Aquatic Botany 110 (2013) 61–66

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Genetic diversity and gene flow in *Zostera marina* populations surrounding Long Island, New York, USA: No evidence of inbreeding, genetic degradation or population isolation

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ARTICLE INFO

Article history:
Received 25 September 2012
Received in revised form 8 May 2013
Accepted 21 May 2013
Available online 30 May 2013

Keywords:
Zostera marina
Genetic population structure
Long Island
Microsatellite
Inbreeding

ABSTRACT

Since the 1930s, eelgrass around Long Island, New York, USA, has experienced significant ecological and anthropogenic disturbances reducing areal coverage of the species. Patchiness, low density or isolation of these remaining populations increase susceptibility of this aquatic angiosperm to extinction. The loss of genetic diversity and patch connectivity, may contribute to lower fitness of eelgrass thus affecting recovery potential. Previous studies of eelgrass populations around Long Island report genetically isolated populations with low diversity. In contrast, this study found neither the evidence of inbreeding nor indications of genetic degradation for the same populations. Measures of genetic diversity such as average alleles ($A = 7.59$) and fixation index ($F = 0.02$) suggest no significant impediments to genetic connectivity among populations sampled. Gene flow ($N_m = 4.58$) and bottleneck analyses suggest the major disturbances of the past have not strongly affected population structure in the Long Island system. These findings have significant implications for both management and restoration. Locally, eelgrass populations in Long Island waters are unlikely to decline through genetic erosion or inbreeding processes alone. Plants from within these populations possess adequate genetic diversity to undertake restoration activities. On a larger geographic scale, the ability of these plants to maintain such high levels of genetic diversity and connectivity despite the significant areal losses historically provides optimism for the recovery potential of this species despite recent global losses.

1. Introduction

Zostera marina L. (eelgrass) is an ecologically and economically important marine angiosperm. The current species range spans the northern hemisphere (Green and Short, 2003) across large physiochemical gradients. The species is important because it controls primary production, oxygenation, sediment stabilization and habitat complexity in shallow coastal ecosystems (Moore and Short, 2006). Over the last century, there have been global losses of seagrass coverage (Waycott et al., 2009), most without indications of recovery (Rasmussen, 1977; Dennison et al., 1987; Rozsa, 1994). In addition, there are increasing concerns that the rates of loss are accelerating (Orth et al., 2006; Waycott et al., 2009). Recognizing the importance of *Z. marina* in coastal ecosystems, understanding the mechanisms of areal loss and developing potential strategies to mitigate these changes, are of management priority.

Eelgrass (*Z. marina*) populations in the coastal waters of Long Island, New York, USA have experienced a series of ecological disturbances during the past century. During the 1930s, there were catastrophic *Z. marina* population losses due to a wasting disease that decimated eelgrass meadows on both sides of the Atlantic. It is estimated that 80% of the eelgrass coverage was lost along the east coast of North America (Cottam and Munro, 1954). In 1985, Long Island's eelgrass meadows were further impacted by the first recorded bloom of a 'brown tide' forming pelagophyte, *Aureococcus anophagefferens* (Gobler and Sunda, 2012). The annual reoccurrence of these blooms during the subsequent three decades has substantially altered the abundance and distribution of eelgrass on Long Island through presumed light stress brought on by rampant water column production. During this time, these blooms further reduced

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http://dx.doi.org/10.1016/j.aquabot.2013.05.003

areal coverage of eelgrass meadows by 40% within the Great South Bay and Peconic Estuary (Cosper et al., 1987; Dennison et al., 1987). Collectively, these significant losses in *Z. marina* coverage may have severely impacted the capacity of remaining populations to maintain genetic diversity (Frankel and Soulé, 1981; Lande, 1988). Loss of genetic diversity characteristically leads to inbreeding which is particularly problematic through the expression of deleterious alleles that reduce the fitness and the adaptive capacity of the plant. Additionally, low hydrologic connectivity between populations (Wilson et al., 1991) may reduce the dispersal potential, gene flow and long-term recovery in subpopulations of *Z. marina* (Kendrick et al., 2012).

High resolution, co-dominant genetic markers are now widely used tools for determining population genetic structure (Bachmann, 1994), and have been successfully applied to the study of *Z. marina* (Bachmann, 1994; Reusch, 2001). The investigation of eelgrass genetic diversity has been made possible through the availability of numerous microsatellite markers (Reusch et al., 1999). Previous genetic analysis has shown that eelgrass populations across broad geographic ranges have maintained moderate to high levels of genetic diversity even within populations that have endured large scale losses (Ferber et al., 2008; Ort et al., 2012; Wyllie-Echeverria et al., 2010). Across broad geographic scales a diversity of population genetic structure exists, including rare cases of highly clonal populations (Reusch et al., 1999; Olsen et al., 2004).

Given the ecological importance of eelgrass in New York, management agencies have made repeated efforts to restore their populations (South Shore Estuary Reserve, Peconic Estuary Program; New York State Seagrass Taskforce 2009). Unfortunately, the majority of these restoration efforts have been unsuccessful. Choice of restoration strategies, in particular the use of transplanted fragments over broadcast seeds and the selection of donor populations will have a significant impact on total effective population size and genetic diversity in the coming years (Williams and Orth, 1998; Williams, 2001; Reynolds et al., 2012). Long-term success of such efforts will be measured by the ability of restored populations to be self-sustaining and to increase the connectivity of presently fragmented subpopulations (Reynolds et al., 2012). Low genetic diversity, however, will jeopardize both population maintenance and recovery through the deleterious effects of inbreeding. Therefore, it is critical to quantify the current level of genetic diversity and connectivity between populations within this system prior to effective restoration.

Historical eelgrass losses in Long Island waters (Rasmussen, 1977; Cosper et al., 1987; Dennison et al., 1987), limited geographic connectivity (Wilson et al., 1991) and Campanella et al. (2010a,b) suggest that eelgrass populations around Long Island may be highly clonal, existing as spatially segregated populations. They suggested that evidence pointed to historical bottlenecks for eelgrass populations in Long Island. This would imply that significant genetic erosion has occurred, making the populations quite vulnerable to ongoing or future disturbances exhibiting low population resilience. Because of the potential vulnerability to genetic degradation, knowledge of population structure is critical to the development of management strategies to protect, maintain, and restore eelgrass populations in this region. The purpose of this study was to evaluate current genetic diversity of eelgrass within Long Island waters.

2. Materials and methods

Two different sampling designs were employed to collect *Z. marina* ramets from meadows throughout Long Island (Fig. 1). The first was a stratified random sampling method (hexagon tessellation) designed to locate and sample 188 sites throughout the Fire Island National Seashore in Great South Bay. In addition, nineteen other meadows located in Peconic Bay and Long Island Sound were sampled in a similar way. A second methodological design was used in Shinnecock Bay to collect 31 samples from a rectangular spatial array (3 transects) where sequential samples were collected at 5 m intervals. In total, this study sampled genetic material from 293 eelgrass ramets within the South Shore Estuaries, Peconic Estuary and NY waters of Long Island Sound.

Genetic samples were acquired by collecting whole plants and storing them at −20 °C in plastic, gas impermeable bags. Upon returning to the lab, the two youngest leaves were removed, cleaned of epiphytes, dried with paper towels, and placed in labeled 40 ml plastic scintillation vials filled with silica bead desiccant. DNA was extracted from these dry tissue samples using manual grinding in a mortar and pestle and a modified Qiagen DNeasy plant mini kit protocol (Qiagen Pty. Ltd., Valencia, CA). Aliquots of extracted DNA were used in two separate multiplex PCR amplifications on an MJ Research™ PTC-200 thermocycler using *Z. marina* specific, 5′-fluorochrome (standard labels Hex, Tet, Fam) labeled polymorphic microsatellite markers GA2, CT3, CT35, CT17H, GA6, GA3, CT19, and CT20 (Reusch, 2001). Amplification of the microsatellites was accomplished through 20 μL polymerase chain reactions (PCR) containing approximately 30 ng of template DNA, 0.5 U of Bioline Immolase DNA Taq (Bioline Pty. Ltd., CA), 1.5 μL 10x Bioline Immobuffer (160 mM $(NH_4)_2$ SO_4, 670 mM Tris–HCl pH 8.3, 0.1% Tween-20), 2.5 mM $MgCl_2$, 0.133 mM each dNTP, BSA at a concentration of 0.1 μg/μL, and 0.33 mM fluorescently labeled forward (FAM, HEX, or TET) and reverse microsatellite primers. Thermal cycling protocols consisted of a 7 min 94 °C denaturing step followed by 30 cycles of at annealing temperature of 50 °C. For all cycles denaturing steps were conducted at 94 °C and extension temperature was 72 °C. PCR products were analyzed using a DNA sequencer MEgaBACE™ 1000 (GE Healthcare). The software MEgaBACE Genetic Profiler 2.2 (GE Healthcare) was used to assign alleles for each sample at each locus relative to an internal size standard (MEgaBACE™ ET400-R size standard, GE Healthcare, Buckinghamshire, UK).

Estimates of genetic diversity and population genetic structure were conducted using a suite of genetic analysis software packages. Standard tests for expected versus observed heterozygosity (Hardy Weinberg Equilibrium), allelic diversity, population differentiation (*F*-statistics), gene flow (F_{st}) and isolation by distance were analyzed using GenAlEx 6.41 (Peakall and Smouse, 2006). Estimates of gene flow based on private alleles were calculated in Genepop 4.0.10 (Raymond and Rousset, 1995; Rousset, 2008) and implemented via Genepop on the web (http://genepop.curtin.edu.au/index.html). Clonal identity and the spatial distribution of clones were identified using Geneclone 2.0 (Arnaud-Haond and Belkhir, 2007). Measures of population differentiation as hierarchical f-statistics (Weir and Cockerham, 1984) were assessed using Genodive 2.0b22 (Meirmans and van Tienderen, 2004) using 9999 jacknife replicates across loci to generate standard errors for pairwise population comparisons. Fstat 2.9.3 (Goudet, 1995) was used to calculate allelic richness (A_r) using rarefaction to equivalent population size ($N = 20$; the smallest population size from LIS). The fixation index (F_{st}) ranges from 0 to 1 where 0 indicates no genetic differentiation among groups (i.e. the groups are acting as a single genetically connected population) and 1 indicating fixed differences between populations.

Small spatial scale genetic structure within and among populations was evaluated using spatial autocorrelation across various equidistant size classes (GenAlEx 6.41; Peakall and Smouse, 2006, Geneclone 2.0; Arnaud-Haond and Belkhir, 2007). In GenAlEx each spatial autocorrelation run consisted of 9999 permutations and 9999 bootstraps. In Geneclone 2.0 each population was run independently using 100 distance classes, genets with resampling,