

Short communication

The effect of boat propeller scarring intensity on genetic variation in a subtropical seagrass species

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Abstract

We report here the effect of one form of disturbance, boat propeller scarring, on genetic variation in the subtropical seagrass *Halodule wrightii*. We developed an amplified fragment length polymorphism assay to measure genetic variation in plots representing four levels of scarring intensity: reference (0% scarring), low (1–5%), moderate (5–15%) and severe (>15%). Although we found severely scarred plots to have the lowest, and moderately scarred plots to have the highest, mean genetic diversity estimates (H_e , P), differences among scarring levels were found to be non-significant ($\alpha=0.05$). Analysis of molecular variance also showed no significant effect of scarring intensity. While propeller scarring can cause significant habitat loss, scarring intensities of up to 20% may not yet have seriously affected those factors (population size, flowering density, recruitment, gene flow) that strongly influence population genetic variation. The relatively recent occurrence of this type of disturbance, however, could mean that any long-term effects have yet to be detected.

Keywords: disturbance; genetic variation; propeller scarring; seagrass.

Disturbance, fragmentation and habitat loss from natural or anthropogenic causes are serious issues for seagrass ecosystems (Short and Wyllie-Echeverria 1996, Duarte 2002, Orth et al. 2006). Anthropogenic disturbance, in particular, has been implicated in numerous incidences of seagrass loss,

ranging from the fragmentation of once continuous beds to the elimination of seagrasses from specific bays (Pulich and White 1991, Robblee et al. 1991, Onuf 1994). The effects of these losses on coastal ecosystems are considerable, and are expected to increase as coastal populations continue to grow (Orth et al. 2006).

Most studies of disturbance or fragmentation have focused either on the cause or their effect on habitat size, function or biodiversity (Saunders et al. 1991, Short and Wyllie-Echeverria 1996, Fahrig 2003). Disturbance and fragmentation, however, can also alter population genetic variation through effects on population size, isolation, dispersal, or recruitment, among other factors (Young et al. 1996, DiBattista 2008). These effects are not always negative. For example, while many instances of disturbance are correlated with reduced population size, allelic diversity, or heterozygosity (Young et al. 1996, Jacquemyn et al. 2003, Lienert and Fischer 2003, DiBattista 2008) others have been correlated with increased allelic and/or genotypic diversity (Coffroth and Lasker 1998, Kudoh et al. 1999, Hammerli and Reusch 2003), a result frequently attributed to increased recruitment or dispersal. This has important implications for seagrass ecosystems, where genetic diversity is an important factor in resilience and recovery from disturbance (Hughes and Stachowicz 2004, Reusch et al. 2005).

One form of disturbance that fragments seagrass meadows is propeller scarring produced by shallow draft recreational boats (Zieman 1976, Dunton and Schonberg 2002). Scarring physically removes plants, which may have detrimental effects on genetic variation. If a significant number of genets is removed, it could result in a reduced effective population size, leading to decreased allelic diversity over time through random genetic drift. Scarring may also reduce sexual reproduction (heterozygosity) and increase differentiation among beds if flowering shoots are removed and the potential for pollen-mediated gene flow is diminished (Young et al. 1996, Hammerli and Reusch 2003, Reusch 2003). Conversely, propeller scarring may have beneficial effects on genetic variation if the removal of genets is limited and swaths created in the seagrass canopy lead to increased recruitment, genet intermingling and subsequent sexual reproduction (Duarte et al. 2006, Reusch 2006). Scarring also breaks the rhizome connections between ramets, possibly destroying the putative advantages of larger clones, allowing smaller ones to be more competitive (Hammerli and Reusch 2003, Diaz-Almela et al. 2007). Though less probable, propeller scarring may also contribute to gene flow among beds by uprooting and

dispersing vegetative fragments (Harwell and Orth 2002, Campbell 2003).

We undertook this study to examine the effect of propeller scarring on genetic variation in the subtropical seagrass *Halodule wrightii* (Ascherson) from Redfish Bay, Texas, USA (Figure 1). We developed an amplified fragment length polymorphism (AFLP) assay (Vos et al. 1995) to measure genetic variation from 120 samples distributed among replicated plots representing four levels of propeller scarring: reference (0% removal of vegetation from scarring), low (1–5%), moderate (5–15%) and severe (>15%) (Figure 2). The AFLP assay produced a total of 160 reproducible (98%) markers that were used to score each sample. Scores indicated each sample to be a unique genotype. Marker scores were also used to calculate estimates of population genetic diversity, such as the proportion of polymorphic loci (P), mean expected heterozygosity (H_e) and Jaccard similarity coefficient (J) (Table 1). While severely scarred plots had the lowest, and moderately scarred plots the highest, mean



Figure 1 *Halodule wrightii*: study area in Redfish Bay, Texas, USA. Sampling sites (each ~0.2 km²) are numbered. Four 10×25 m sampling plots were established in each of six sites: 1) 0% removal of vegetation from propeller scars (reference); 2) 1–5% scarring (low); 3) 5–15% scarring (moderate); and 4) >15% scarring (severe). Scarring intensity was determined from maps (Dunton and Schonberg 2002), aerial surveys (2003) and intensive ground truthing (2003–2004).

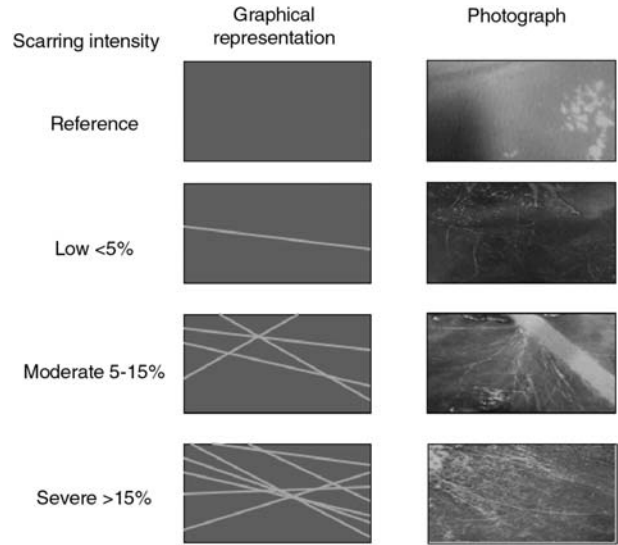


Figure 2 *Halodule wrightii*: graphical and photographic representations of scarring intensities (modified from Sargent et al. 1995). Reference (0% scarring); low (1–5%); moderate (5–15%); and severe (>15%). All photographs taken from Redfish Bay, Texas, USA. Five (leaf) samples were taken from each plot, spaced 5 m apart, for a total of 20 samples per site (30 per scarring level). DNA was extracted from leaf tissue using the Dneasy Plant miniprep kit (Qiagen, Valencia, CA, USA). The AFLP assay was developed using the AFLP amplification kit for regular genomes from Applied Biosystems (Foster City, CA, USA). Selective amplification was performed using *Mse* I-CT and *Eco*RI-ACA primers. AFLP Product bands were separated and scored using a Beckman-Coulter (Fullerton, CA, USA) CEQ 8000 Genetic Analyzer and fragment analysis software program.

values for both P and H_e (Table 1), ANOVA indicated these differences were non-significant ($\alpha=0.05$, data not shown). Similarity among samples within plots (J), a crude estimate of inbreeding, was found to be lowest for moderately scarred plots though, again, differences among scarring levels were not significant. Analysis of molecular variance (AMOVA, Excoffier et al. 1992) also found that scarring intensity had

Table 1 *Halodule wrightii*: genetic diversity and similarity estimates for four levels of propeller scarring in Redfish Bay, Texas, USA.

Scarring level	P	H_e	J
Reference	0.39±0.05	0.13±0.02	0.64±0.05
Low	0.38±0.07	0.13±0.02	0.64±0.07
Moderate	0.44±0.06	0.15±0.03	0.55±0.06
High	0.33±0.05	0.12±0.02	0.64±0.05

P , proportion of polymorphic loci; H_e , mean expected heterozygosity; J , Jaccard coefficient of similarity.

Binary (1,0) AFLP marker scores were used to calculate genetic diversity and similarity estimates using the GenAlEx™ and NTSYSpc™ software packages (Rohlf 2000, Peakall and Smouse 2005). Values represent means±SE for each scarring level, calculated from average values for plots from six replicate sites (5 samples/plot, 30 samples total per scarring level).

Table 2 *Halodule wrightii*: analysis of molecular variance (AMOVA) table for examining effect of propeller scarring intensity on genetic variation.

Source	df	SS	Variance component	% Total	p-Value
Between scarring levels	3	79.0	0.0	0	0.855
Among plots within scarring levels	20	589.9	2.9	17	0.001
Within plots	96	1423.6	14.8	83	0.001

Binary (1,0) AFLP marker scores were used for AMOVA calculations using the GenAEx™ software package (Peakall and Smouse 2005).

no significant effects (Table 2). The location of sampling plots (sites), however, was significant and accounted for 17% of the variation.

The role of location in explaining genetic variation led us to examine genetic differentiation at two levels: between sites in Redfish Bay (0.75–6 km apart) and between plots within sites (25–100 m apart). With one exception, we found significant ($p \leq 0.05$) genetic differentiation between all sites, indicating genetic structure at the kilometer scale in Redfish Bay (Table 3). Differentiation estimates between plots within sampling sites were generally not significant, with individual exceptions, although these did not correspond to any particular scarring intensity (data not shown).

Our results indicate that propeller scarring has a weak effect on *Halodule wrightii* genetic variation in Redfish Bay, at least at the scale examined (up to 20% removal of vegetation). Most likely this is due to a lack of effect on population size (e.g., removal of ramets vs. genets), flowering density, or on some type of balance between removal and recruitment. For example, Reusch (2006) found that while experimental removal of *Zostera marina* cover increased the occurrence of new genotypes, clonal diversity did not significantly change over a 2-year period. Also, while there is little information on the lifetime of individual scars, preliminary data from Redfish Bays indicate they may recover in as little as two years in *H. wrightii* meadows (Texas Parks and Wildlife Department, personal communication). Studies with other species indicate that regrowth into disturbed areas occurs primarily through clonal processes (Olesen et al. 2004, Hammerstrom et al. 2007). The relatively quick recov-

ery through putatively clonal processes may preclude any long-term effects on population size or flowering density as long as new scars are not being created at equivalent or greater rates.

Scarring is also a relatively recent phenomenon that has increased with coastal population growth and the rise in popularity of recreational motorboats (last 25–50 years). Therefore, insufficient time may have elapsed to detect an effect. In this regard, it is interesting to note the results of a study by Lowe et al. (2005). In a meta-analysis of the effects of habitat loss and degradation on neotropical trees, they showed that while most studies found no significant effects on genetic variation, a majority did find significant, negative impacts on inbreeding, reproductive output and fitness. Thus, while disturbance in the short-term may exhibit negligible effects on measurable genetic variation, it does not mean that damage has not occurred.

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References

Campbell, M.L. 2003. Recruitment and colonisation of vegetative fragments of *Posidonia australis* and *Posidonia coriacea*. *Aquat. Bot.* 76: 175–184.

Coffroth, M.A. and H.R. Lasker. 1998. Population structure of a clonal gorgonian coral: the interplay between clonal reproduction and disturbance. *Evolution* 52: 379–393.

Diaz-Almela, E., S. Arnaud-Haond, M. Vliet, E. Álvarez, N. Marbà, C. Duarte and E. Serrão. 2007. Feed-backs between genetic structure and perturbation-driven decline in seagrass (*Posidonia oceanica*) meadows. *Conserv. Genet.* 8: 1377.

DiBattista, J. 2008. Patterns of genetic variation in anthropogenically impacted populations. *Conserv. Genet.* 9: 141–156.

Duarte, C.M. 2002. The future of seagrass meadows. *Environ. Conserv.* 29: 192–206.

Duarte, C.M., J.W. Fourqurean, D. Krause-Jensen and B. Olesen. 2006. Dynamics of seagrass stability and change. *In:* (A.W.D.

Table 3 *Halodule wrightii*: genetic differentiation (Φ_{ST}) estimates between scarring test sites in Redfish Bay, Texas, USA.

Site	1	2	3	4	5	6
1	0					
2	0.175	0				
3	0.074	0.082	0			
4	0.161	0.092	0.045	0		
5	0.157	0.113	0.056	0.070	0	
6	0.166	0.094	0.076	0.068	0.017*	0

All values significant at $p < 0.05$ (* $p = 0.08$). Genetic differentiation estimates based on Φ_{ST} statistic, analogous to Wright's F_{ST} when binary data are used (Excoffier et al. 1992). A Mantel test revealed no significant relationship between Φ_{ST} values and physical distance between sites. All calculations were performed with the GenAEx™ software package (Peakall and Smouse 2005).

- Larkum, R.J. Orth and C.M. Duarte, (eds) *Seagrasses: biology, ecology and conservation*. Springer, Dordrecht, pp. 271–294.
- Dunton, K.H. and S.V. Schonberg. 2002. Assessment of propeller scarring in seagrass beds of the south Texas coast. *J. Coast. Res.* 37: 100–110.
- Excoffier, L., P.E. Smouse and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479–491.
- Fahrig, L. 2003. Effects of habitat fragmentation of biodiversity. *Annu. Rev. Ecol. Syst.* 34: 487–515.
- Hammerli, A. and T.B. Reusch. 2003. Inbreeding depression influences genet size distribution in a marine angiosperm. *Mol. Ecol.* 12: 619–629.
- Hammerstrom, K.K., W.J. Kenworthy, P.E. Whitfield and M.F. Merello. 2007. Response and recovery dynamics of seagrasses *Thalassia testudinum* and *Syringodium filiforme* and macroalgae in experimental motor vessel disturbances. *Mar. Ecol. Prog. Ser.* 345: 83–92.
- Harwell, M.C. and R.J. Orth. 2002. Long-distance dispersal potential in a marine macrophyte. *Ecology* 83: 3319–3330.
- Hughes, A.R. and J.J. Stachowicz. 2004. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proc. Natl. Acad. Sci. USA* 101: 8998–9002.
- Jacquemyn, H., F. Van Rossum, R. Brys, P. Endels, M. Hermy, L. Triest and G. De Blust. 2003. Effects of agricultural land use and fragmentation on genetics, demography and population persistence of the rare *Primula vulgaris*, and implications for conservation. *Belg. J. Bot.* 136: 5–22.
- Kudoh, H., H. Shibaike, H. Takasu, D.F. Whigham and S. Kawano. 1999. Genet structure and determinants of clonal structure in a temperate deciduous woodland herb, *Uvularia perfoliata*. *J. Ecol.* 87: 244–257.
- Lienert, J. and M. Fischer. 2003. Habitat fragmentation affects the common wetland specialist *Primula farinosa* in north-east Switzerland. *J. Ecol.* 91: 587–599.
- Lowe, A.J., D. Boshier, M. Ward, C.F.E. Bacles and C. Navarro. 2005. Genetic resource impacts of habitat loss and degradation: reconciling empirical evidence and predicted theory for neotropical trees. *Heredity* 95: 255–273.
- Olesen, B., N. Marba, C. Duarte, R. Savelle and M. Fortes. 2004. Recolonization dynamics in a mixed seagrass meadow: the role of clonal versus sexual processes. *Estuar. Coast.* 27: 770.
- Onuf, C.P. 1994. Seagrasses, dredging and light in Laguna Madre, Texas, USA. *Estuar. Coast. Shelf Sci.* 39: 75–91.
- Orth, R.J., T.J.B. Carruthers, W.C. Dennison, C.M. Duarte, J.W. Fourqurean, K.L. Heck, A.R. Hughes, G.A. Kendrick, W.J. Kenworthy, S. Olyarnik, F.T. Short, M. Waycott and S.L. Williams. 2006. A global crisis for seagrass ecosystems. *BioScience* 56: 987–996.
- Peakall, R. and P.E. Smouse. 2005. *GenAlEx6: genetic analysis in Excel – population genetic software for teaching and research*. Australian National University, Canberra. <http://www.anu.edu.au/BoZo/GenAlEx/>.
- Pulich, W. and W.A. White. 1991. Decline of submerged vegetation in the Galveston Bay system: chronology and relationships to physical processes. *J. Coast. Res.* 7: 1125–1138.
- Reusch, T.B.H. 2003. Floral neighbourhoods in the sea: how floral density, opportunity for outcrossing and population fragmentation affect seed set in *Zostera marina*. *J. Ecol.* 91: 610–615.
- Reusch, T.B.H. 2006. Does disturbance enhance genotypic diversity in clonal organisms? A field test in the marine angiosperm *Zostera marina*. *Mol. Ecol.* 15: 277–286.
- Reusch, T.B.H., A. Ehlers, A. Hammerli and B. Worm. 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proc. Natl. Acad. Sci. USA* 102: 2826–2831.
- Robblee, M.B., T.R. Barber, P.R.J. Carlson, M.J. Durako, J.W. Fourqurean, L.K. Muehlstein, D. Porter, L.A. Yarbro, R.T. Zieman and J.C. Zieman. 1991. Mass mortality of the tropical seagrass *Thalassia testudinum* in Florida Bay (USA). *Mar. Ecol. Prog. Ser.* 71: 297–299.
- Rohlf, F.J. 2000. *NTSYSpc Numerical taxonomy and multivariate analysis system*. Department of Ecology and Evolution, State University of New York, Stony Brook, NY.
- Sargent, F., T. Leary, D. Crewz and C. Kruer. 1995. *Scarring of Florida's seagrasses: assessment and management options*. Florida Marine Research Institute, St. Petersburg, pp. 43.
- Saunders, D.A., R.J. Hobbs and C.R. Margules. 1991. Biological consequences of ecosystem fragmentation: a review. *Conserv. Biol.* 5: 18–32.
- Short, F.T. and S. Wyllie-Echeverria. 1996. Natural and human-induced disturbance of seagrasses. *Environ. Conserv.* 23: 17–27.
- Vos, P., R. Hogers, M. Bleeker, M. Reijmans, T.v.d. Lee, M. Hornes, A. Friters, J. Pot, J. Paleman, M. Kuiper and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.* 23: 4407–4414.
- Young, A.G., T. Boyle and T. Brown. 1996. The population genetic consequences of habitat fragmentation for plants. *Trends Ecol. Evol.* 11: 413–418.
- Zieman, J.C. 1976. The ecological effects of physical damage from motor boats on turtle grass beds in Southern Florida. *Aquat. Bot.* 2: 127–139.

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