Characterization and ecological implication of eelgrass life history strategies near the species' southern limit in the western North Atlantic

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ABSTRACT: Eelgrass *Zostera marina* L. populations located near the species southern limit in the western North Atlantic were assessed monthly from July 2007 through November 2008. We identified (1) dominant life history strategies and local environmental conditions in southern *Z. marina* populations, (2) quantified differences in reproductive phenology between populations and different local environmental conditions, and (3) compared reproductive strategies to established annual and perennial life history paradigms. Observed populations expressed both life history strategies with one *Z. marina* population completely losing aboveground biomass and reestablishing from seeds (annual model) while another population retained aboveground biomass throughout the year (perennial model). A third life history strategy, characterized here as a mixed-annual population, was also observed after some seedlings were found to reproduce both sexually and asexually during their first year of growth thereby not conforming to any currently established life history paradigm. Development of multiple life history strategies within this region may be in response to stressful summer water temperatures associated with the southern edge of the species' range. We suggest that neither annual nor perennial life history strategies always provide a superior mechanism for population persistence as perennial populations can be susceptible to multiple consecutive years of stress, and annual populations are unable to fully exploit available resources throughout much of the year. The mixed-annual strategy observed here represents another possible life history model which may provide the mechanism necessary for Z. *marina* populations to persist during times of environmental transition.

KEY WORDS: *Zostera marina* · Life-history · Annual · Perennial · Seed bank · Biomass

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INTRODUCTION

Eelgrass *Zostera marina* is circumglobally distributed, ranging in latitude from warm temperate waters to near arctic conditions (Setchell 1929, den Hartog 1970, Green & Short 2003, Moore & Short 2006). *Z. marina* displays a tolerance to wide ranging temperature and salinity conditions and has distinct life history strategies allowing the species to exploit intertidal to subtidal zones and from open ocean environments to the interior of brackish water estuaries (Setchell 1929, Harrison 1993, Meling-López & Ibarra-Obando 1999, van Katwijk & Wijgergangs 2004).

The most common life-history strategy observed in *Zostera marina* populations is the perennial form where plants persist for several years primarily through asexual growth (den Hartog 1970; Tomlinson 1974; Lincoln et al. 1990). Perennial Z. *marina*

populations have also been documented to express behavior similar to the biennial life history model (Setchell, 1929). After germinating, seedling growth in biennial populations occurred via clonal expansion only and flowering stem development and fruiting were not observed until after a period of 1 to 2 yr of growth (Setchell 1929; Thayer et al. 1984). More recently an annual life history strategy has been described for *Z. marina* populations, where mature plants consist of reproductive (flowering) shoots only and all plants complete their life cycle (seeds germinate, flower, produce seeds) and die within 12 mo (Keddy & Patriquin, 1978, Gagnon et al. 1980, De Cock 1981, Harlin et al. 1982, Phillips et al. 1983a, Robertson & Mann 1984, Santamaría-Gallegos et al. 2000).

Although they have been documented throughout the species' geographic distribution, annual forms of *Zostera marina* are primarily found in areas characterized by stressful environmental conditions such as ice scour (Robertson & Mann 1984), extreme temperatures (Phillips et al. 1983a, Santamaría-Gallegos et al. 2000), and interactions between physical disturbances such as grazers and strong storms (van Lent & Verschuure 1994). Phillips et al. (1983a) suggested that the large annual population in the Gulf of California may have developed in response to recurring high summer water temperatures experienced at the southern limit of the species distribution. At Bahia Kino, where the most extensive *Z. marina* meadows in the Gulf of California occur, water temperatures reach 32°C in July, resulting in 100% mortality of *Z. marina* shoots (Phillips & Backman 1983). In this meadow, the entire population is sustained year to year by the production of seeds from annual plants (Meling-López & Ibarra-Obando 1999).

In the western Atlantic, the southern limit of *Zostera marina* distribution occurs in North Carolina where there is an estimated 80 937 ha of submerged aquatic vegetation including *Z. marina*, widgeon grass *Ruppia maritima* and shoal grass *Halodule wrightii* (Street et al. 2005). With the warm climate and proximity of the Gulf Stream, temperatures in North Carolina coastal waters reach ≥30°C in summer and mortality of *Z. marina* is high during this period (Thayer et al. 1984). *Z. marina* dominated meadows lose most of their aboveground biomass by early fall in response to the high thermal stress experienced at this southerly latitude (Kenworthy 1981, Thayer et al. 1984). Flowering frequency in the population is also relatively high (11 to 32%; Thayer et al. 1984, Fonseca et al. 1985) and seed-

lings are ubiquitous throughout the system (authors' pers. obs.). Long-term observations in seagrass meadows in Back and Core Sounds in Carteret County, North Carolina suggest that there are both perennial and annual populations (K. Kenworthy pers. obs.). Recent observations (authors' pers. obs.) in a well studied site in the Newport River Estuary (Thayer et al. 1977, 1984, Penhale 1977) indicated that a formerly perennial *Z. marina* meadow was expressing characteristics of an annual population: 100% shoot mortality was observed in the early fall (October to November) of 2004, 2005, and 2006, yet each year the meadow was reestablished by seedlings (authors' pers. obs.). Although the occurrence of annual populations at the southern limit in the Atlantic does not seem as prevalent as in the Pacific populations in the Gulf of California, given the similarities of thermal stressors between these geographical regions there is reason to predict that *Z. marina* populations in North Carolina should benefit from a strategy with a higher proportion of sexual reproduction.

It is the goal of this paper to (1) investigate dominant life history strategies and local environmental conditions in southern western Atlantic *Zostera marina* populations; (2) quantify differences in reproductive phenology (biomass and density of vegetative and reproductive shoots, total seed production, total seed bank density, and viable seed bank density) between observed life history strategies; and (3) compare reproductive strategies within these thermally stressed populations to established life history paradigms.

MATERIALS AND METHODS

Study sites

We selected 2 sites in North Carolina for our study (Fig. 1). Phillips Island (NC1; 34° 43′ N, 76° 41′ W) is located on a shallow small semi-enclosed shelf in the Newport River Estuary in Carteret County. The seagrass bed is dominated by *Zostera marina* with small isolated patches of *Ruppia maritima.* Morgans Island (NC2, 34° 66′ N, 76° 52′ W), located ~14 km southeast of Phillips Island in Back Sound on a shallow open shelf, is a mixed bed of *Z. marina* and *Halodule wrightii* with a minor amount of *R. maritima.* Both sites are shallow, with mean lower low water (MLLW) depths <0.25 to 0.5 m (Penhale 1977, Thayer et al. 1977). Within each site a 30 × 30 m area was delineated for sampling.
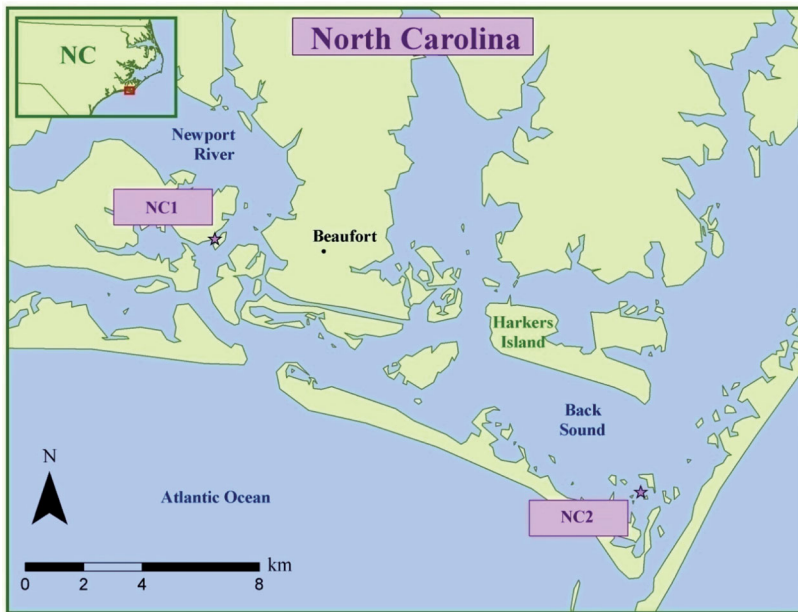
Fig. 1. Sampling sites (★; NC1, NC2) for study of eelgrass *Zostera marina* life history strategies in North Carolina

Study site characterization

Bottom water temperature (°C) was recorded at each site every 15 min throughout the study period with 3 HOBOware Pro (Onset Computer) water temperature sensors deployed on the sediment surface. Salinity (PSU) and dissolved oxygen (DO; mg l^{-1}) were measured monthly from August 2007 to October 2008 with a Yellow Spring Instruments model 650 sonde. During each site visit, three 500 ml water samples were collected by hand, filtered (Gelman Supor, 0.45 µm), and frozen until analyzed for dissolved inorganic nitrogen (DIN; NO_x, NH_4^+) and dissolved inorganic phosphorus (DIP; PO_4^{3-}) with a Lachat Instruments auto analyzer (Liao 2002, Knepel & Bogren 2002, Smith & Bogren 2002). Water samples were also filtered and analyzed for chlorophyll *a* (Strickland & Parsons 1972) and total suspended solids (TSS). TSS was quantified from a well-mixed sample of known volume. The sample was filtered through a GF/F filter and the residue retained on the filter was dried to constant weight at 103 to 105°C and reported as mg l^{-1}.

At each site, 5 sediment cores (10.4 cm diameter, 10 cm depth) were collected in July and December 2007 and in June and September 2008 to quantify organic content and sediment exchangeable nutrients. The upper 6 cm of the core was removed then subdivided. Percent organic matter was determined by drying a sediment sub-section at 60°C until a constant dry weight (DW) was reached. Samples were then weighed, combusted at 500°C for 5 h, and weighed again. Percent organic matter was calculated as the difference in weights (Erftemeijer & Koch 2001). Sediment exchangeable nutrients were extracted with a volume KCl (2 M) equal to twice the sediment volume, shaken on a rotary shaker for 1 h at room temperature, centrifuged 6 min at 1252 g, filtered (Gelman Supor, 0.45 µm), and frozen in sterile Whirlpak® bags until analyzed. NH_4^+ was determined by the technique of Solorzano (1969). DIN (NO_x) and DIP (PO_4^{-3}) were determined on a Lachat Instruments auto analyzer (Liao 2002, Knepel & Bogren 2002, Smith & Bogren 2002). Additionally, in December 2007, percent sand, silt, and clay fractions were determined using a wet sieve method (Plumb 1981).

Zostera marina characterization

Five *Zostera marina* biomass cores (22 cm diameter, 10 cm depth) were haphazardly collected monthly from both NC1 and NC2 from July 2007 through November 2008. Samples were sieved (1.0 cm mesh) and washed clean of sediment in the field and all plant material was immediately transported back to the lab for processing. Biomass samples were sorted as reproductive shoots or non-reproductive shoots, and as surviving shoots or newly germinated seedlings. Reproductive shoots were defined as erect shoots that contained multiple rhipidia (De Cock 1981) and seedlings were determined by the presence of a seed coat or by a curved rhizome base (Setchell 1929, Taylor 1957b). Vegetative shoot density, reproductive shoot density, and the number of seeds per reproductive shoot were recorded. Vegetative shoots were then separated from the rhizome directly below the leaf sheath into aboveground and belowground biomass. Only living belowground biomass, whitish to light brown in color and attached to shoots, was processed. Belowground biomass was analyzed as total belowground biomass and not divided by reproductive state because some plants produced both vegetative and reproductive shoots. All biomass samples were dried at 60 °C to a constant weight.

Seed production and seed bank characteristics

The maximum potential number of seeds produced at each site was calculated monthly from August 2007 through October 2008 as the product of the average number of seeds per reproductive shoot and the average number of reproductive shoots m^{-2} (van Lent & Verschuure 1994). At each site, 5 sediment cores (4 cm diameter, 10 cm length) were collected in July and December 2007 and again in June, September, October and November 2008 to quantify total and viable sediment seed bank densities. All cores were wet-sieved (0.5 mm mesh) to separate the seeds; all seeds were counted and stored overnight in ambient seawater at 4°C. The percentage of seeds retained within the sediment seed bank was quantified using the difference between the potential and measured seed bank densities.

Viability of all collected seeds was tested using tetrazolium staining (Lakon 1949, AOSA 1981, McFarland & Shafer 2011). Seed embryos were removed from their seed coats and soaked in a 1% tetrazolium chloride solution for 24 h before examination on a dissecting scope at 10× magnification (Lakon 1949, AOSA 1981, Conacher et al. 1994). Seeds with a pink to brown stained cotyledon and axial hypocotyl were considered viable (Taylor 1957a, Harrison 1993). The percentage of viable seeds retained within the sediment seed bank was quantified compared to the total number of seeds collected in the seed bank at each site.

Statistical analyses

Water column TSS and sediment organic content and percentages of sand, silt and clay between sites and over time were analyzed separately with nonparametric statistics as the data were non-normally distributed (TSS and sediment organic content: Friedman's Chi Square; percent sand, silt and clay: Kruskal-Wallis and Kolmogorov-Smirnov Tests; SAS System for Windows). Monthly averaged water column temperature, salinity, DO, chlorophyll a, DIN, DIP and sediment DIN and DIP data were transformed when necessary and analyzed with repeated measures ANOVA to compare the effects of time, site and the interactions of these factors (Zar 1996).

Repeated measures ANOVA (PROC GLM; SAS System for Windows) was employed to test the effects of site and time on vegetative and reproductive shoot aboveground biomass, total belowground biomass, the proportion of reproductive shoots, and the proportion of viable seeds in the seed bank. Prior to analysis, data were transformed when necessary (biomass: square root transformation; proportion data: arcsine square root transformation), normality was confirmed, and homogeneity of variance was verified with Cochran's test (Zar 1996).

Differences in vegetative and reproductive shoot density, seed production, and seed bank density between sites were analyzed using negative binomial regression with time and site as factors (PROC GENMOD; SAS System for Windows) (Allison 1999). Negative binomial regression is a generalized form of Poisson regression which corrects for overdispersion in count data (Allison 1999). For all significant ($p < 0.05$) model terms odds ratios were calculated using Wald chi square statistics (SAS System for Windows). Likelihood ratio tests for all parameter estimates were also calculated and compared to the Wald Chi Square Statistics (Allison 1999).

RESULTS

Study site characterization

Water column temperature varied significantly over time across both sites ($p < 0.001$). Temperature was significantly greater at NC1 than NC2 during the spring and summer months ($p < 0.001$; Fig. 2A). Daily temperature (mean ± SE) ranged from $7.2 ± 0.1°C$ to $30.3 ± 0.1°C$ at NC1 and from $4.8 ± 0.1°C$ to $29.8 ± 0.1°C$ at NC2. When comparing temperatures collected every 15 min across the summer months (June to August), temperatures were above 25°C for 88.5% and 93.1% of the time at NC1 and NC2, respectively (Fig. 3).

Water column DO and salinity varied significantly over time across both sites ($p < 0.001$ for all) and were significantly greater at NC2 than NC1 ($p < 0.001$; Fig. 2B, C). Water column chlorophyll a (chl a) was significantly greater at NC1 than NC2 ($p < 0.001$) and va ried significantly over time with highest values observed during summer months ($p < 0.001$; Fig. 2D). TSS followed a similar pattern to chlorophyll a and was significantly greater at NC1 than NC2 ($p < 0.001$; Fig. 2E). Water column NO_x and NH_4^+ were similar between sites (NO_x p = 0.369; NH_4^+ p = 0.099) but were significantly different over time although no distinct seasonal patterns among years were evident (NO_x p = 0.042; NH_4^+ p = p < 0.001; Fig. 2F, G). Water column PO_4^{3-} was also significantly greater at NC1 ($p = 0.008$) and differed significantly over time with lowest values in early winter ($p = 0.008$; Fig. 2H).

Sediment organic content and percentages of sand, silt and clay were the only sediment characteristics
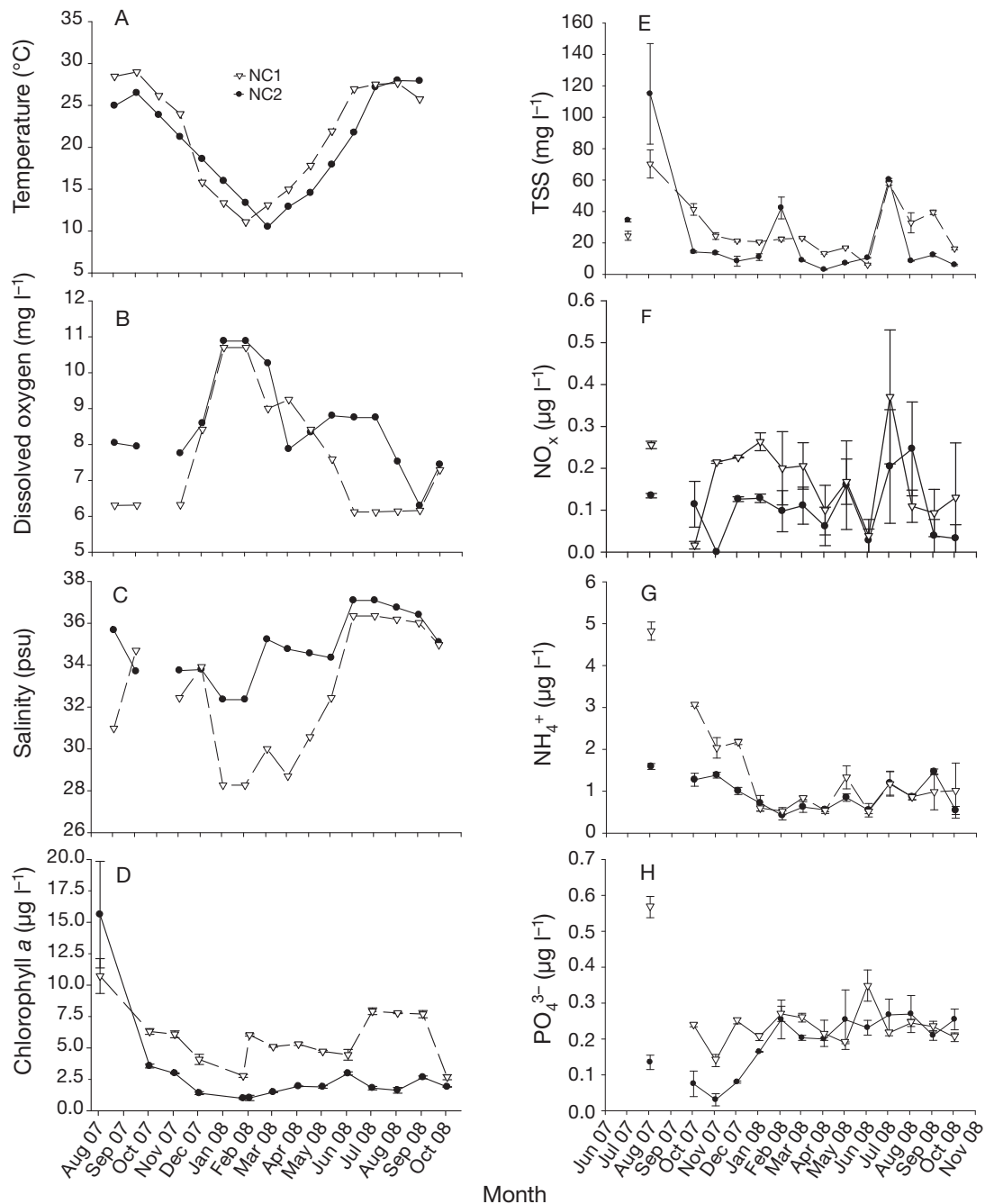
Fig. 2. Monthly values (mean ± SE; n = 3) at 2 sites (NC1 and NC2) in North Carolina for (A) water temperatures (°C); (B) dissolved oxygen (mg l^{-1}); (C) salinity (psu); (D) chlorophyll *a* (µg l^{-1}); (E) total suspended solids (TTS; mg l^{-1}); (F) NO$_x$ (µg l^{-1}); (G) NH$_4^+$ (µg l^{-1}); and (H) PO$_4^{3-}$ (µg l^{-1}). Note differences in *y*-axes

to differ significantly between sites. NC1 had finer grained sediment composition (silt p = 0.004; clay p = 0.007; Table 1) and significantly greater sediment organic content (p < 0.001) than NC2. Organic content did not change significantly over time (p = 0.789). Sediment NH$_4^+$ (p = 0.069) and PO$_4^{3-}$ (p = 0.887) did not differ significantly between sites but did differ significantly over time (p < 0.001 for both).

Zostera marina characterization

There were no perennial plants at NC1 and all *Zostera marina* shoots originated from seedlings. At NC2, perennial plants surviving through summer contributed to population growth in the fall and in the next spring. There was no significant difference in vegetative aboveground biomass between sites (p =
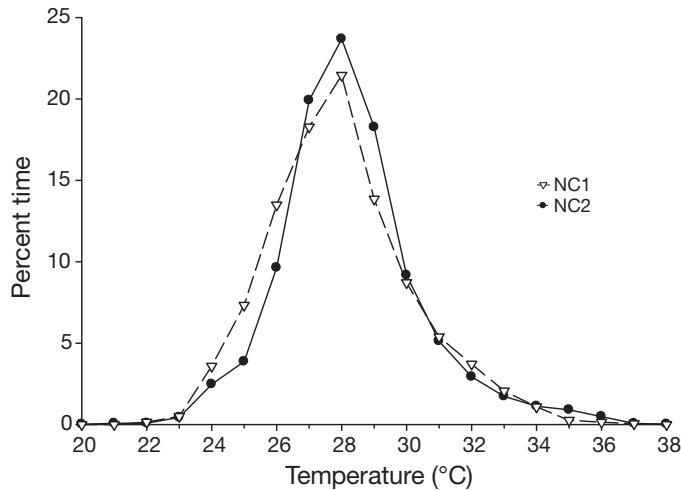
Fig. 3. Frequency distribution of 15-min water temperature measurements at 2 sites (NC1 and NC2) in North Carolina during June, July, and August 2008

Belowground biomass was significantly greater at NC2 compared to NC1 (p < 0.001) and varied significantly over time (p < 0.001; Table 2B; Fig. 4C). Belowground biomass was completely absent November 2007 and reached a seasonal low in November 2008 at NC1 while reaching a seasonal

0.648; Table 2A), although there was a difference over time (p < 0.001; Table 2A). Maximum aboveground vegetative biomass for both sites occurred in July in both 2007 and 2008 (Fig. 4A). Aboveground vegetative biomass (all values mean ± SE) was completely absent from NC1 in November 2007 and again in September and October 2008 while biomass decreased to the lowest recorded value, 13.80 ± 5.00 g DW m^{-2}, in October 2007 at NC2 (Fig. 4A).

Reproductive aboveground biomass at both sites varied over time (p < 0.001; Table 2A), reaching a maximum in March 2008 then decreasing until completely disappearing in July 2008 (Fig. 4B). Total reproductive shoot biomass and the proportion of reproductive to total shoot biomass was significantly greater at NC1 (maximum 30.93 ± 2.41 g DW m^{-2}) than NC2 (maximum 15.66 ± 2.93 g DW m^{-2}; p = 0.003; Table 2A).

Table 2. *Zostera marina.* Results of repeated measures, ANOVA for biomass of (A) vegetative and reproductive shoots aboveground and (B) total belowground for 2 sites in North Carolina over the 2007–2008 growing season. *p < 0.05

Factor	df	Type III SS	Mean SS	*F* value	p
(A) Aboveground Vegetative					
Between Sites					
Site	1	0.11	0.11	0.22	0.648
Error (site)	8	4.04	0.51		
Within Sites					
Time	15	291.19	19.41	31.58	<0.001*
Time × Site	15	56.92	3.79	6.17	0.003*
Error (time)	120	73.78	0.61		
Reproductive					
Between Sites					
Site	1	2.65	2.65	17.52	0.003*
Error (site)	8	1.21	0.15		
Within Sites					
Time	4	3.23	0.81	10.1	<0.001*
Time × Site	4	0.83	0.21	2.59	0.055
Error (time)	32	2.56	0.08		
(B) Belowground Total					
Between Sites					
Site	1	30.79	30.79	1010	<0.001*
Error (site)	4	0.12	0.03		
Within Sites					
Time	15	20.06	1.40	26.51	<0.001*
Time × Site	15	11.88	0.79	14.95	<0.001*
Error (time)	60	3.18	0.05		

Table 1. Sediment data (monthly mean ± SE) for 2 sites in North Carolina (NC1 and NC2)

Site and date	% organic matter	NH$_4^+$ (µM)	PO$_4^{3-}$ (µM)	% sand	% silt	% clay
NC1						
Jul 2007	3.0 ± 0.2	14.7 ± 2.9	0.9 ± 0.1	—	—	—
Dec 2007	3.3 ± 0.3	8.9 ± 2.9	0.8 ± 0.2	79.7 ± 1.0	14.3 ± 0.7	6.0 ± 0.5
Jun 2008	3.5 ± 0.8	42.1 ± 17.0	0.9 ± 0.3	—	—	—
Sep 2008	2.8 ± 0.1	21.3 ± 7.5	0.2 ± 0.1	—	—	—
NC2						
Jul 2007	1.3 ± 0.1	20.4 ± 6.4	1.5 ± 0.5	—	—	—
Dec 2007	1.3 ± 0.2	12.7 ± 0.8	0.6 ± 0.2	90.6 ± 1.9	6.5 ± 1.4	3.0 ± 0.5
Jun 2008	0.7 ± 0.1	15.2 ± 1.6	3.8 ± 1.2	—	—	—
Sep 2008	1.2 ± 0.2	14.3 ± 2.4	0.0 ± 0.0	—	—	—

low in October 2007 and November 2008 at NC2 (Fig. 4C). The proportion of belowground biomass to total biomass was also greater at NC2 compared to NC1.

Vegetative shoot density was 2.5 times greater at NC2 than NC1 throughout the study period (p <
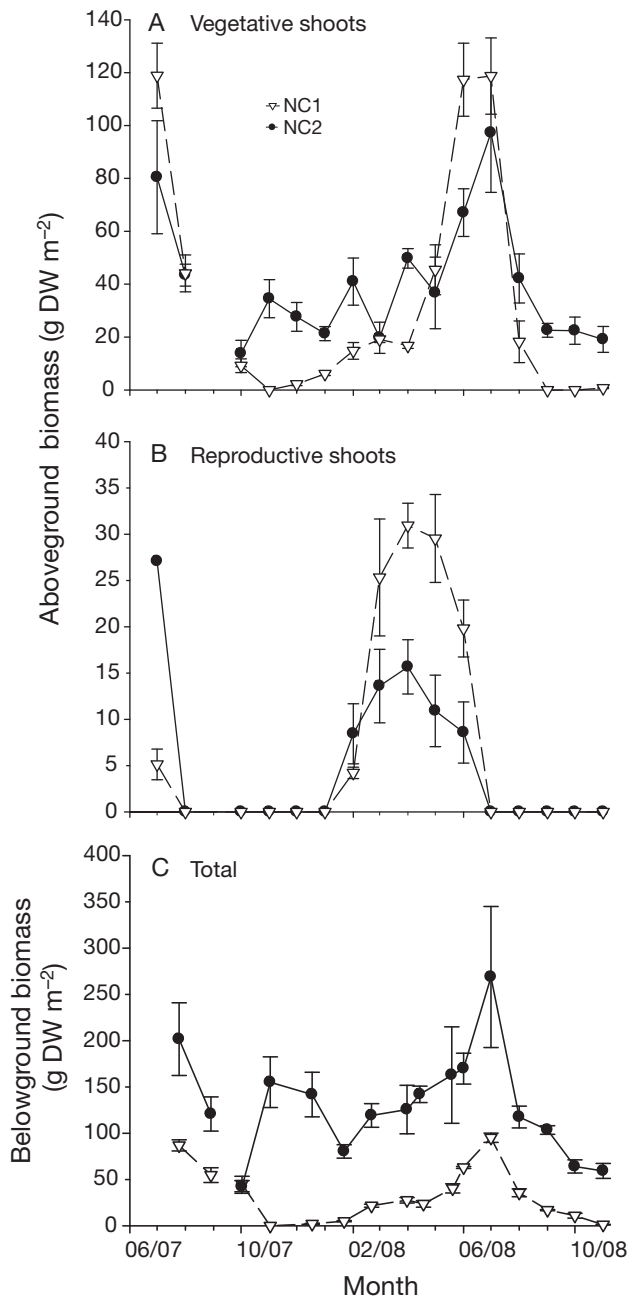

Fig. 4. *Zostera marina.* Monthly biomass at 2 sites (NC1 and NC2) in North Carolina. (A) Aboveground vegetative shoots; (B) aboveground reproductive shoots; and (C) belowground biomass (both vegetative and reproductive) during the 2007 to 2008 growing season. Means ± SE (n = 5)

0.001; Table 3). NC1 maximum abundance occurred in February, then decreased over time resulting in a complete loss of shoots after September 2008. Density increased again with germination of new seedlings in November 2008. NC2 maximum vegetative shoot density occurred in April, 2 mo later than at NC1. While exhibiting seasonal declines, *Zostera marina* density at NC2 was never below 531 ± 248 shoots m^{-2} (Fig. 5A).

Reproductive shoot densities were similar between sites (p = 0.889; Table 3). NC1 flowering shoot density reached a maximum in March 2008 (603 ± 157 reproductive shoots m^{-2}; Fig. 5B), while NC2 density peaked in May 2008 (463 ± 224 shoots m^{-2}). The proportion of reproductive shoots, ranged from 33 ± 3% at NC1 to 26 ± 13% at NC2 of total shoots during the period of maximum reproductive shoot density (March to May 2008; Fig. 5C).

Seed production and seed bank characteristics

The average number of rhipidia per reproductive shoot, the number of seeds per rhipidia, and the potential seed production per area were similar between sites (Tables 3 & 4). Although not significantly different, potential seed production was numerically greater at NC1 (61 563 seeds m^{-2}) compared to NC2 (41 146 seeds m^{-2}). This is reflected in the seed bank were total density was 3.4 times greater at NC1 than NC2 (p < 0.0001; Table 3). Seeds were always present in the sediment at both sites, although <1% of the estimated seeds produced in 2008 were retained within the sediment seed bank at NC2 and <2% were retained in NC1. Seed bank viability was variable, but on average, densities of viable seeds were 27.2 times greater at NC1 than NC2 (p = 0.003; Table 3). No viable seeds were observed in the seed bank following the period of maximum germination (Table 5).

DISCUSSION

Life history strategies

Different *Zostera marina* populations have been characterized as either perennial, biennial, or annual forms (Setchell 1929, Keddy & Patriquin 1978, Thayer et al. 1984). An alternative model was described for *Z. marina* in the southwestern Netherlands where a bed with attributes of both perennial and annual life histories was documented

Table 3. *Zostera marina.* Negative binomial regression model analyzing the effects of site on vegetative shoot density, reproductive shoot density, maximum estimated number of produced seeds (N_{ps}), total seed bank density (N_{sb}), and viable seed bank density (N_{vb}). Odds ratios calculated based on the parameter estimates. *p < 0.05

Parameter	df	Est	SE	X^2	p	Odds ratio	Wald 95% CL Low	High
Vegetative								
Intercept	1	4.28	0.08	2745.61	<0.001*			
Site	1	−0.9	0.12	56.95	<0.001*	0.41	0.32	0.51
Dispersion	1	0.47	0.06					
Reproductive								
Intercept	1	2.43	0.17	214.88	<0.001*			
Site	1	0.03	0.22	0.02	0.889	1.03	0.67	1.58
Dispersion	1	0.51	0.11					
N_{ps}								
Intercept	1	5.61	0.23	600.26	<0.001*			
Site	1	0.54	0.32	2.84	0.092	1.72	3	246.53
Dispersion	1	1.25	0.21					
N_{sb}								
Intercept	1	0.28	0.18	2.52	0.112			
Site	1	1.22	0.23	29.78	<0.001*	3.42	2.2	5.31
Dispersion	1	0.86	0.21					
N_{vb}								
Intercept	1	4.23	1.05	16.14	<0.001*			
Site	1	3.3	1.13	8.62	0.003*	27.18	3	246.53
Dispersion	1	7.66	3.53					

Table 4. *Zostera marina.* Sexual reproductive effort at sites NC1 and NC2 during the 2007 to 2008 growing season. Means ± SE across all months when reproductive shoots were present (February to July)

Sexual reproductive output	NC1	NC2
No. of reproductive shoots m^{-2}	330 ± 51	243 ± 57
No. of rhipidia per shoot	3.5 ± 0.2	3.5 ± 0.4
No. of seeds per rhipidia	11 ± 1	10 ± 1
Maximum no. of seeds produced m^{-2}	12 699 ± 1982	8515 ± 2003

suggesting that some populations exist with a continuum of strategies that include perennial and annual life histories as end members (van Lent & Verschuurre 1994). Our observations in North Carolina suggest that the occurrence of a similar alternate life history strategy for *Z. marina* populations at the southern limit of the species' distribution in the western North Atlantic.

Within a previously described perennial *Zostera marina* bed at NC1 (Penhale 1977, Thayer et al. 1977, 1984), we documented a population with a combination of perennial and annual characteristics. Following a complete loss of biomass, the population was reestablished by seedlings. A portion of the newly established seedlings reproduced asexually through clonal growth, similar to populations with a perennial life history. However, some seedlings produced only reproductive shoots, a characteristic of annual plants. In addition to the senescence of reproductive shoots in the late spring, there was a second complete loss of vegetative shoot biomass at NC1 after just 1 growing season. After the loss of flowering shoots, the surviving plants continued to grow asexually, suggesting that the complete loss of biomass in the fall was due to other factors (i.e. temperature, bioturbation). Thus the *Z. marina* population described at NC1 displayed a mixture of perennial and annual life histories which we characterized as a 'mixed-annual' life history strategy.

Table 5. *Zostera marina.* Seed bank density and percentage of viable seeds at sites NC1 and NC2. Means ± SE

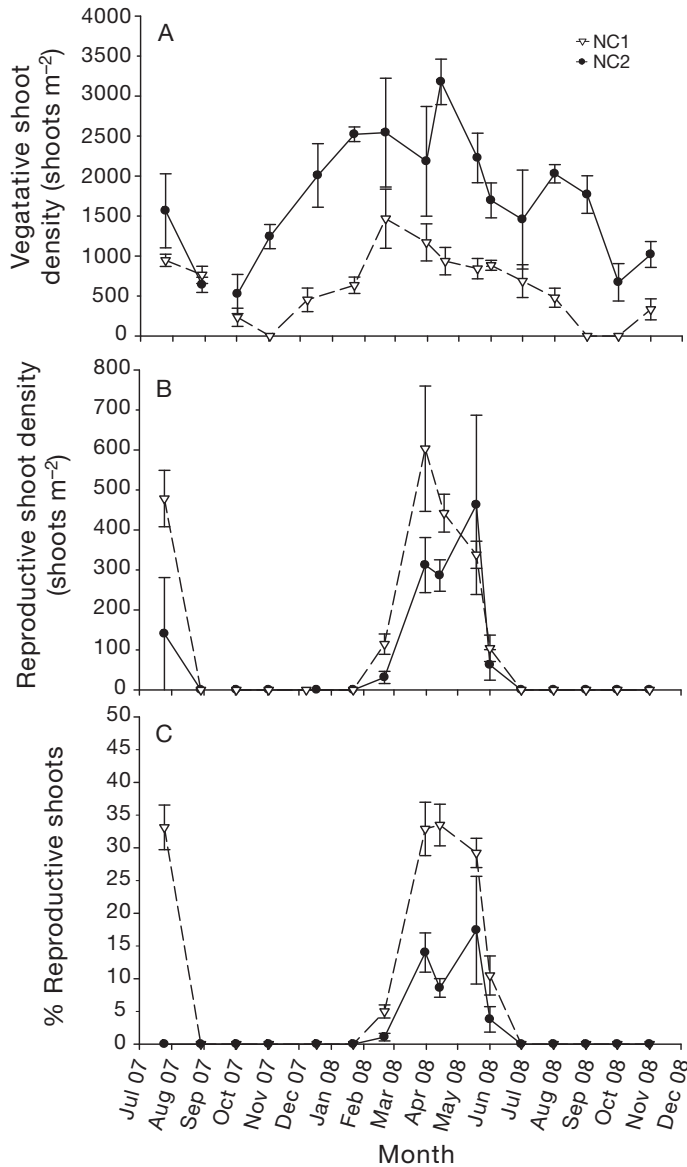| Date	Density (m^{-2}) NC1	NC2	% viable NC1	NC2
Jul 2007	190 ± 97	211 ± 74	33 ± 21	13 ± 13
Dec 2007	232 ± 84	211 ± 100	0 ± 0	0 ± 0
Jun 2008	906 ± 246	147 ± 42	18 ± 9	0 ± 0
Nov 2008	232 ± 91	147 ± 26	0 ± 0	0 ± 0

Fig. 5. *Zostera marina.* Monthly densities at 2 sites (NC1 and NC2) in North Carolina. (A) vegetative shoots, (B) reproductive shoots, (C) percentage of reproductive shoots. Means ± SE (n = 5)

Attributes of a mixed-annual life history

Zostera marina seedlings in the mixed-annual bed at NC1 flowered within 6 mo of germination. This was much earlier than perennial populations within the region which exhibit biennial characteristics and do not flower until their second year of growth (Silberhorn et al. 1983, Phillips et al. 1983b, Thayer et al. 1984). They also produced a greater number of flowering shoots per m^{-2} (maximum 603 ± 157 shoots m^{-2}) than established perennial beds nearby at NC2 (79 ±

110 to 463 ± 224 shoots m^{-2}; Phillips et al. 1983b, this study). In addition, a larger proportion of reproductive shoots (33 ± 3%) were found in the mixed-annual population compared to perennial beds (<10 to 28% of total shoots; Jacobs & Pierson 1981, Silberhorn et al. 1983, Thayer et al. 1984, Olesen 1999). This is, however, significantly less than typical annual meadows where 100% of the shoots flower (Keddy & Patriquin 1978, Robertson & Mann 1984, Meling-López & Ibarra-Obando 1999), and more similar to average densities of reproductive shoots produced in perennial populations.

The greater effort put into sexual reproduction in mixed-annual beds compared to perennial populations may come at the cost of bed maintenance and expansion through asexual reproduction. Energy necessary for vegetative expansion of *Zostera marina* is stored in non-structural carbohydrate reserves which are primarily found in the rhizomes (Burke et al. 1996). Belowground biomass (means ± SE) in the mixed-annual bed (95.08 ± 5.04 g DW m^{-2}) was significantly lower than perennial beds in North Carolina (268.81 ± 76.17 g DW m^{-2}; Thayer et al. 1984) and in the lower Chesapeake Bay (155 g DW m^{-2}; Orth & Moore 1986) and more similar to annual populations observed in the Gulf of California at Punta Chueca (51 ± 17 g DW m^{-2}; Meling-López & Ibarra-Obando 1999) and in Bahía Concepción (26 g DW m^{-2}; Santamaría-Gallegos et al. 2000). Robertson & Mann (1984) hypothesized that the lower belowground biomass found in annual compared to perennial populations was related to the shorter development time in the abbreviated lifecycle of annual populations. Like annual beds, the mixed-annual bed NC1 grows for only a portion of the year and has less time to develop belowground biomass than perennial beds. More energy is allocated to the development of reproductive shoots resulting in a greater proportion of biomass in aboveground compared to belowground structures, mainly as flowers and seeds.

Survival of *Zostera marina* beds that die back annually is dependent on the production of flowers and viable seeds. The mixed-annual population yielded more seeds than the perennial site but had a lower maximum potential seed abundance (61563 seeds m^{-2}) than reported in annual populations in Nova Scotia (78224 seeds m^{-2}; Keddy 1987) and in the Sea of Cortez, Mexico (100376 seeds m^{-2}; Meling-López & Ibarra-Obando 1999). This reduced number of seeds may have severe negative consequences for true annual populations as the seed bank may not contain enough seeds to allow for a sufficient number of shoots to germinate and re-establish

the bed. However, by reproducing both sexually and asexually newly germinated seedlings in the mixed-annual bed may grow and become established more rapidly than seedlings which only produce reproductive shoots (Keddy & Patriquin 1978; van Lent & Verschuure 1994). Therefore, while seed production in mixed-annual beds is limited compared to annual populations, the ability to reproduce both sexually and asexually may compensate for the lack of seed material through relatively rapid expansion and establishment.

In areas with stressful environmental conditions, the production of a greater number of viable seeds may be more beneficial than maintaining year round biomass. This is especially important at the southern limit of *Zostera marina* distribution in North Carolina where water temperatures can exceed the upper limit of the optimum range of the species (25°C) for a high percentage of July and August (Penhale 1977, Thayer et al. 1977, 1984). Water temperatures above 25°C can significantly reduce photosynthetic rates, increase respiration, inhibit leaf growth, and increase *Z. marina* mortality (Marsh et al. 1986, Nejrup & Pedersen 2008, Hosokawa et al. 2009; Höffle et al. 2011). Large scale loss of perennial *Z. marina* populations in the Chesapeake Bay was attributed to stressful environmental conditions including water temperatures exceeding 30°C for only a few weeks in 2005 (Moore & Jarvis 2008). Large scale seed germination and seedling establishment contributed to recovery in Chesapeake Bay (Jarvis & Moore 2010), highlighting the importance of seeds for recovery from large scale declines (Plus et al. 2003, Greve et al. 2005, Lee et al. 2007).

In addition to seed production, another very important factor in considering the potential importance of the mixed-annual life history is the timing of flowering during the life cycle of the plants. Perennial populations that include plants with biennial characteristics do not flower until at least their second year of growth (Setchell 1929, Silberhorn et al. 1983, Thayer et al. 1984). Thus, when a biennial population experiences a high rate of mortality the potential for seed production in the next growing seasons is diminished. This population may be re-established by seeds produced during the previous growing season, but there may be few or no 'second year' biennial plants to produce flowers and replenish the seed bank. Since the seed bank is transient and can only be replenished by the production of new flowers, seed stocks will be even more depleted if there is a second stress event during the next growing season (Jarvis & Moore 2010). Seed banks function to

replenish populations annually and as a recovery mechanism following large scale disturbances (Plus et al. 2003, Greve et al. 2005, Jarvis & Moore 2010). If the seed bank is depleted by consecutive years of stressful conditions resulting in large scale loss of biomass in perennial beds, this may result in the complete loss of the effected population (Jarvis & Moore 2010). Populations expressing the mixed-annual life history strategy have seedlings that are able to flower during their first year ensuring the seed bank is replenished with viable seeds. Therefore the mixed-annual population is able to persist in areas with stressful environmental conditions which may otherwise limit or exclude perennial populations.

Regardless of the strategy by which seeds are produced, they must remain viable in the sediment seed bank until conditions are favorable for germination, after which seedlings can replace adults within the established population (Baker 1989, Murdoch & Ellis 2000). At NC1 and NC2 the seed bank density declined so that by the fall of 2008, following the period of maximum germination for this region (Silberhorn et al. 1983, Thayer et al. 1984), <2% of that year's estimated seed production remained viable in the sediment. Similar large losses of seeds, ranging from 25 to 78% of total seed production, have been reported for both annual and perennial *Zostera marina* beds (Santamaría-Gallegos et al. 2000, Harwell & Orth 2002b, Morita et al. 2007). These losses may be the result of dispersal (Harwell & Orth 2002a, Källström et al. 2008), decay (Morita et al. 2007), predation (Fishman & Orth 1996), or germination (Harper 1977).

The persistence of vegetative shoots after the senescence of reproductive shoots is another feature of the mixed-annual life history strategy that distinguishes it from annual populations. Annual populations of *Zostera marina* produce only reproductive shoots which senesce at the end of the flowering period (Keddy & Patriquin, 1978, Gagnon et al. 1980, De Cock 1981, Harlin et al. 1982, Phillips et al. 1983a, Robertson & Mann 1984, Santamaría-Gallegos et al. 2000). The complete loss of biomass and survival of the population in seed form may provide a mechanism to escape stressful environmental conditions via the seed bank (Phillips et al. 1983a, Robertson & Mann 1984, van Lent & Verschuure 1994, Santamaría-Gallegos et al. 2000). However, if stressful environmental conditions are ameliorated, annual beds are not capable of maintaining a population after the loss of reproductive shoots. The vegetative shoots in the mixed-annual population documented

here continued to persist for up to 3 mo following the last observation of reproductive shoots. Vegetative reproduction by mixed-annual plants enables the population to perenniate and expand when the external factors responsible for mortality and complete loss of biomass are absent.

SUMMARY AND CONCLUSIONS

Growing near its southern range limit in the western North Atlantic, populations of *Zostera marina* express a range of life histories including both perennial and annual forms as well as a combination of both strategies. A mixed-annual life history described for NC1 illustrates an alternate mechanism for persistence through both short-term and extended periods of environmental stress. Reproducing both sexually and asexually during the first year of growth maximizes inter-annual survival of *Z. marina* populations by ensuring maintenance of the existing bed through clonal propagation as well as providing a source of seeds to replenish the population the next growing season (Table 6). We hypothesize that the mixed-annual life history improves the probability for *Z. marina* populations to persist in stressful environments. Rather than relying on a single life history

strategy, some populations in North Carolina can use a range of strategies to overcome the high rate of mortality in a thermally stressed environment.

We suggest that neither annual nor perennial or biennial life history strategies always provide a superior mechanism for population persistence. For example, perennial meadows are able to maintain their populations with clonal reproduction, but are susceptible to multiple consecutive years of large scale loss due to a lack of viable seed bank replenishment (Jarvis & Moore 2010; Table 6). Annual meadows are able to overcome stressful environmental conditions through the annual production of reproductive shoots and a viable seed bank, but these populations are not able to fully exploit available resources throughout the entire growing season due to a lack of vegetative reproduction (Keddy & Patriquin 1978, Phillips et al. 1983a, Hootsmans et al. 1987, Meling-López & Ibarra-Obando, 1999; Table 6). *Zostera marina* is a species that has evolved over millions of years in ephemeral coastal environments (Les et al. 1997, Waycott et al. 2006) and we hypothesize that the species has developed a variety of life history strategies that range between perennial and annual depending on environmental conditions.

Seagrass meadows are being lost from coastal ecosystems at a global rate of 110 km^2 yr^{-1} (Waycott et al.

Table 6. *Zostera marina.* Description of life history strategies expressed in beds near the species' southern limit in the western North Atlantic under both stressed and non-stressed conditions. For this table, stress is assumed to occur at the end of the growing season after flowers have senesced and seeds have been produced

Life history	Year 1	Year 2	Year 3
Stress			
Perennial	All plants die; viable seeds in seed bank from previous year flowering	Bed recovers from seedlings; within year all plants die; no viable seeds remain in seed bank	No bed recovery
Annual	All plants die; viable seeds remain in seed bank from previous year flowering	Bed recovers from seedlings; flowers; within year all plants die; viable seeds remain in seed bank	Bed recovers from seedlings and persists
Mixed-annual	All plants die; viable seeds remain in seed bank from previous year flowering	Bed recovers from seedlings; flowers; within year all plants die; viable seeds remain in seed bank	Bed recovers from seedlings and persists
No stress			
Perennial	Plants persist; viable seeds in seed bank from previous year flowering	Bed persists from vegetative growth and seedlings; flowers; plants persist; viable seeds remain in seed bank	Bed persists from largely vegetative
Annual	All plants die; viable seeds remain in seed bank from previous year flowering	Bed re-grows from seedlings; flowers; all plants die; viable seeds remain in seed bank	Bed recovers from seedlings and persists
Mixed-annual	Annual plants die; viable seeds in seed bank from previous year flowering	Bed persists from vegetative growth and seedlings; flowers; annual plants die; viable seeds remain in seed bank	Bed persists from vegetative growth and seedlings

2009) and are predicted to continue declining as conditions become increasingly stressful due to global climate change (Short & Neckles 1999, Harley et al. 2006). This period of unprecedented environmental transition may result in large scale shifts in the distribution of species (Rogers & McCarty 2000, Scavia et al. 2002) and, in the most extreme cases, greater mortality of sensitive species at local and regional levels (Rogers & McCarty 2000, Najjar et al. 2010). The mixed-annual life history described here represents one possible life history strategy within this range, which may provide the mechanism necessary for *Zostera marina* populations to persist during times of environmental transition. Understanding how the life history strategies of seagrasses may affect their distribution and persistence of a species on local and regional scales is vital for effective prediction of responses to environmental and anthropogenic stressors and ultimately the management of these coastal resources.

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