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A genetic assessment of a successful seagrass meadow (*Posidonia australis*) restoration trial

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Summary

Seagrass meadows are in decline globally. Although numerous experimental methods have been implemented to restore meadows, few have been successful in the long term. Poor decisions on the sourcing of transplants from donor sites, including poor genetic integration and/or low genetic diversity, may impact on restoration success. However, despite evidence to suggest a positive association between genetic diversity and ecological resilience, there is usually little or no input from genetic data to inform on the genetic management of ecological restoration. Cockburn Sound has seen a 77% decline in seagrass cover since 1967. A transplant trial was conducted between 2004 and 2008 with sprigs of *Posidonia australis* being planted into a bare sand area. Survival was monitored annually, and in 2012, we compared genetic diversity in this transplant area with the original donor site. Genetic diversity in the restored meadow was very high and comparable to the donor site, with no genetic differentiation detected. The high level of genetic diversity and choice of site may have played an important role in the success of this restoration trial. The observed natural recruits around the site after establishment of transplants suggest that local restoration efforts may improve seafloor habitat and facilitate natural expansion of the meadow.

Keywords: genetic diversity; marine restoration; microsatellite DNA; *Posidonia australis* ; provenance; seagrass; transplant

Introduction

Seagrass meadows are under threat globally, mostly through anthropogenic impacts (Waycott *et al*. 2009). While many species go largely unnoticed by the majority of the population, the ecosystem services provided by these 'forests of the marine world' cannot be replaced. A number of techniques have been trialled in an attempt to develop efficient and cost-effective methods to regenerate seagrass meadows, including mechanically transplanting large sods, transplanting sprigs, seed broadcasting and improving seafloor habitat (Seddon 2004; Bastyan & Cambridge 2008; Marion & Orth 2010). Cost is a prohibitive factor for many of these methods, while availability of plant material and impact on existing meadows are prohibitive for others. The use of vegetative transplants has been the most widely used method, but restored areas are small, long-term success has not been good, and donor meadows can be negatively impacted.

Restoration can occur at several levels of intensity. At some sites, water pollutants have been the major cause of decline; once these have been removed, natural recruitment of plants may occur through vegetative growth and recruitment of individuals from seed, with little

additional effort required. However, a more difficult situation for long-term restoration is where the natural physical landscape (of the seafloor) has been altered or secondary effects alter wave action and sand bank formation on a local scale, such that new recruits are unable to establish (or even translocated meadow clumps or sprigs are washed away). This is a much greater challenge in the marine environment than such reconstructive efforts carried out in terrestrial restoration sites. In the absence of natural recruitment, sprigs or seedlings may need to be sourced from a donor site some distance away. It is at this point where an understanding of levels of genetic diversity and spatial genetic structure can contribute to improved restoration outcomes, by identifying the most genetically appropriate source material for restoration sites. Here, we make a retrospective assessment of the genetic management of a successfully restored seagrass meadow in Cockburn Sound, Western Australia.

Methods

Cockburn Sound is a natural embayment approximately 16 km long and 7 km wide, to the west of the southern end of the Perth metropolitan area. The embayment is protected by Garden Island and a reef system to the west that provides shelter from the main oceanic currents and winter storms. Cockburn Sound has seen a 77% decline in seagrass cover since 1967, largely due to the effects of eutrophication, industrial development and sand mining (Kendrick *et al*. 2002). In small, localised areas, natural recruitment has been very successful, while other parts have not been able to recruit and recover naturally.

A transplant trial was conducted between 2004 and 2008, as part requirement of a seagrass loss compensation measure mitigating the impacts of shell sand dredging. The transplant site

consisted of a total of 3.2 hectares of bare sand at 2.2–4.0 m depth on Southern Flats, Cockburn Sound (32°15.091′S°115° 43.418′E). Donor material was sourced from a naturally occurring seagrass meadow on Parmelia Bank, at the northern end of Cockburn Sound, approximately 16 km away from the recipient site. Plagiotropic mature pieces of a dominant local seagrass, *Posidonia australis* Hook.f., with well-developed rhizomes and roots were collected from the leading edge of seagrass meadows. Sprigs (15–20 cm length) were harvested from the donor material after it was brought to the surface. Each sprig was then tied to a purpose-designed wire staple (30 cm in length) using biodegradable cable ties. Sprigs were kept under water as much as possible, and wire pegs were collated into groups of five before being secured with string to enable accurate quantification, transport, handling and planting at the recipient site. The sprigs were planted into a bare sand area at 50-cm shoot spacing by SCUBA divers, using metal quadrats as a guide, by gently excavating enough sediment to receive the rhizome/root material and securing the sprig within the sediment using the attached wire staple. Sprig survival was periodically monitored in 10 × 10 m representative subplots (15–20 plots per hectare to take into account possible variability between planting days) over a period of 5 years.

Shoot material was collected from established plants for microsatellite DNA genotyping from the donor site in 2004 and from the 2007/2008 plantings in the restoration site in January 2012 (Table 1). DNA was extracted from shoot meristem and genotyped using seven polymorphic markers (Sinclair *et al*. 2009). Genetic (clonal) diversity within meadows was assessed using the software GENALEX v6.1 (Peakall & Smouse 2006). GENALEX was also used to visually represent the genetic relationships between all sampled multilocus genotypes (MLGs) via a principal coordinate analysis (PCA), as well as calculate genetic subdivision among the two sites with *F*-statistics.

Results

Multilocus genotypes for seven loci were obtained for 94 samples from the donor and restoration sites for P. australis. High levels of diversity were detected, almost identical between the donor site and the restoration site, although the restoration site contained a large number of private alleles (Table 1). This is perhaps reflected in that no MLGs were shared between sites. The spatial arrangement of MLGs in the PCA (Fig. 1) shows the restoration site was sourced from the same genetic provenance (FST = 0.007 ± 0.002).

Discussion

The restored seagrass meadow is a restoration success, having grown well to fill in gaps to become a healthy, self-sustaining meadow, with first flowering in July 2010, 3 years after initial transplant. Our genetic sampling was carried out from mature shoots only, thus meaning that they were collected from original donor material, rather than newly seedling recruits. Seagrasses are generally regarded as highly clonal; however, clonal richness for *P. australis*, based on microsatellite DNA markers, varies extensively among meadows within the Cockburn Sound area (R = 0.1–0.96, Sinclair *et al*. unpublished). Levels of genetic diversity were very high in the restored meadow and nearly identical to the donor meadow on Parmelia Bank. The lack of shared MLGs between the restored and donor meadows indicates that transplants were not sourced from the exact location of our original sampling. The high level of genetic diversity in the restored meadow reflects the high level of diversity in the donor meadow and young age of the meadow.

This has been a retrospective look at genetic diversity within a small successfully restored *P. australis* meadow, and the first estimate of genetic diversity in a transplant programme for this widespread Australian species. Our results show that high genetic diversity in the donor site was captured in the donor material used for this restored meadow. While we cannot directly attribute the success of this site to genetic diversity alone, a healthy, now reproducing, restored meadow is well on the way to becoming a self-sustaining meadow and attracting new recruits within the meadow as well as in the surrounding sandy areas. This may suggest that once some transplants become established, the seafloor may stabilise enough to allow natural recruitment from dispersing seeds within or from other meadows, or the plants provide a natural trap for seeds to settle and establish. (We note that *Posidonia* 'seeds' germinate prior to dispersal and bear a plumule and radical and hence are sometimes referred to as 'seedlings'.) A major contributing factor may also be the result of good site selection.

As with many restoration projects, the current project was conducted on a small scale. Scaling up is very labour intensive and with significant impacts on donor sites (see Seddon 2004), although some positive developments are being made (Verduin *et al*. 2012). The refining of field techniques (e.g. through improved local site selection for transplants) and the use of genetic approaches to select and manage appropriate genetic material can improve the success of restoring *P. australis* meadows, at least on a small scale. Areas adjacent to Cockburn Sound have healthy seagrass meadows that produce very large numbers of fruit (containing germinating seeds) annually. Genetic and ecological data on dispersal capabilities of *P. australis* indicate that seeds are capable of dispersing tens of kilometres (Kendrick *et al*. 2012; Ruiz-Montoya *et al*. 2012). Despite very few reports of natural recruitment in the literature for this species (e.g. Cambridge *et al*. 2006; Bastyan &

Cambridge 2008), we suggest that natural recruitment through long-distance dispersal of seed may help to ensure the long-term viability of restored seagrass meadows initially established with transplants in south-west Western Australia.

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Table 1. Summary of sampling and genetic diversity indices for the sampled donor and restoration sites: MLG = number of unique multilocus genotypes; clonal richness R = (MLG−1)/(N−1), where G is the number of distinguishable genets and N is the number of sample units; the total number of alleles (Na); private alleles (p[I]); observed heterozygosity (Ho); expected heterozygosity (He)

Sample site	*N*	Area (m^2)	Distance between shoots	MLG	R	Na	*p*[I]	H_o	H_e
Donor site	47	900	20.3	46	0.98	40	7	51.3	53.1
Restoration site	47	1963	20.0	45	0.96	44	11	47.8	50.0

Figure 1. Principal coordinate analysis (PCA) showing the spatial overlap of multilocus genotypes (MLGs) for the donor site (closed symbol) and the restoration site (open symbol: RS). No MLGs were shared between sites.