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Numerous mitigation transplants of the eelgrass *Zostera marina* in southern California shuffle genetic diversity and may promote hybridization with *Zostera pacifica*

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ABSTRACT

Intensive human pressures along the southern California coast have led to >50 mitigation transplants of eelgrass over the past 30 years. We analyzed diversity and population structure of *Zostera marina* and *Zostera pacifica* at 36 locations to identify potential management units and further develop transplant guidelines. Normalized allelic diversity of *Z. marina* was uniformly moderate to high (4.78; 3.48–6.44) and nearly two-fold higher than mainland *Z. pacifica* (2.70; 1.74–4.89). More than half of the *Z. marina* populations exhibited strongly significant inbreeding coefficients coupled with strong linkage disequilibrium attributable to transplant effects; neither attribute was found in *Z. pacifica*. Both species were characterized by high genotypic diversity and an absence of large clones. A Bayesian analysis of population structure suggested 6 potential management units for *Z. marina* and 3 for *Z. pacifica;* some units included disjunct locations associated with transplants. Hybridization between *Z. marina* and *Z. pacifica* was documented at Newport Bay Entrance Channel and south San Diego Bay. The presence of two species requires management plans for each, as well as avoidance of potential transplant-induced hybridization. Although transplant admixtures elevate diversity, shuffling among locations may potentially reduce the genetic potential necessary to ensure rapid adaptation, even though overall transplant success has been successful. Given that transplants will continue (from both plants and seeds), we recommend that the current requirement for "two additional distinct donor sites" be restricted to within a management unit for small, routine mitigations and expanded to among-management units for wholesale *de novo* restorations.

1. Introduction

Zostera marina (narrow-leaved eelgrass) is the most widely distributed seagrass in temperate, northern hemisphere regions of both the Pacific and Atlantic. Along the eastern Pacific coast, it extends from Arctic Alaska to southern Baja California Mexico where it forms meadows in fjords, bays, lagoons and portions of the open coast characterized by soft sediments (Green and Short, 2003). *Zostera pacifica* (wide-leaved eelgrass) is restricted to the California Channel Islands and the adjacent mainland north to at least Monterey Bay and south to San Diego Bay (Watson, 1891; Engle and Miller, 2003; Coyer et al., 2008). Recent reviews of the biology, morphology and conservation of *Zostera* species can be found in Larkum et al. (2006), Waycott et al. (2006, 2009), Procaccini et al. (2007) and Short et al. (2011).

The maximum extent of eelgrass in southern California is less than 5000 acres (~2000 hectares) based upon available information from large-scale surveys. San Diego Bay and Mission Bay collectively comprise approximately 90% of the known mapped extent of eelgrass. However, a number of coastal embayments have experienced limited eelgrass monitoring and open coast populations have not been comprehensively assessed; thus, significant potential for greater eelgrass habitat probably exists. Furthermore, distinction between the two eelgrass species has not been a focus of regional eelgrass monitoring (Bernstein et al., 2011).

Many bays and lagoons along the Southern California Bight have undergone multiple eelgrass transplants as compensatory mitigation following filling, dredging and placement of structures. Eelgrass restoration has also occurred as a component of various large-scale wetland and lagoon restoration efforts with >50

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mitigation transplants documented over the past 30 years (NMFS, 2011). Even though these activities have resulted in an expansion of eelgrass habitat beyond the direct losses authorized by permitted actions (Bernstein et al., 2011), genetic effects on pre-/post-population reestablishment and fitness remain unknown.

The importance of genetic biodiversity for eelgrass health and ecosystem function is now well established. Experiments with *Z. marina* showed that increased genotypic diversity led to: (a) increased growth rates and competitive superiority of some clones and seed production (Williams, 2001; Hammerli and Reusch, 2002); (b) greater biomass production and recovery following grazing (disturbance) by geese (Hughes and Stachowicz, 2004); (c) enhanced shoot density (reflecting habitat quality) and biomass of epiphytic algae (a measure of food resource availability) (Hughes and Stachowicz, 2009a,b); and (d) a "high-disturbance" response and better resilience (Hughes and Stachowicz, 2011). At the community level, seagrass genotypic diversity was strongly correlated with an increase in the biodiversity of the associated community, thus adding complexity and greater insurance effects for resistance and resilience (Reusch et al., 2005; Eklöf et al., 2012). In a word of caution, however, Massa et al. (2013) experimentally showed that effects attributed to genotypic diversity alone need to be dissected and reconsidered to include the embedded allelic diversity, as one may not be a simple proxy for the other. In any case, however, the amount of genetic variation in a population affects its evolutionary potential and capacity to rapidly adapt to new circumstances, a process characterized by the occurrence of local ecotypes. For example, experimental studies have documented differences in gene expression and photosynthetic performance between intertidal and subtidal temperature- and light-ecotypes of *Z. marina* (Oetjen and Reusch, 2007; Bergmann et al., 2010; Oetjen et al., 2010; Franssen et al., 2011, 2014; Winters et al., 2011). In short, whereas ecological factors affecting eelgrass meadows have been well studied (reviewed in Larkum et al., 2006), consideration of evolutionary factors (reviewed in Waycott et al., 2006; Procaccini et al., 2007) is gaining importance in both primary research and improved conservation management because it is increasingly recognized that "evol-eco" processes occur in real time (Spielmann et al., 2004; Allendorf and Luikart, 2007). Finally, the evolutionary dimension of genetic-level diversity is an explicit goal of the International Convention on Biological Diversity (Laikre et al., 2010).

In the present study, we focus on mainland *Z. marina* and *Z. pacifica* populations along the Southern California Bight, from Point Conception to San Diego Bay (including additional sampling from north of Point Conception and south along the Pacific coast of Baja California). The aims were to: (1) establish the current baseline distribution of allelic diversity in *Z. marina* and *Z. pacifica* as an indicator of evolutionary potential for adaptation; (2) assess genotypic diversity (clonal diversity) as a reflection of local meadow persistence, stability and sexual reproduction; (3) compare genetic population structure and gene flow within and among bays and harbors that have experienced one or more mitigation transplants over the past 30 years; (4) determine whether interspecific hybridization has occurred between the two species; and (5) utilize the above "status" information to help define management units and modify mitigation guidelines that will minimize the risk of inadvertently reducing long-term meadow fitness.

2. Materials and methods

2.1. Sample collection

Samples ($n = 48$) of both *Zostera* species were collected from 36 sites (meadows) from Morro Bay, California to Magdalena Bay, Baja California Sur, Mexico: 25 with *Z. marina* and 11 with *Z. pacifica* (Fig. 1, Table 1, Fig. A1). Samples were collected by divers using scuba at all sites in California; samples from Mexico were collected at low tide. In all cases, shoots were collected at intervals of approximately 1.5 m along transects, a standard interval used in genetic baseline studies which facilitates comparisons among studies. Transects were perpendicular to shore where extensive beds were present. However, many areas exhibited fringing eelgrass beds along narrow margins of bays and channels and in these areas, transects ran horizontal to the shore. Each sample was isolated in a separate bag and placed in a cooler until further processing later in the day. Leaves (2–3) from each shoot were blotted dry and cut into 5–10 mm lengths before placement into 1.7-mL plastic tubes filled with silica gel crystals for rapid dehydration and subsequent storage.

2.2. DNA extraction and microsatellite amplification

Template DNA for PCR reactions was obtained from 2 to 3, 5–10 mm pieces of silica-dried leaves. Six microsatellite loci were used for both *Z. pacifica* and *Z. marina*: Zosmar-CT3, CT12, CT19, GA2, GA3, and GA6 (Reusch et al., 1999; Reusch, 2000; Olsen et al., 2004). Locus CT20 is a diagnostic locus, as it does not amplify in *Z. pacifica* (Coyer et al., 2008). Consequently, it was not included when both *Z. marina* and *Z. pacifica* populations were considered simultaneously, but was included when only *Z. marina* populations were evaluated (see Table A1 for *Z. marina* diversity based on 7 loci). The hypervariable loci CT17H and CT35, which are commonly utilized for *Z. marina*, were not used in the present study because their genotypes revealed mosaic alleles in some, but not all populations, suggesting the presence of multiple cell lineages within the same ramet (=somatic mutation) (Reusch and Boström, 2011). DNA extraction was based on a method developed for the seaweed *Fucus* (Hoarau et al., 2007) with subsequent modification for *Zostera* by heating the CTAB mixture to 60 °C (Coyer et al., 2009). PCR amplification and genotyping are described elsewhere (Coyer et al., 2004; Olsen et al., 2004). Genotypes were visualized on an ABI 3730 gene analyzer (Applied Biosystems) and analyzed using GENOTYPER (Applied Biosystems) software.

2.3. Genets and ramets

A genetic individual (genet) consists of many shoots (ramets) that can extend for several meters along a rhizome. Sampled shoots can, therefore, have the same multilocus genotype (MLG) if derived from the same large clone. The relative number of genets and ramets sampled in a given area was distinguished with GENCLONE 2.0 (Arnaud-Haond and Belkhir, 2007). Probabilities of identity by chance (*P*sex (F_{IS})) were calculated for each sample to avoid false assignment of individual ramets sharing the same MLG by chance to the same genet (clone). *P*sex (F_{IS}) accounts for departure from Hardy–Weinberg equilibrium (HWE) and provides the most conservative estimates of clonal identity (Arnaud-Haond and Belkhir, 2007).

All ramets reported as identical were identical due to clonality, not chance ($P < 0.05$). All subsequent analyses utilized genets only, i.e., duplicate MLGs removed.

Clone size was estimated by the spatial resolution of the linear sampling method (i.e., 1.5 m), which provided a coarse minimum value only; shoots were not sampled in a quadrat or mapped. For example, if three consecutive samples had the same MLG, the clone was estimated as minimally 4.5 m in size.

2.4. Data analysis

Allelic richness ($\hat{A}$) is the mean number of alleles^{-locus}. Allelic richness was standardized to $N = 20$ genets (smallest number for