Genetic diversity of *Zostera noltii* populations under various levels of disturbance

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Introduction:

Effects of disturbances on genetic diversity and evaluation of the levels of genetic diversity required for population resilience constitute a major gap in current knowledge of how populations respond to perturbations. This is a major problem in coastal management, as conservation of genetic diversity may be essential to maintain the capacity of these systems to recover from new perturbations.

Recovery of seagrass communities following perturbations may achieve biomass recovery, but the effects on genetic diversity are unknown. Important loss of genetic diversity may result if disturbed areas recover their seagrass cover mostly by means of clonal propagation instead of sexual reproduction. Loss of genetic diversity can in turn decrease the resilience (i.e., the capacity of these systems to recover from perturbations) making these systems even more vulnerable to new perturbations.

The *Zostera noltii* populations in the Ria Formosa lagoon, Portugal, form an ideal model for studying these problems because the seagrass meadows in this area are frequently exposed to various disturbances that cause seagrass fragmentation at a variety of scales (Figure 1). However, it is not known whether genetic diversity might be affected following these perturbations.

Objective:

To investigate the effects of disturbances caused by natural and anthropogenic perturbations on the genetic diversity of the intertidal seagrass *Zostera noltii*. Understanding the effects of perturbations on genetic diversity and population resilience is crucial for predicting the capacity of populations to recover from further disturbances and for establishing policies for biodiversity conservation, because high genetic diversity is thought to be important to maintain the capacity of these systems to recover from new perturbations.

Hypothesis:

Genetic diversity is higher in relatively undisturbed meadows as compared to anthropogenically and naturally perturbated meadows. It is also hypothesized that clone sizes will be larger and higher in number in the "pristine" sites than in the perturbated areas. We further hypothesize that genetic diversity and disturbance level are non-linearly related. This follows the intermediate disturbance hypothesis where there is low genetic diversity at low and high levels of disturbance while it is higher when under intermediate disturbance (Figure 4).

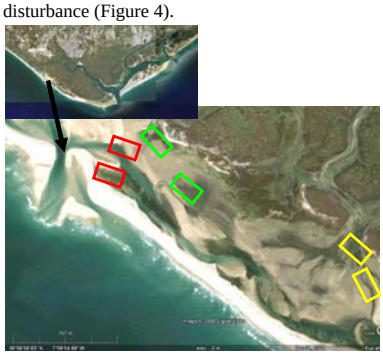

Figure 1. *Zostera noltii* sampling sites in the Ria Formosa Lagoon, Faro, Portugal. Yellow squares are the "pristine" areas, the green squares represent areas under constant antropogenic stress (shelfish harvesting) and the red squares represent natural disturbance by high sediment movement.

Clam harvest in action in a *Zostera noltii* meadow. The sediment is completely turned over and *Z. noltii* plants are uprooted. However, these disturbed areas quickly recover to 100 percent cover. What about the genetic diversity?

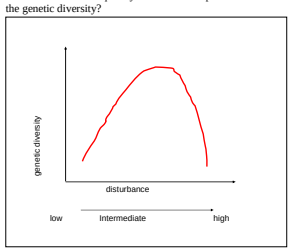

Figure 4. Representation of the intermediate disturbance hypothesis. Low genetic diversity in stable environment with large clones due to exclusion of less competitive genotypes. High diversity with intermediate disturbance resulting from higher sexual vs clonal reproduction and low diversity in the high disturbance areas ultimately leading to global mortality.

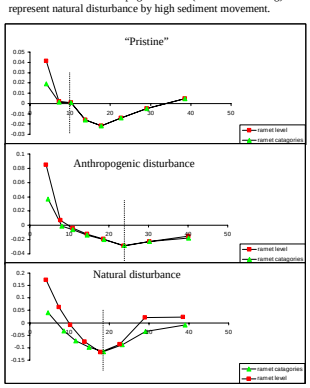

Figure 5. Autocorrelation plots for one of each disturbance levels. The red squares represent calculation when all ramets are taken into account. The green triangles represent analysis with only one ramet from each genet. The distance class where the two lines merge represents the clonal subrange (dashed line). This is the distance range beyond which clonality has negligible effects on genetic structure.

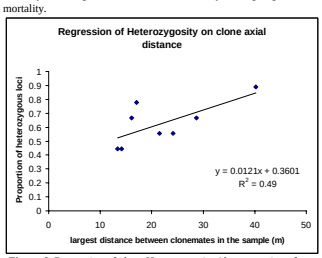

Figure 6. Regression of clone Heterozygosity (the proportion of heterozygous loci) on clone axial distance (the largest distance between clonemates observed in the sample). For this plot data from the six sampling grids were merged.

Methods:

Site selection: Three categories were selected for sampling. A relatively undisturbed ("pristine"), an anthropogenic (clam harverting) and a natural disturbed (sediment movement) area. In each of these 2 sites were selected for sampling (2 additional sietes were selected in the clam digged area).

Sampling: Shoots were collected in a 10 x 50 meter grid with a 2 meter interval. This results in a total of 150 shoots sampled per quadrat. The collected shoots were cleaned with paper towel and preserved in bags with silica gel.

DNA extraction: Only a small part of the shoot containing the meristem was used for DNA extraction. A CTAB extraction protocol was followed.

PCR and genotyping: We used 9 microsatellite loci to estimate genetic diversity and its spatial structure for *Zostera noltii*, in areas with contrasting levels of disturbance. Three multiplex PCR were necessary to amplify the full multi locus genotype. Genotyping was performed on a ABI377 automated sequencer. Genotypes were scored using ABI gene scan software.

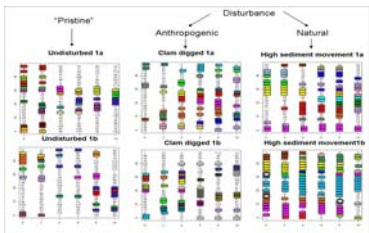

Figure 2. Clonal plots representing the number and size of clones in 10 x 50m quadrats in sites with different disturbance regimes. The minimum distance between consecutive samples is 2m. Colored numbers represent genets with more than 1 ramet. Numbers without color represent unique multi locus genotypes.

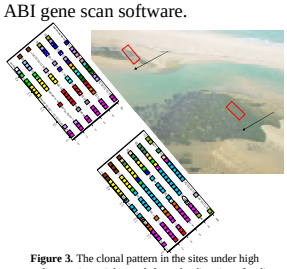

Figure 3. The clonal pattern in the sites under high sedimentation might result from the direction of sediment movement (direction of the arrows). The clones seems to run ahead of the sand movement, therefor all the large clones stretch over the total width of the sampling grid in the direction of the sediment movement to escape burrial.

Table 1. From the number of individuals sampled (N) and the number of multilocus genotypes (G) the genotypic richness (R) was calculated as (G-1)/(N-1). The inbreeding coefficient was calculated using Genetix software and the significance was determined after 100 permutations. The genetic diversity or allelic richness (Â) and the sd Â were calculated using the StandArich package using R software. Allelic Richness was calculated by standardizing for differences in populations size.

Populations	N	G	R	Fis		Â	sd_Â
Undisturbed 1	150	113	0.75	0.07768		7.441	0.259
Undisturbed 2	150	122	0.81	-0.00873	ns	7.728	0.229
High sedimentation 1	150	78	0.52	0.06671		7.336	0.256
High sedimentation 2	150	44	0.29	-0.03889	ns	6.111	0
Clam digged 1a	150	101	0.67	0.01225	ns	7.699	0.255
Clam digged 1b	150	113	0.75	0.0429		7.382	0.3
Clam digged 2a	150	94	0.62	0.02768	ns	7.788	0.177
Clam digged 2b	150	126	0.84	0.04745		7.303	0.24

Results & Conclusions:

The highest level of clonality (low genotypic richness) was found in the sites with high natural disturbance (sediment movement). Clone sizes found in these meadows ranged from ca.2 up to ca.100m^2. The highest number of unique multi locus genotypes were found in the undisturbed sites and in the sites under anthropogenic disturbance (clam harvesting) (Figure 2 and Table 1). The extremely high disturbance caused by sediment movement may cause this pattern by increasing genotype mortality and lowering recruitment/seedling survival.

The clonal subrange ranged from ca. 10 m in the undisturbed sites to ca 20 and ca 25 in the high sedimentation and clam harvesting site respectively (Figure 5.) This could mean that the undisturbed sites rely more on sexual reproduction and recruitment. There is probably high competition for space in these sites. The natural disturbed sites show high clonal reproduction. These sites are under sediment stress and probably invest most energy in clonal growth to keep ahead of the moving sand bar (Figure 3).

There is some preliminary evidence that when the space is cleared due to disturbance the recovery is mediated mostly by clonal response and the most heterozygous clones seem to be the ones in advantage to spread over open areas (Figure 6). This may be due to clonal integration, foraging advantage or other size-related fitness traits.

Our study does not show a clear effect of disturbance on genetic diversity. The genetic richness (Â) shows comparable values except for one of the high sedimentation plots (Table 1). There are no differences in heterozygosity between the different sites (data not shown).

It seems that our Undisturbed and Anthropogenically disturbed sites are not much different. These meadows both appear to be intermediately disturbed resulting in high genotypic and genetic diversity corresponding to the center of the Intermediate Disturbance plot (Figure 4). The meadows under high natural perturbation are more to the right side of the plot resulting in lower genotypic diversity in both and a lower genetic richnes in one of the meadows.

Acknowledgements
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