-Curtis, square root transformed), with SAV species and diel period as factors. North of the causeway, the composition of SAV inhabitants varied significantly between *M. spicatum* and *V. americana* (Fig. 4A; R = 0.656, p = 0.002) and diel period (Fig. 4A; R = 0.406, p = 0.003). Subsequent pairwise comparisons showed that while the composition of fishes and invertebrates did not vary significantly with diel period in *V. americana* above the causeway (R = 0.217, p = 0.102), the composition of these organisms in *M. spicatum* did vary significantly between diel periods (R = 0.596, p = 0.002). South of the cause-
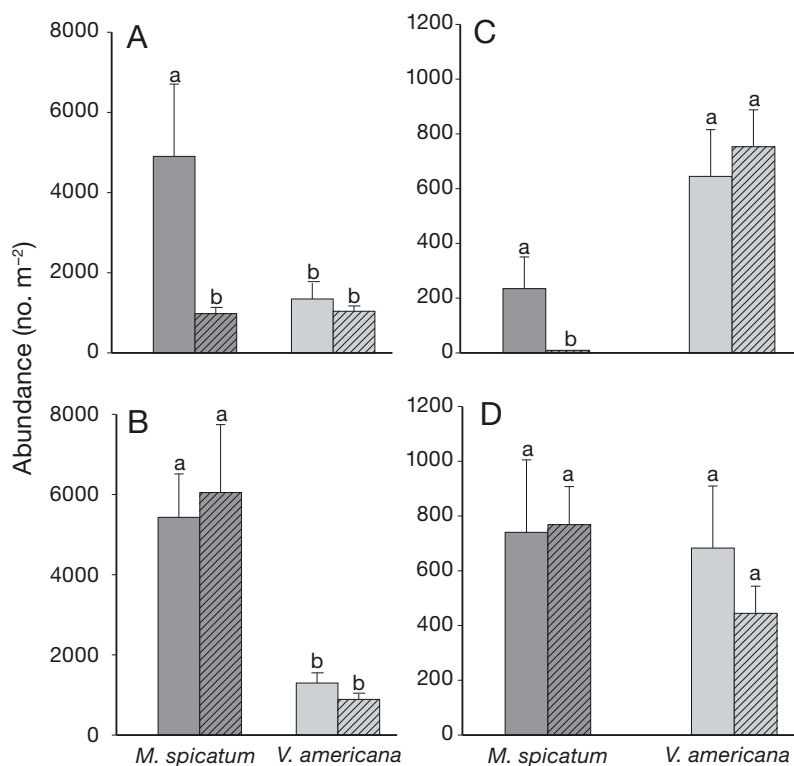
Fig. 4. Abundance (mean ± SD) of (A,B) total organisms and (C,D) benthic-associated organisms collected (A,C) above and (C,D) below the causeway in each submerged aquatic vegetation species (see Fig. 2) during the day (gray) and night (hatched). Different letters indicate statistically signficant differences (p < 0.05)

structure at night above the causeway (28%) than during the day (56%) or at any time south of the causeway (43 to 48%) (Table 2). Similar decreases were demonstrated by other amphipods such as *Grandidierella* sp. and *Corophium* sp. (Tables 1 & 2; Fig. 5). *Neritina usnea* snails also were less prevalent in collections in *M. spicatum* during the night above the causeway, contributing around 11% to community structure during the day to 6% at night (Table 2). Faunal decreases in *M. spicatum* above the causeway at night were also apparent for abundant organisms such as mysid shrimps (*Taphromysis* sp., *Mysidopsis* sp.) and *C. sapidus*.

While the abundance of many organisms decreased at night in *M. spicatum* above the causeway, other organisms increased in abundance. These included insects of the order Ephemeroptera and family Coenagrionidae, *Palaemonetes* grass shrimp, *Physella* sp. snails, and rainwater killifish *Lucania parva* (Tables 1 & 2; Fig. 5).

way, a significant shift in community structure was also detected between *V. americana* and *M. spicatum* communities (Fig. 4B; R = 0.768, p = 0.001); however, no differences in assemblages were found with diel period (Fig. 4B; R = −0.01, p = 0.466).

These shifts in community structure were driven primarily by changes in the abundance of several key organisms (Fig. 5). Species such as blue crabs *Callinectes sapidus* and some amphipods (*Grandidierella* spp. and *Corophium* sp.) were more abundant in *V. americana* than in *M. spicatum*. In *M. spicatum*, community composition was largely dominated by the amphipod *Gammarus* spp. and aquatic insects. Diurnally, the abundance of these gammarid amphipods, the most abundant of all organisms collected, declined significantly in *M. spicatum* at night above the causeway (Tables 1 & 2; Fig. 5). Abundance in *M. spicatum* above the causeway decreased from a mean of over 4000 amphipods m^{-2} during the day to just 400 at night. No such decline was detected in *M. spicatum* south of the causeway, where densities were consistently around 4000 m^{-2} in both day and night-comparisons. SIMPER analysis confirmed that amphipods contributed less to *M. spicatum* community

Relationship between faunal communities and environmental conditions

The BIOENV analysis indicated that a single environmental variable, DO (ρ = 0.386), provided the best explanation for the variance in community structure (Table 3). Combinations of DO, temperature, and salinity explained even less variance in the data (ρ ranging from 0.178 to 0.344). The combination of DO and temperature (ρ = 0.344) and DO and salinity (ρ = 0.318) produced the subsequent best explanations for faunal distribution patterns. As a result, we suggest that of all the environmental variables we documented, these communities are most heavily influenced by changes in DO among the different macrophytes and locations.

DISCUSSION

While a number of disturbances such as oil spills, high-intensity storm events, and overfishing have affected GOM estuarine communities (Palaseanu-Lovejoy et al. 2013, Able et al. 2015, McCann et al.
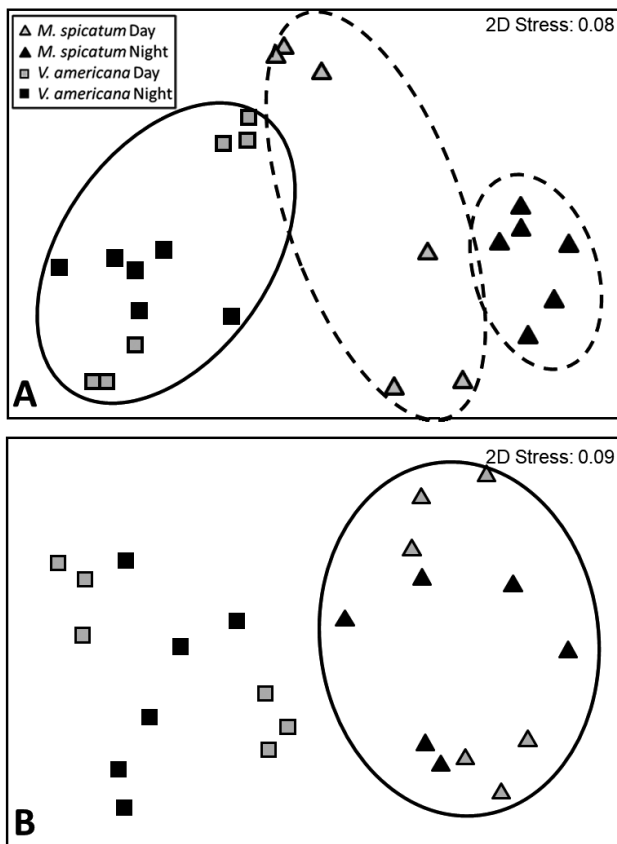
Fig. 5. Nonmetric multidimensional scaling plot illustrating changes in community structure in beds of 2 submerged aquatic vegetation (SAV) species (*Myriophyllum spicatum*, *Vallisneria americana*) (A) above and (B) below the causeway. Solid ellipses indicate significant differences in SAV species, and dashed ellipses indicate significant differences in diel period

2017), changes to freshwater inflow quality and quantity are among the most widespread. Hydrological alterations have modified the structure and function of numerous estuarine ecosystems throughout the southeastern United States, including the Mississippi River system (Rabalais et al. 2002), the Apalachicola River basin (Havens et al. 2013), and the Florida Everglades (Fennema et al. 1994). In the MTD, previous research has documented that the area north of the manmade causeway, a hydrological barrier, experiences fewer extreme physical forces, both in terms of maximum wave energy and mean current velocity, than do areas south of the causeway (Martin & Valentine 2012). This causeway also forms an impediment to water mixing and creates more opportunity for stratification, leading to hypoxic events. Additionally, the presence of this causeway has been shown to have altered biological production and nutrient exchange between the upstream water-

Table 1. Densities (mean ± SE; ind. m⁻²) of the most abundant organisms found in each area, submerged aquatic vegetation species (see Fig. 2), and diel period

| Species | Above causeway | | | | Below causeway | | | |
	M. spicatum Day	*M. spicatum* Night	*V. americana* Day	*V. americana* Night	*M. spicatum* Day	*M. spicatum* Night	*V. americana* Day	*V. americana* Night
Gammarus spp.	4226.8 ± 1457.1	401.8 ± 39.3	431.6 ± 166.1	138.1 ± 46.5	3995.8 ± 795.0	4844.1 ± 1508	323.2 ± 33.0	231.0 ± 69.8
Grandidierella sp.	191.7 ± 96.3	1.2 ± 0.69	554.2 ± 177.0	559.5 ± 81.0	683.3 ± 225.8	704.8 ± 119.2	634.5 ± 206.3	402.4 ± 84.2
Taphromysis sp.	247.0 ± 98.8	8.3 ± 2.7	104.8 ± 43.8	6.6 ± 2.1	11.9 ± 3.6	40.5 ± 12.7	52.4 ± 25.2	22.6 ± 8.1
Neritina usnea	54.2 ± 11.7	20.8 ± 3.5	78.0 ± 28.2	88.1 ± 8.7	26.2 ± 3.0	29.8 ± 9.1	81.0 ± 17.4	92.9 ± 14.8
Corophium sp.	5.4 ± 4.3	0.0 ± 0.0	60.1 ± 26.2	158.9 ± 47.6	7.1 ± 3.7	1.8 ± 1.1	14.9 ± 4.1	10.1 ± 3.8
Ephemeroptera	1.8 ± 1.11	155.4 ± 29.7	0.0 ± 0.0	0.0 ± 0.0	80.4 ± 34.5	9.5 ± 4.0	4.2 ± 2.4	0.0 ± 0.0
Coenagrionidae	24.4 ± 8.2	127.4 ± 18.3	1.2 ± 0.7	3.0 ± 2.1	55.4 ± 24.3	63.1 ± 32.5	4.8 ± 1.6	5.4 ± 2.3
Littoridinops sp.	40.5 ± 32.5	87.5 ± 32.4	0.0 ± 0.0	1.2 ± 0.7	50.0 ± 21.9	48.8 ± 20.9	28.0 ± 14.6	10.7 ± 5.8
Palaemonetes spp.	7.1 ± 2.2	47.6 ± 13.2	16.7 ± 5.7	19.1 ± 6.3	20.2 ± 7.4	16.1 ± 5.8	8.3 ± 1.8	17.9 ± 6.4
Lucania parva	19.1 ± 8.5	27.4 ± 4.7	0.0 ± 0.0	0.0 ± 0.0	33.3 ± 21.1	25.0 ± 13.1	0.0 ± 0.0	0.0 ± 0.0
Mytilopsis leucophaeata	23.8 ± 8.6	8.3 ± 2.7	11.3 ± 4.6	1.2 ± 0.7	33.9 ± 10.9	52.4 ± 11.7	12.5 ± 4.4	1.8 ± 0.7
Mysidopsis sp.	15.5 ± 7.0	7.1 ± 5.9	7.1 ± 5.3	13.7 ± 7.3	256.0 ± 132.6	72.0 ± 38.0	8.3 ± 2.7	17.9 ± 9.4
Physella sp.	3.0 ± 1.0	40.5 ± 19.1	0.0 ± 0.0	0.0 ± 0.0	28.0 ± 13.5	7.7 ± 2.3	1.2 ± 1.1	0.6 ± 0.5
Callinectes sapidus	8.3 ± 3.0	0.0 ± 0.0	12.5 ± 2.8	15.5 ± 3.9	3.6 ± 2.2	6.6 ± 2.0	10.1 ± 4.0	7.7 ± 2.6
Diptera	7.1 ± 1.7	6.6 ± 4.8	7.1 ± 3.7	0.0 ± 0.0	19.1 ± 4.7	31.6 ± 8.4	2.4 ± 1.1	3.0 ± 1.3
Melita sp.	0.0 ± 0.0	0.0 ± 0.0	15.5 ± 5.9	0.0 ± 0.0	44.0 ± 27.1	16.1 ± 6.7	77.4 ± 33.4	28.6 ± 14.2

Table 2. SIMPER analysis. The species contributing most to the assemblage structure in each habitat (area, submerged aquatic species [*Myriophyllum spicatum*, *Vallisneria americana*], diel period) are shown. Numbers indicate percent contribution to the Bray-Curtis similarity index. (−) no contribution

| Species | Above causeway | | | | Below causeway | | | |
| | *M. spicatum* | | *V. americana* | | *M. spicatum* | | *V. americana* | |
	Day	Night	Day	Night	Day	Night	Day	Night
Gammarus spp.	56.09	27.9	19.29	12.98	47.57	43.29	26.5	21.14
Neritina usnea	10.99	5.61	13.06	16.22	4.37	4.04	12.18	14.78
Taphromysis sp.	6.92	−	5.22	−	−	4.39	2.89	4.73
Coenagrionidae	5.63	14.84	−	−	3.15	2.87	−	−
Grandidierella sp.	4.69	−	29.48	37.53	16.59	21.02	25.71	29.17
Lucania parva	2.74	6.17	−	−	−	−	−	−
Diptera larvae	2.67	−	−	−	3.06	3.7	−	−
Mytilopsis leucophaeata	2.3	−	−	−	3.3	4.9	−	−
Corophium sp.	−	−	8.07	14.28	−	−	4.69	2.99
Callinectes sapidus	−	−	6.43	5.66	−	−	2.17	3.79
Palaemonetes spp.	−	6.23	4.31	4.31	2.23	2.34	3.81	3.69
Melita sp.	−	−	2.38	−	−	−	5.36	5.27
Ephemeroptera	−	15.14	−	−	2.69	−	−	−
Littoridinops sp.	−	9.04	−	−	2.72	2.48	−	−
Physella sp.	−	2.79	−	−	−	−	−	−
Caenidae	−	2.7	−	−	−	1.69	−	−
Mysidopsis sp.	−	−	−	−	2.77	−	−	2.42
Mulinia sp.	−	−	−	−	2.34	−	−	−
Edotia montosa	−	−	−	−	−	−	3.36	−
Rhithropanopeus harrissi	−	−	−	−	−	−	2.25	3.91
Trichoptera	−	−	−	−	−	−	2.18	−

Table 3. Correlation coefficients from the BIOENV analysis demonstrating the relationship between environmental variables and the biological similarity matrix. DO: dissolved oxygen

No. of variables	Correlation coefficient	Selection(s)
1	0.386	DO
2	0.344	Temperature, DO
2	0.336	Salinity, DO
3	0.318	Salinity, temperature, DO
3	0.256	Salinity, DO, depth
3	0.25	Temperature, DO, depth
2	0.237	DO, depth
1	0.23	Salinity
2	0.182	Salinity, temperature
2	0.178	Salinity, depth

shed and downstream estuary (Goecker et al. 2009). Despite the small differences in salinity, faunal communities south of the causeway are generally dominated by estuarine residents, while freshwater and brackish organisms dominate the structure of faunal SAV assemblages north of the causeway, presumably the result of restricted recruitment due to this hydrological barrier (Rozas et al. 2013, this study).

Here, we documented the nocturnal occurrence of low DO events in *Myriophyllum spicatum* beds in the area north of the causeway. While DO concentrations were generally low in *M. spicatum* above the causeway during day (~1.8 mg l^{-1}), they were less than half of that at night (~0.8 mg l^{-1}). Plant and microbial respiration, coupled with the lack of mixing, likely leads to these patterns. In comparison, the native *Vallisneria americana*, which has a less expansive canopy and most often has less aboveground biomass (Martin & Valentine 2012), maintained high levels of DO in each area. The increase in nightly incidences of extremely low DO in *M. spicatum* north of the causeway leads to substantial changes to the shelter-seeking nocturnal community that develops in *M. spicatum*. These changes suggest that there are important ecological consequences that can only be detected by sampling at night, and future evaluations of invasive species should also consider diel variation in ecosystem services.

Many studies have indicated that organisms can develop strong behavioral responses to hypoxia and will actively avoid areas of low DO (Kramer 1987, Breitburg 2002). In transects conducted in the Chesapeake Bay, Ludsin et al. (2009) reported that bay anchovies *Anchoa mitchilli* reduced foraging time in benthic waters when hypoxia was present and spent more time in well-oxygenated surface waters. Likewise, sampling across multiple DO conditions in the Neuse River estuary, North Carolina, demonstrated that increasing pinfish *Lagodon rhomboides*, bay

anchovies *A. mitchilli*, and flounder *Paralichthys dentatus* and *P. lethostigma* abundance was correlated to higher DO concentrations, with benthic organisms such as blue crabs *Callinectes sapidus* and flounders demonstrating the strongest avoidance response (Bell & Eggleston 2005). In the GOM, the dead zone that develops seasonally at the terminus of the Mississippi River routinely experiences DO concentrations below 2 mg l^{-1} (Rabalais et al. 2002), and trawling surveys indicate that many mobile organisms, including shrimp and finfishes, aggregate along the edges of the most hypoxic waters (Craig 2012). In addition, experiments giving common representatives of southeastern United States estuarine fauna (including *Leiostomus xanthurus*, *L. rhomboides*, *Micropogonias undulatus*, *Brevoortia tyrannus*, *Mugil curema*, *Fundulus heteroclitus*, *Farfantepenaeus aztecus*) a choice between high and low DO revealed that all fauna can detect, and avoid, concentrations of DO of 1 mg l^{-1} (Wannamaker & Rice 2000), although some organisms may exhibit acclimatization seasonality in their tolerance to such settings (Love & Rees 2002). Our findings agree with these previous studies in that many organisms found in *M. spicatum* during the day north of the causeway or during any period south of the causeway respond by avoiding low DO areas. These include organisms such as blue crabs *C. sapidus*, which decreased from over 8 m^{-2} during the day to 0 at night in *M. spicatum* north of the causeway (Table 1). Somewhat surprisingly, organisms with limited mobility also tended to avoid *M. spicatum* when it became hypoxic, including amphipods *Gammarus* spp. and *Grandidierella* sp., gastropods *Neritina usnea*, and crustaceans *Taphromysis* sp. (Table 1).

Some organisms, however, exhibited an unexpected trend with increases in density in *M. spicatum*'s hypoxic waters. These include some insect larvae (Ephemeroptera and Coenagrionidae) which use specialized breathing structures to take advantage of atmospheric oxygen, crustaceans such as *Palaemonetes* spp., air-breathing freshwater snails such as *Physella* sp., and rainwater killifish *Lucania parva*, which has morphological adaptations to utilize the well-oxygenated waters at the surface. In these instances, we hypothesize that these organisms may be occupying *M. spicatum* as a refuge from predators or taking advantage of adaptations to forage in such environments. Previous research has demonstrated that other fish, such as fathead minnows *Pimephales promelas*, utilize sublethal hypoxic conditions as a refuge from predators such as yellow perch *Perca flavescens* (Robb & Abrahams 2002). Jellyfish polyps

settling in the northern GOM dead zone exhibit similar increases in survival (Miller & Graham 2012). Other studies have indicated yellow perch (Roberts et al. 2012) and central mudminnows *Umbra limi* (Rahel & Nutzman 1994) may engage in short-term foraging forays into hypoxic waters, perhaps to feed on prey that have reduced movement, a common response to low DO conditions (Kramer 1987).

Traditional evaluations of effects from invasive species, such as *M. spicatum* and other habitat-forming species, have often failed to account for diel variability in associated environmental metrics as well as faunal presence. While we know from various marine, estuarine, and freshwater ecosystems that nighttime can be very important for many organisms (reviewed in Hammerschlag et al. 2017), this work suggests that failing to account for this variability may lead to erroneous conclusions. Here, community structure in *M. spicatum* was dependent on diel hypoxic patterns that are triggered by the lack of water movement impeded by hydrological modification and suggests that restoration of natural flow patterns may not only deter *M. spicatum* colonization and proliferation (Martin & Valentine 2012) but also prevent hypoxia that develops in these habitats, which may also impact adjacent areas.

We acknowledge a number of shortcomings in the current study and suggest that these knowledge gaps be prioritized in future research efforts. Due to constraints on instrumentation, we were only able to place 1 continuous monitoring device in each area/ SAV species. A greater understanding of the spatial extent of *M. spicatum*-induced hypoxia could contribute significantly to a better understanding of the landscape-level impacts of these events. Sampling using the suction technique used here, while quantitative, integrates collection of organisms over the entire water column. Important variability in the use of the stratified water column may reveal additional complexity in the response to hypoxic conditions. Nevertheless, this study provides an important first step in understanding the role of estuarine SAVs on a diel basis as well as how anthropogenic impacts in the form of hydrological modifications may alter the productivity of these ecosystems.

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