New insights into the population genetics of partially clonal organisms: when seagrass data meet theoretical expectations

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Abstract

Seagrass meadows are among the most important coastal ecosystems, in terms of both spatial extent and ecosystem services, but they are also declining worldwide. Understanding the drivers of seagrass meadow dynamics is essential for designing sound management, conservation, and restoration strategies. However, the poor knowledge of the effect of clonality on the population genetics of natural populations severely limits our understanding of the dynamics and connectivity of meadows. Recent modelling approaches have described the expected distributions of genotypic and genetic descriptors under increasing clonal rates. Here, in light of these recent theoretical developments, we revisited population genetic data for 165 meadows of four seagrass species. Contrasting shoot life span and rhizome turnover led to the prediction that the influence of asexual reproduction will increase along a gradient from *Zostera noltii* to *Zostera marina, Cymodocea nodosa* and *Posidonia oceanica*, which should be reflected by an increasing departure from Hardy-Weinberg equilibrium (F_{is}) and decreasing genotypic diversity (R). This meta-analysis provides a nested validation of this hypothesis at both the species and meadow scales through a significant relationship between F_{is} and R within each species. By empirically demonstrating the theoretical expectations derived from recent modelling approaches, this work calls for the use of Hardy-Weinberg equilibrium (F_{is}) rather than the strongly sampling-sensitive genotypic index R to assess the importance of clonal reproduction, at least when the impact of selfing on F_{is} can be neglected. The results also emphasize the need to revise our appraisal of the extent of clonality and its influence on the dynamics, connectivity and evolutionary trajectory of partial asexuals in general, including in seagrass meadows, to develop the most accurate management strategies.

Keywords: seagrass, mating system, clonal growth, dispersal, marine meadows

Introduction

Clonality is a life history trait spread across the Tree of Life (Halkett et al., 2005) that characterizes the species forming the basis of most important terrestrial and marine ecosystems. The drastic decline in many ecosystems engineered by partially clonal species (Carlsson Callaghan, 1994; Carpenter et al., 2008; Polidoro et al., 2010; Waycott et al., 2009) emphasizes the need to identify the drivers and life history traits, including reproductive strategies, underlying demographic declines or the colonization of new areas during range collapses, shifts, or expansions (Aitken et al., 2008; Callaghan et al., 1992; Cornelissen et al., 2014; Pecuchet et al., 2018; Yu et al., 2016). Although population genetics can provide essential indirect evidence, the use of population genetics has been limited thus far by the lack of clear theoretical predictions on the effect of partial asexuality on the distribution of genetic polymorphism, hampering the comprehensive analysis and interpretation of population genetics data.

The use of molecular markers has led to major improvements in the study of the architecture, dynamics, and evolution of clonal organisms, particularly since the development of molecular and analytical methods to detect clonality (Halkett et al., 2005; Tibayrenc et al., 1990) and to assess clonal membership (Arnaud-Haond et al., 2007b; Tibayrenc et al., 1990). These improvements have allowed the identification in natural populations of clonal replicates (i.e., ramets) of distinct "genetic individuals" (i.e., genets) through the recognition of clonal lineages (multi-locus genotypes, MLGs, or multi-locus lineages, MLLs). This has permitted the estimation of not only genetic (i.e., allelic richness A and heterozygosity H) but also genotypic (i.e., indices based on the number of MLGs or MLLs) diversity.

The simplest index of genotypic richness, G, responds to the number of MLGs or MLLs (Arnaud-Haond et al., 2007b). Since this specific unit of evolution (i.e. the genotype: Ayala, 1998) became accessible, genotypic diversity has been the subject of a growing number of studies aiming to investigate the resistance of natural populations in diverse environmental conditions (Hughes et al., 2008; Massa et al., 2013; Reusch et al., 2005). Such information is essential for understanding the evolution and dynamics of natural populations, including populations of environmental engineers (Callaghan et al., 1992; Cornelissen et al., 2014), such as corals and seagrasses, that form the basis of essential and declining coastal ecosystems (Carpenter et al., 2008; Hughes Stachowicz, 2009; Orth et al., 2006). G naturally increases with the sample size in terms of the number of ramets (Ellstrand & Roose 1987; Dorken & Eckert 2001). A variety of richness and diversity metric indices have thus been used to describe clonality in natural populations; some of these indices, such as the Shannon and Simpson indices, were borrowed from biodiversity literature, while others were simple indices ($Pd =$

G / N and R = (G-1) / (N-1)) based on the ratios of different genotypes (G) to the sample size (N) (Arnaud-Haond et al., 2007b). However, the versatile and inconsistent use of these indices, combined with a lack of a common standardized sampling strategy, have prevented sound biological comparisons of the extent of clonal reproduction and its consequences on the ecology and evolution of the diversity of studied organisms (Arnaud Haond et al., 2007a). For approximately a decade, R has been more consistently and widely used than other indices. Initially, thought to allow comparative studies, R is also sometimes considered a *proxy* for the relative influence of sexual *versus* clonal reproduction and the consequences of such a reproductive strategy for the dynamics of natural populations, the mutation models, the main unit targeted by natural selection and drift (genotypes or alleles), and the main drivers of migrations (propagules or adults/fragments for plants, fungi and several invertebrates such as corals). Unfortunately, the strong dependence of R on sampling strategy and density has also been clearly demonstrated in two previous studies (Arnaud-Haond et al., 2007c; Gorospe et al., 2015). In fact, those two studies, one based on a subsampling approach applied to two seagrass species and the other based on the exhaustive genotyping of a coral reef, showed a complete lack of stabilization of R with increasing sampling size and density. Basically, the index declines as new samples are added. This unfortunate property results in an inability to derive any equivalence between the R value and the extent of clonal reproduction, c, thus jeopardizing the management and conservation strategies for populations of partially asexual organisms and the ecosystems these populations support. In fact, only two recent programmes allow the computation of c from genetic data, and their use relies on the important but seldomly fulfilled requirement of temporal samples; these programmes are CloNcaSe (Ali et al., 2016), a method used for organisms with cyclical parthenogenesis, and ClonEstiMate (Becheler et al., 2017), a Bayesian method for samplings ideally separated by one generation.

As for genetic diversity indices, they are seldom used in ecological studies to appraise the influence of clonality. This may be partly because pioneer mathematical models suggest clonal rate has a very limited influence on the genetic composition of populations reported through F_{is} (de Meeûs et al., 2007) and linkage disequilibrium (Navascues et al., 2010) indices. These models suggest a departure from HWE towards heterozygote excess and linkage disequilibrium would be a signature of nearly exclusive clonality (Halkett et al., 2005). However, almost exclusively clonal lineages are exceptions, resulting in the interpretation of negative F_{is} values (heterozygous excess), when not overlooked or hastily discarded through over-conservative corrections for multiple tests (Bonferroni correction, Rice, 1989), as indicative of high clonality rates (Halkett et al., 2005). Such an interpretation path has been followed to interpret heterozygote excess in organisms such as pea aphids (Delmotte et al., 2002; Halkett et al., 2005b) and cultivated algae (Sousa et al., 1998; Guillemin et

al., 2008) as signature of clonal dominance, while the lack of a departure from HWE, together with high R, has been interpreted as a clue for predominant sexual reproduction (Krueger-Hadfield et al., 2011). More recent mathematical models have suggested the occurrence, at equilibrium, of larger inter-locus variance in F_{is} values and an expected departure from Hardy-Weinberg equilibrium (HWE) as the rate of clonality increases (Stoeckel Masson, 2014), even for modest rates of clonality(Reichel et al., 2016; Stoeckel et al., 2019). These findings were more recently used to enlarge the interpretation of deviations from HWE as indicative of partial (though not necessarily extreme) clonality in organisms such as invasive algae (Krueger-Hadfield et al., 2017)

Taken together, the severe sampling bias associated with R and the expected influence of partial clonality on genetic parameters may explain the paradoxical observation of heterozygote excess in partially clonal organisms (Reichel et al., 2016), despite the often elevated values for clonal richness, which are possibly partly due to a low sampling density (Arnaud-Haond et al., 2007c; Gorospe et al., 2015). Seagrasses are a good example of this paradox. On the one hand, moderate to high levels of clonal richness, R, together with limited but significant values of genetic differentiation (F_{st}), whether at the ramet or at the genet level, have led some authors to propose a strong influence of sexual reproduction on the dynamics and evolution of seagrass meadows, implying important recombination rates and large-scale dispersal (Kendrick et al., 2012; McMahon et al., 2014). On the other hand, the maximum values that F_{st} can reach are strongly limited by the use of highly polymorphic molecular markers (Hedrick, 1999a, 2005; Jost, 2008). In addition, elevated values for clonal richness often appear with significant but often-overlooked heterozygote excesses, thus paradoxically suggesting a high incidence of clonality (Reichel et al., 2016; Stoeckel et al., 2019).
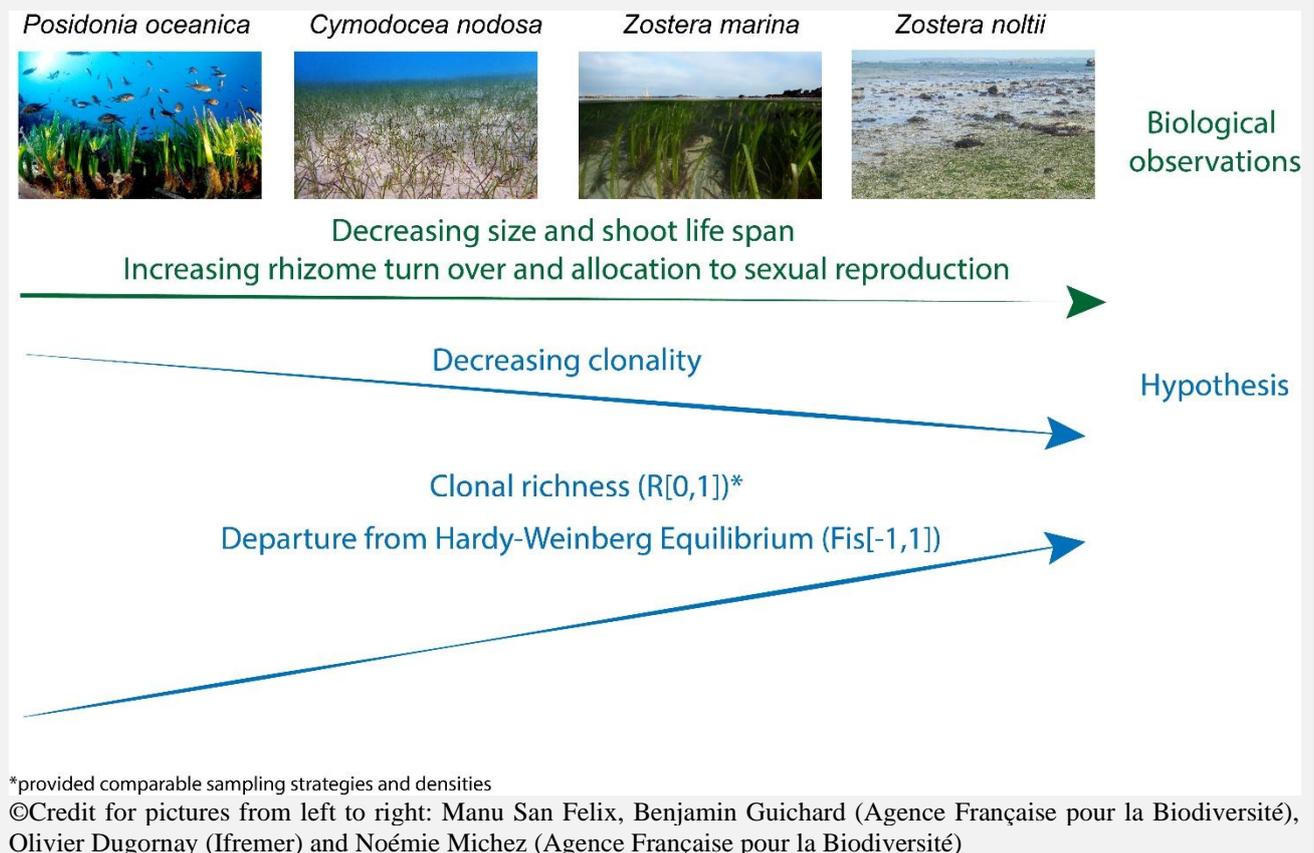
To resolve this paradox and better understand the meaning of R and F values as they relate to the extent of clonal reproduction and the importance of dispersal, we propose a re-analysis of previously published seagrass data in light of recent modelling developments describing the effect of increasing c values on the genotypic and genetic descriptors (including R and F_{is}) of populations (Stoeckel et al., 2019). These four seagrasses are the main species structuring coastal ecosystems along the Atlantic and Mediterranean coasts and exhibit a gradient from long-lived species exhibiting a slow turnover of shoots and low to moderate reproductive output, namely, *Posidonia oceanica* and *Cymodocea nodosa,* to shorter-lived species, namely, *Zostera marina* and *Zostera noltii*, which exhibit an increased shoot turnover and allocation to sexual reproduction (see Box1, Material and Methods section and Table 1). Considering the expected influence of clonality based on the knowledge of clonal growth and the allocation of the four seagrass species and more recent theoretical predictions of increasing heterozygote excess with increasing levels of clonality, we expected a progressive increase in clonal diversity and a progressive decrease in heterozygosity along this

gradient from *P. oceanica* to *Z. noltii*, starting with negative F_{is} values (heterozygote excess) and moving towards null (or slightly positive when heterozygote deficiency occurs due to inbreeding) F_{is} values for the shortest-lived species (Box 1).

Box 1: Main biological features of the four studied seagrasses and the associated hypothesis about the clonal architecture and genetic signatures.

Studies of the growth and dynamics of the four main European seagrasses, which inhabit coastal habitats from the inter- and subtidal compartments (*Z. marina* and *Z. noltii*) to water depths reaching depths up to and occasionally greater than 50 m (*C. nodosa* and *P. oceanica*), have shown marked discrepancies in the shoot growth, turnover and life span of these species, as well as investments in sexual reproduction.

This implies differences in the relative incidence of clonal reproduction in the short and long terms, affecting the pattern of space occupation, as well as the temporal dynamics of shoots and meadows at both ecological and evolutionary time scales. These are summarized in the scheme below, together with a hypothesis about the expected clonal signature tested in the present work and parameters describing the clonal richness (*R*) and genetic composition (F_{is}) of meadows.

*provided comparable sampling strategies and densities

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Here, we aimed to test these theoretical expectations by using a meta-analysis of population genetics data published on these four seagrass species to answer the following questions:

1) Do estimates of genotypic diversity deliver informative values that can be used to assess comparative investments in clonal *versus* sexual reproduction among species submitted to a similar sampling scheme?

2) Does departure from HWE in natural meadows reliably reflect the relative importance of clonal *versus* sexual reproduction among species with different investments in clonal growth and different life spans?

3) Do the two families of parameters (genotypic and genetic) provide congruent qualitative estimates at both the interspecific and intraspecific levels?

Material and methods

Studied species

Seagrasses reproduce clonally through rhizome elongation, and the rhythm and shape of this process differ among the studied species and largely scale with plant size (Duarte, 1991a; Marba Duarte, 1998). *P. oceanica* and *C. nodosa* are known to exhibit more stable rhizome connections, a longer shoot life span and a lower shoot turnover than the other species (Diaz-Almela et al., 2008; Duarte, 1991a; Marba et al., 1996; Marba et al., 1998). In contrast, *Zostera* species exhibit the fastest growth but a more limited occupation of space (Marba et al., 1998; Sintes et al., 2006), as well as a much shorter life span.

Plant size thus appears to be strongly related to turnover time and module (i.e., rhizome internodes, leaf clusters and roots) longevity (Duarte, 1991a). These parameters of clonal growth are accompanied by a relatively parallel trade-off between clonal architecture and sexual reproductive output. The events and success of flowering are highly temporally and spatially heterogeneous for *P. oceanica* and *C. nodosa*, which seldom produce more than several tens of seeds per square metre; in contrast, highly profuse episodes of annual flowering in *Zostera* can lead to the production of thousands of seeds per square metre (Marba et al., 1996; Marba et al., 2004). Finally, both *Zostera sp*. and *P. oceanica* are hermaphrodites, while *C. nodosa* is dioecious (Larkum & Den Hartog, 1989).

These descriptions of clonal architecture and sexual reproduction features allow us expect a decreasing influence of clonality from *P. oceanica* to *C. nodosa*, *Z. marina* and *Z. noltii* (see Table 1). Although no numerical estimates of shoot life span and rhizome turnover are available for the smallest and shorter-lived species *Z. noltii*, it is expected to exhibit a much higher turnover than the other species, in line with its habitat, which is characterized by relatively unstable environmental conditions (Duarte, 1991b). The fact that *C. nodosa* is an obligate outcrosser while other species can theoretically practice selfing may however result in different interferences between sexual reproduction and clonality on the distribution of heterozygosity (F_{is}) in this species compared to the other three, and is susceptible to affect the gradient expected on the basis of the sole influence of clonality (Box 1).

Interestingly, these species also often exhibit a gradient on the shore (Duarte, 1991b). *P. oceanica* and *C. nodosa* reach depths up to 40 to 50 metres (with P. oceanica dominating at larger

depth, den Hartog, 1970), while the two *Zostera* species are most often encountered in intertidal areas, with *Z. noltii* sometimes exposed to desiccation and stressful conditions (Massa et al., 2009). All four species have been the focus of large-scale genetic surveys for phylogeographic studies in the past few decades. Datasets are thus available that can be used to test expectations about the investment of the species in clonal reproduction and to screen for signatures of effects of clonal reproduction on their genetic composition.

Genetic datasets

The *P. oceanica* dataset contains 36 samples of approximately 40 units each, with a total of 1424 shoots or connected shoots representing sampling units (SUs) ranging from Spain to Cyprus, a range of over 4000 km of coastline. For each sampling site, the SUs were collected according to randomly defined coordinates in a quadrat 80 metres long and 20 metres wide. Seven microsatellite markers were used: Po15, Po5, Po5-40, Po5-49, Po5-10, Po4-3 and Po5-39. The database of genotypes was constructed in a previous meta-analysis (Arnaud-Haond et al., 2014). Three meadows that were initially studied for the impact of farms on the genetic composition of the species (the populations Amathous, Acqua Azzura and Agias Nicholaos; Diaz Almela et al., 2007) were discarded to avoid introducing bias.

The *C. nodosa* dataset (Alberto et al., 2008) consisted of 47 meadows containing approximately forty SUs each, with a total of 1586 SUs from Cyprus to the Canary Islands and Madeira. For each meadow, the SUs were selected randomly in a quadrat 60 metres long by 14 metres wide. Eight microsatellite markers were used: Cn2-38, Cn2-14, Cn2-24, Cn4-19, Cn2-16, Cn2-18, Cn4-29 and Cn2-45 (Arnaud-Haond et al., 2014).

The first dataset for *Z. marina* consisted of 13 quadrats sampled in 7 meadows with 30 SUs per quadrant, resulting in a total of 390 SUs. The meadows were on the Brittany coast from Saint-Malo to Arradon. For each sampling site, SUs were randomly selected in two quadrats 20 metres long by 30 metres wide in 2009 (Becheler et al., 2013; the same sampling was performed in 2011, but in order to avoid partially duplicate information, only the first time series was included). A total of 9 microsatellite markers were used: GA35, GA2, GA17H, GA23, GA12, GA19, GA20, GA16 and GA17D (Becheler et al., 2014; Becheler et al., 2010). The second dataset consisted of 14 meadows from Greenland to Southern Iberia, in which 427 SUs at least 1–1.5 m apart from each other were collected haphazardly (Diekmann Serrão, 2012a) and genotyped with 8 microsatellite markers: GA6, GA3, CT17H, CT19, CT3, CT20, GA2 and CT35 (Diekmann Serrão, 2012b). We removed genotypes with missing loci and populations with few SUs (for Greenland: Kobbefjord, with one SU; for Southern Iberian: Arrabida, with 4 SUs, and Sado, with 6 SUs), leaving 11 meadows and 403 SUs.

The *Z. noltii* dataset included 33 meadows sampled across the entire geographic range of the

species and genotyped with 9 microsatellite markers: ZnB1, ZnH10, ZnB3, ZnB8, ZnH8, ZnD6, ZnE7, ZnF8 and ZnF11 (Coyer et al., 2004). Different sampling protocols were used and are detailed in the original publication; these protocols include the random collection of SUs within quadrats 60 metres long by 20 metres wide, 10 metres long by 15 metres wide and 10 metres long by 25 metres wide, as well as sampling along a linear transect every metre. One sampling site (BSea3) represented by a single SU was excluded from the initial dataset; thus, 32 meadows were kept for a total of 1117 SUs.

Genetic and genotypic indices

RClone 1.0.2 (Bailleul et al., 2016) and GenClone (Arnaud-Haond Belkhir, 2007a) in *R* statistical software (R Core Team, 2015) were used to manage the different datasets and to compute the genetic and genotypic indices of interest. We chose to examine the F_{is} genetic index, for which models delivered several theoretical predictions, the genotypic clonal richness and index *R* the supposed inferred sexual reproduction rate. We also aimed at comparing results with the unbiased estimator of the complement of the Simpson index (estimator *L*) of heterogeneity known as one of the index less sensitive to sample size (Lande, 1996), and modified to vary positively with heterogeneity (Pielou, 1969) between 0 and about 1-(1/G):

$$D* = 1 - L = 1 - \sum_{i=1}^{G} \left[\frac{n_i(n_i - 1)}{N(N-1)} \right]$$

where n_i is the number of sampled units with the MLL_i.

R and D* were computed for each population, and F_{is} was computed for each population with (ramet level) and without (genet level) replicates.

The relationship between *R* or *D** and the F_{is} were considered at both the intra- and inter-species levels. The Pearson correlation index between *R* and the F_{is} was computed, and the significance was assessed. Linear models predicting the F_{is} based on *R* were constructed, and the determination coefficient r^2 and the F test were used to assess the quality of each model.

Results

Clonal richness, R

The mean genotypic index R increased gradually from *P. oceanica* to *C. nodosa*, *Z. marina* and *Z. noltii* (Table 1, Figure 1), with the variance (reflected by the maximal and interquartile ranges) of these values decreasing from *P. oceanica* to *Z. noltii*. Only *C. nodosa* slightly departed from this trend, with a mean R lower than that of *P. oceanica* and the greatest maximal ranges of R values among the seagrasses. As for the Simpson index D^* (Table 1; Figure S1), the values were constrained to a narrower range and the ranking of values among the four species was thus not very informative, although there were clearly lower values for the longer lived species than for *Zostera* sp.

Inbreeding coefficient, F_{is}

The F_{is} values and their means progressively increased from *P. oceanica* to *Z. noltii* (Table 1; Figure 1). Only *Z. noltii* showed a slightly positive mean F_{is}. The interquartile values were nearly strictly negative for *P. oceanica* and *C. nodosa*, with increasingly positive F_{is} values from *Z. marina* to *Z. noltii*. This progression of the F_{is} interquartile values was even clearer when the datasets were analysed without replicates (Figure 1) than with replicates (Figure S1).

Table 1: Summary of the clonal growth features (Duarte, 1991) for each of the four seagrass species, the average compiled values of genotypic heterogeneity (D^*) and diversity ($\bar{R}$) and departure from Hardy-Weinberg equilibrium ($\overline{F_{is}}$) and the linear correlations between the two latter (*: p<0.05; **: p<0.01;***: <0.001), which were obtained from the dataset including all genotyped ramets (with replicates, $\overline{F_{is}}_{\_AR}$) and the dataset including only multi-locus lineages, or genets (without replicates, $\overline{F_{is}}_{\_OG}$).

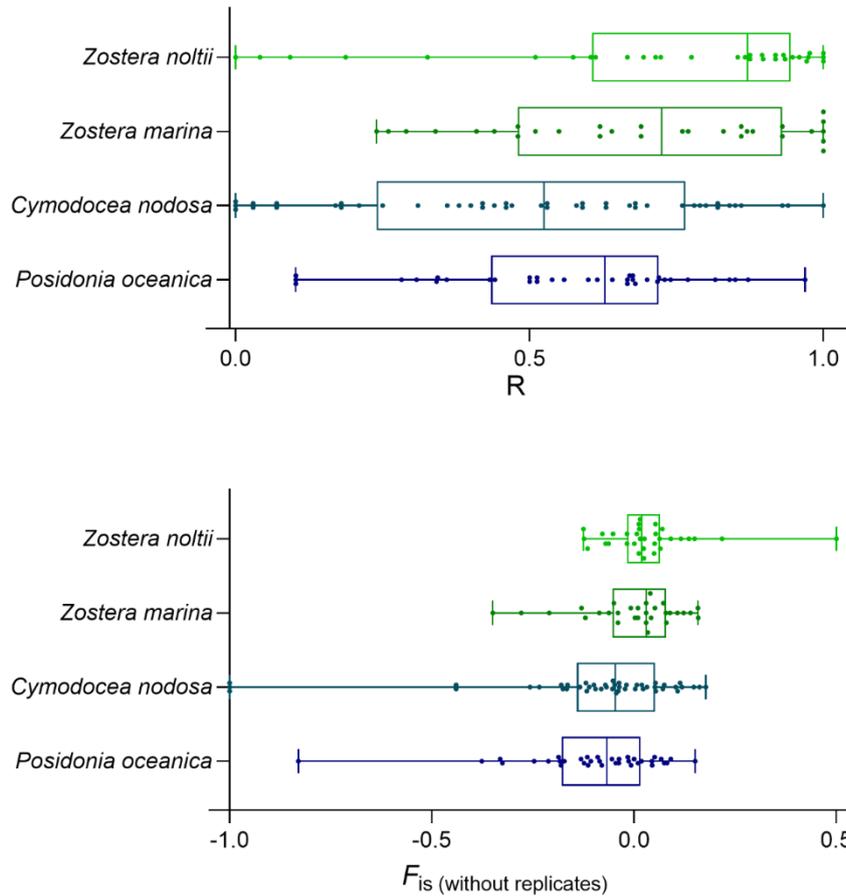|	rhizome turnover	shoot lifespan	D^*	$\bar{R}$	with replicates (ramet level)		without replicates (genet level)	
					$\overline{F_{is}}_R$	correlation coefficient	$\overline{F_{is}}$	correlation coefficient
Posidonia oceanica	0.09	11.98	0.88	0.57	-0.085	0.63***	-0.108	0.73***
Cymodocea nodosa	0.14	2.40	0.78	0.50	-0.075	0.76***	-0.002	0.48***
Zostera marina	2.19	1.52	0.98	0.71	-0.105	0.46***	-0.091	0.39**
Zostera noltii			0.97	0.75	0.012	0.17	0.015	0.14

Figure 1: Boxplot showing the average departure from Hardy-Weinberg equilibrium (F_{is}, with replicates, see Figure S1 for the same results at the ramet level) and genotypic richness (R) values over all studied meadows for each of the four seagrass species: *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera marina* and *Z. noltii*

Relationship between clonal indices and F_{is}

At the intra-species level (Figure 2, Table 1), the relationships between R and F_{is} were also positive and significant for all but the *Z. noltii* datasets. At the ramet level, the relationships ranged from highly positive (*P. oceanica* and *C. nodosa*) to slightly positive (*Z. marina*) and nearly null (*Z. noltii*). Similar results, though slightly weaker, were obtained without replicates (Figure S2, Table 1), with the exception of a stronger correlation for *P. oceanica* at the genet level than at the ramet level.
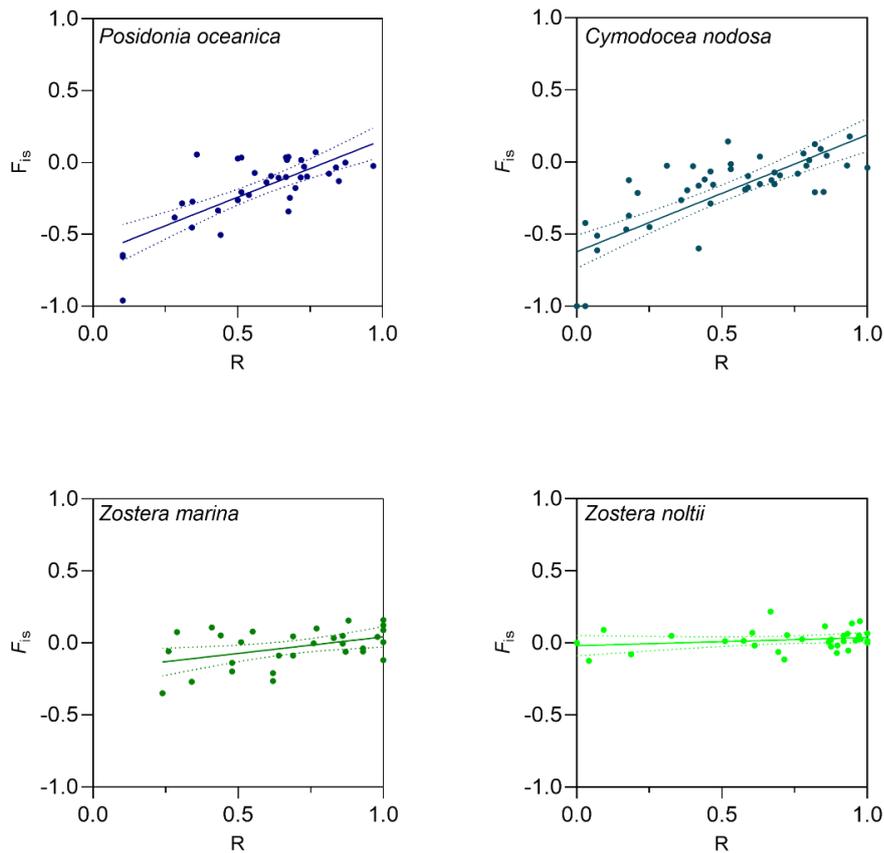
Figure 2: Relationships between the level of genotypic richness (R) and departure from Hardy-Weinberg equilibrium (F_{is}, at the genet level, i.e., without replicates; see Figure S2 for the ramet level data) at the meadow scale for each of the four seagrass species

In analyses both with (at the ramet level) and without (at the genet level) replicates, R was a good predictor of F_{is} in linear models (Table 1), with the exception of *Z. marina* when replicates were excluded and *Z. noltii* for datasets either with or without replicates. The F-tests were significant, with relatively high and significant r^2 indices for the three other species. At the inter-species level (Figure 3), the relationships between R and F_{is} were always positive and strong (Table 1). When considering only the genets (i.e., no replicates), the results were similar (Figure S3), but the correlations and the linear model were weaker than those obtained at the genet level (Table 1).

As for the index of heterogeneity D*, no relationship was observed for the three shorter lived species (with non-significant p-values and r^2 comprised between 0.00 and 0.05), probably due to the narrow range of values obtained. Only for *P. oceanica* both correlations were significant (r^2=0.71 and 0.18 respectively, with p<0.01 in both cases), with a noticeably lower correlation at the genet level.
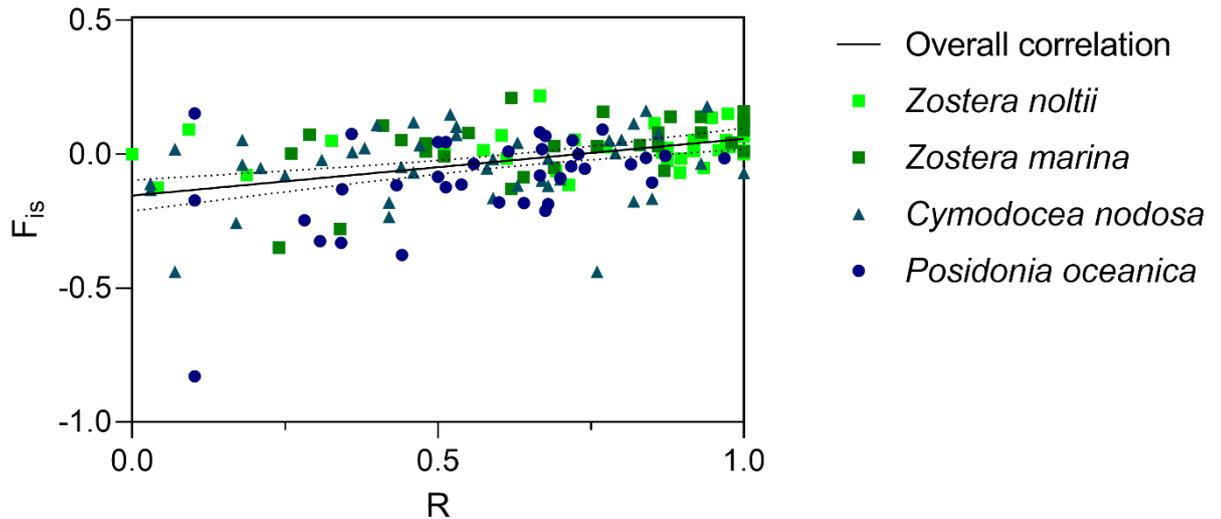
Figure 3: Overall relationships between the genotypic richness (R) and departure from Hardy-Weinberg equilibrium (F_{is}, at the genet level, i.e., without replicates; see Figure S3 for the ramet level data) at the meadow scale for each of the four seagrass species

Discussion

Here, we conducted a comparative re-analysis of genetic data from four species. The good ecological knowledge of the clonal architecture of these species allowed the testing and validation of predictions obtained from recent mathematical developments. Our analyses confirmed nearly all expectations derived from recent mathematical predictions about the relationships between 1) the turnover and longevity of shoots, 2) the prevalence of clonality, and 3) the consequential genotypic signatures and, above all, the genetic composition of natural meadows. When assessing the balance between clonal and sexual reproduction, by giving more weight to the genetic composition signature (F_{is}) than to the genotypic index, which is highly sensitive to sampling (i.e., R), this analysis unravelled an apparent paradox reported in the literature: the frequent observation of apparently high genotypic richness, which suggests a high rate of sexual input, and systematic departure from HWE towards heterozygote excess, which suggests a very high rate of asexual reproduction. The results were in line with recent theoretical developments (Stoeckel et al., 2019), showing a barely discernible signature of c on R and a much clearer signature on F_{is}. This trend was clear at both the inter-species and intra-species (among-meadow) levels, showing a nested pattern at both scales. Consequently, these results illustrate the need for a revised framework for the interpretation of molecular data from partial asexuals such as seagrasses, which may have important consequences for conservation and management strategies in the context of global change and increasing restoration needs (Carpenter et al., 2008; Hughes et al., 2008; Orth et al., 2006).

Fitting empirical data to model predictions

Our results support the ecological hypothesis that the importance of the clonal multiplication of ramets for the growth and maintenance of populations (compared to sexual input through seedlings) increases with increasing module lifespan.

First, the mean genotypic richness R gradually increases with decreasing longevity and increasing turnover of modules, from *P. oceanica* to *Z. noltii* (Figure 1). Although unable to provide a reliable quantitative assessment of the rates of clonality due to large subsampling bias (Arnaud-Haond et al., 2007b; Becheler et al., 2017; Gorospe et al., 2015; Stoeckel et al., 2019), R may remain useful in comparative studies, and shows a broader range to discriminate scenario than the Simpson index of heterogeneity D*. With a comparable sampling scheme and effort, R can help assess the relative importance of clonality among natural populations. Such comparability was true here for *P. oceanica* and *C. nodosa* and partly for the *Zostera* species. It is thus important to bear in mind that the use of R here was by no means an attempt to estimate c but was for comparing the values of meadows sampled with similar sampling scales and strategies.

In fact, the high diversity of *Z. noltii* in this analysis may be influenced both by the short life span (and high turnover) of its shoots and the slightly different sampling designs used to gather the data analysed for the species. Indeed, the *Z. noltii* datasets were collected by sampling along mostly linear transects, while the datasets of the other three species were randomly or haphazardly sampled in a standardized area appropriated for their known clonal architecture, which should have minimized bias (Pielou, 1966) and enabled comparative analysis. Linear transects result in significantly higher genotypic diversities than other sampling methods due to the strong edge effect (Arnaud-Haond et al., 2007b). It is thus not possible to rigorously disentangle the effect of high rhizome turnover and low shoot longevity from the putative edge effects on the ranking of *Z. noltii* in terms of genotypic richness. However, the expected ranking for F_{is} stands (Figure 1), suggesting life history traits had a stronger influence than sampling bias on these results.

This importance of sampling is reflected in a different way by the slight deviation from this general trend for *C. nodosa*. Despite having a slightly higher rhizome turnover than *P. oceanica* (Duarte, 1991a), *C. nodosa* shows lower mean R (and interestingly also lower mean D*) values and a comparable distribution of F_{is} values with even a tendency towards more extreme cases of heterozygote excess (Figure 1, Figure S1). Several hypotheses can explain this result. First, despite a slightly higher rhizome turnover and a much shorter shoot life span in *C. nodosa* than in *P. oceanica*, the establishment and dynamics of *C. nodosa* meadows may rely more on clonal reproduction than those of *P. oceanica* meadows. Indeed, *C. nodosa* exhibits irregular sexual reproductive events dependent on environmental conditions but also exhibits a much faster clonal extension rate than *P.*

oceanica (Marba et al., 1996; Marba et al., 2004). Moreover, although *C. nodosa* and *P. oceanica* were collected with an identical sampling strategy, the *C. nodosa* dataset contained more highly clonal meadows than the *P. oceanica* dataset, which may be partly due to its obligate dioecy (leading to colonizers, if lonely, to rely on clonal growth), as well as the inclusion of sampling sites located at the limit of its distribution (Billingham et al., 2003). In fact, repeating the same analysis in Mediterranean populations delivered higher mean R (and D^*) values, which were comparable only to those of *P. oceanica*, and a slightly less negative F_{is}, providing a better fit to the initial predictions.

Second, the increasing departure from HWE towards negative F_{is} (heterozygote excess, Figure 1 and 2) from *Z. noltii* to *P. oceanica* also supports the prediction of mathematical models (Stoeckel et al., 2014). As the prevalence of clonality increases, the trajectory of the population towards equilibrium slows down for both positive and negative F_{is} values, with less positive than negative F_{is} values (Reichel et al., 2016). In fact, F_{is} values are usually negative for *P. oceanica* and *C. nodosa*, and an increasing proportion of positive values are observed for *Z. marina* and *Z. noltii*. The tendency towards heterozygous excess for the first two species is even clearer when the data are considered at the genet (without replicates, Figure 2) rather than the ramet scale (Figure S2), with only interquartile F_{is} values equal to or greater than 0.

Interestingly, this inter-species pattern is also observed at the nested, intra-species level (Figure 2), showing that the relationship between estimates of clonal richness R and F_{is} transcends the species boundary and applies within species at the meadow scale. This phenomenon is reflected by a positive correlation between F_{is} and R among meadows for *P. oceanica*, *C. nodosa* and *Z. marina* (Table 1), again supporting the predictive power of population genetics models that explicitly take partial clonality into account. Using a standard sampling strategy, many more clonal meadows (i.e., those exhibiting lower R values) tend to exhibit higher departures from HWE towards heterozygote excess (negative F_{is} values), again in line with theoretical predictions.

Finally, three of the four species are hermaphrodites with no existing data suggesting self-incompatibility. Nevertheless, all of the species show heterozygote excess at the ramet level, and this excess is maintained at the genet level to a different extent for two of the species. Sexual reproduction significantly departing from random mating due to selfing or inbreeding could pull F_{is} towards positive values reflecting heterozygote deficiency, wiping off at least part of the signature of clonality. Here, if any inbreeding or selfing would occur in populations of the three hermaphroditic species, negative F_{is} still suggest a predominant influence of clonal over sexual reproduction. The occurrence of such departure to random mating may however explain the fact *C. nodosa*, which is the only obligate outcrosser, is also the species that slightly departs from the global ranking expected among species by showing extreme heterozygote excess similar to or higher than the values obtained for the longer-lived *P. oceanica*.

Considering the accumulated ecological and physiological knowledge of the rhizome growth and dynamics of these species, our comparison of theoretical predictions, which come from a 'simple' Wright-Fisher-like model extended to explicitly include clonality, with the population genetics data gathered on four seagrass species, shows remarkable congruence. This result highlights the need i) to extend population genetics theory to predict the dynamics of genetic diversity while accounting for various reproductive systems and ii) to expand the production and interpretation of empirical data to enhance our understanding of the main drivers of demography and connectivity in natural populations, as well as the possibility of achieving these goals.

Implications for understanding clonal versus sexual prevalence and its influence on the dynamics and evolution of natural populations

Species ranges are the result of multiple ecological and evolutionary drivers, among which genetic drift, selection and dispersal are essential processes strongly influenced by both environmental factors and demography (Gaggiotti, 2017). The accurate use of molecular markers and population genetics tools and models thus requires a good theoretical understanding of the ways by which evolutionary forces, including the reproductive system, drive the temporal and spatial dynamics of genetic polymorphism to in turn infer demographic dynamics and history from molecular data. New theoretical works are needed to explore a broader range of life history traits (including for example the genetic architecture under the combined effect of clonality and haplo-diploidy or selfing), but also target the development of estimators of c from genetic data. In fact, current indices based on classical ecological (i.e. non-exhaustive) sampling mostly provide imperfect proxies of the relative importance of clonal *versus* sexual reproduction (Stoeckel et al., 2019). Understanding the respective roles of clonal and sexual recruitment and dispersal in determining local demography and spatial connectivity is essential for forecasting the evolution of these features in the context of global change and future range shifts. This knowledge is also a prerequisite for defining accurate management measures, and the effort to obtain this knowledge has been a strong incentive underlying population genetic studies of seagrass during the past few decades (Alberto et al., 2005; Arnaud-Haond et al., 2007b; Arnaud-Haond et al., 2012; Kendrick et al., 2012). Although the importance of clonal growth in the colonization, expansion, and maintenance of meadows has long been acknowledged (Duarte, 1991b; Kendrick et al., 2012), recent studies have proposed migration *via* the production and dispersal of seeds as a possible central driver of the dynamics and persistence of seagrasses (Kendrick et al., 2012; McMahon et al., 2014). This hypothesis is rooted in the observation of moderate to elevated levels of genotypic and genetic diversity, together with limited genetic differentiation and isolation by distance, in a panel of species that contains the four species targeted here and the Australian *Posidonia australis*, the Pacific *Zostera pacifica* and the Atlantic *Thalassia*

testudinum (Kendrick et al., 2012).

Despite being represented by a limited number of species (approximately 70), seagrasses are characterized by diverse life history traits (Hemminga Duarte, 2000; McMahon et al., 2014), as illustrated by the panel of four species examined here. The hypothesis proposed by Kendrick *et al.* (2012) may thus apply to some seagrass species, particularly short-lived species producing a large number of seeds (Phan et al., 2017), especially if one also accounts for the dispersal of vegetative fragments (McMahon et al., 2014). However, the prevalence of clonality is associated with a decrease in the loss of diversity due to the influence of drift (Reichel et al., 2016), which may partly explain the elevated levels of genetic diversity observed in well-established meadows. Those high levels of alpha diversity also intrinsically limit the maximum possible estimates of beta diversity (genetic differentiation as estimated through Fst, Charlesworth, 1998; Gregorius, 2010; Hedrick, 1999b) and their saturation, which can be well described by the disruption of isolation by distance patterns over large scales (Kendrick et al., 2012).

Part of the observations that led to the hypothesis of the central role of seed dispersal may thus reflect the prevalence of clonal reproduction, regardless of whether high connectivity exists. In fact, similar observations of high diversity and limited differentiation with no large-scale pattern of isolation by distance led to the exploration of mutation patterns across the distribution range of the long-lived species *P. oceanica* and *C. nodosa* at the distribution range scale (Arnaud-Haond et al., 2014). Disentangling the spatial distribution pattern of polymorphism revealed the accumulation of somatic mutations through clonal propagation had a stronger influence than the pattern of dispersal of sexual propagules, implying the influence of clonal reproduction was stronger than that of sexual reproduction on the dynamics and evolution of meadows at large spatial (distribution range) and temporal (evolutionary) scales (Arnaud-Haond et al., 2014).

The results presented here thus support the co-existence of complex trade-offs between clonal and sexual reproduction and dispersal among seagrass species, which may be better understood by specifically taking into account the large diversity of life history traits these species express (McMahon et al., 2014). Disentangling these cases individually and accurately assessing the level of genotypic diversity, as well as the influence of genotypic diversity on the resistance of natural populations (Hughes et al., 2008; Massa et al., 2013; Reusch et al., 2005), are challenging but are also extremely important for designing sound management and restoration strategies in a fluctuating environment (McMahon et al., 2017), representing a major research axis to develop in the future. Marine connectivity research has been enriched in recent years by the improvement of predictive (oceanographic modelling, Robert Sponaugle, 2009) and molecular (next-generation sequencing and high-density genome scan) tools (Riginos et al., 2016; Selkoe et al., 2016). The former can be readily enriched through the extensive knowledge gained by seagrass ecologists on the broad diversity of life

history traits determining the timing and extent of the production and dispersal of sexual and clonal propagules (Duarte, 1991a; McMahon et al., 2014). The latter will certainly benefit research on seagrass population genetics by allowing a finer-grained snapshot of the distribution of polymorphisms and the various origins of mutations at nested spatial scales to better disentangle the relative impact of different evolutionary forces on their emergence and spatial spread. Finally, the integration of oceanographic and molecular information in new integrative Bayesian frameworks of analysis is currently underway (Gaggiotti, 2017) and represents a promising path for grasping the diversity and complexity of seagrass strategies in terms of demography and dispersal.

Conclusion

The data re-analysed here on four seagrass species support both i) the ecological hypothesis of higher clonality in longer-lived, lower-turnover species and ii) the mathematical hypothesis of greater deviation from HWE towards an excess of heterozygotes at higher levels of clonality. This re-analysis also supports iii) the statistical prediction that, with the usual sampling density and grain size (although useful in comparative studies with rigorously standardized sampling strategies), the use of the genotypic diversity index R to infer c will systematically lead to gross underestimates of its true prevalence (Arnaud-Haond et al., 2007b; Gorospe et al., 2015; Stoeckel et al., 2019). The results, which were obtained for three hermaphroditic species and an obligate outcrosser, also suggest that the screening of F_{is} would greatly help to assess the importance of clonal reproduction for many species, including depending on the importance of clonal reproduction and selfing, self-fertilizing species. These results, in line with theoretical predictions, thus support the use of the signature of heterozygote excess (a negative F_{is}), which is less prone to large sampling bias than genotypic richness (R), to assess the extent of clonal *versus* sexual reproduction. Non-negative F_{is} values ~~of~~ may however not be interpreted as the absence of partial clonality, particularly in those cases where other forces (species exhibiting potential for high selfing rates, or datasets prone to null alleles for example) may counterbalance the effect of clonality on F_{is}. By emphasizing this often-overlooked signature of clonal prevalence in the literature, the results also call for a revision of our perception of the balance between sexual and clonal reproduction in natural populations of partial asexuals such as seagrass meadows. Recent empirical and theoretical developments now provide directions towards improved estimates of these essential parameters to provide sound interpretations of population genetics data, elucidate the short-term drivers of the natural dynamics and the resistance of meadows, forecast meadow resilience or shifts in their distribution range under global change, and define sound conservation strategies. Finally, this meta-analysis is the first attempt to revisit empirical data in light of recent theoretical developments (Stoeckel et al., 2019), calling for similar research on important clonal species such as other ecosystem engineers, important crops, invasive species and pathogens.

Acknowledgements

Author Contributions

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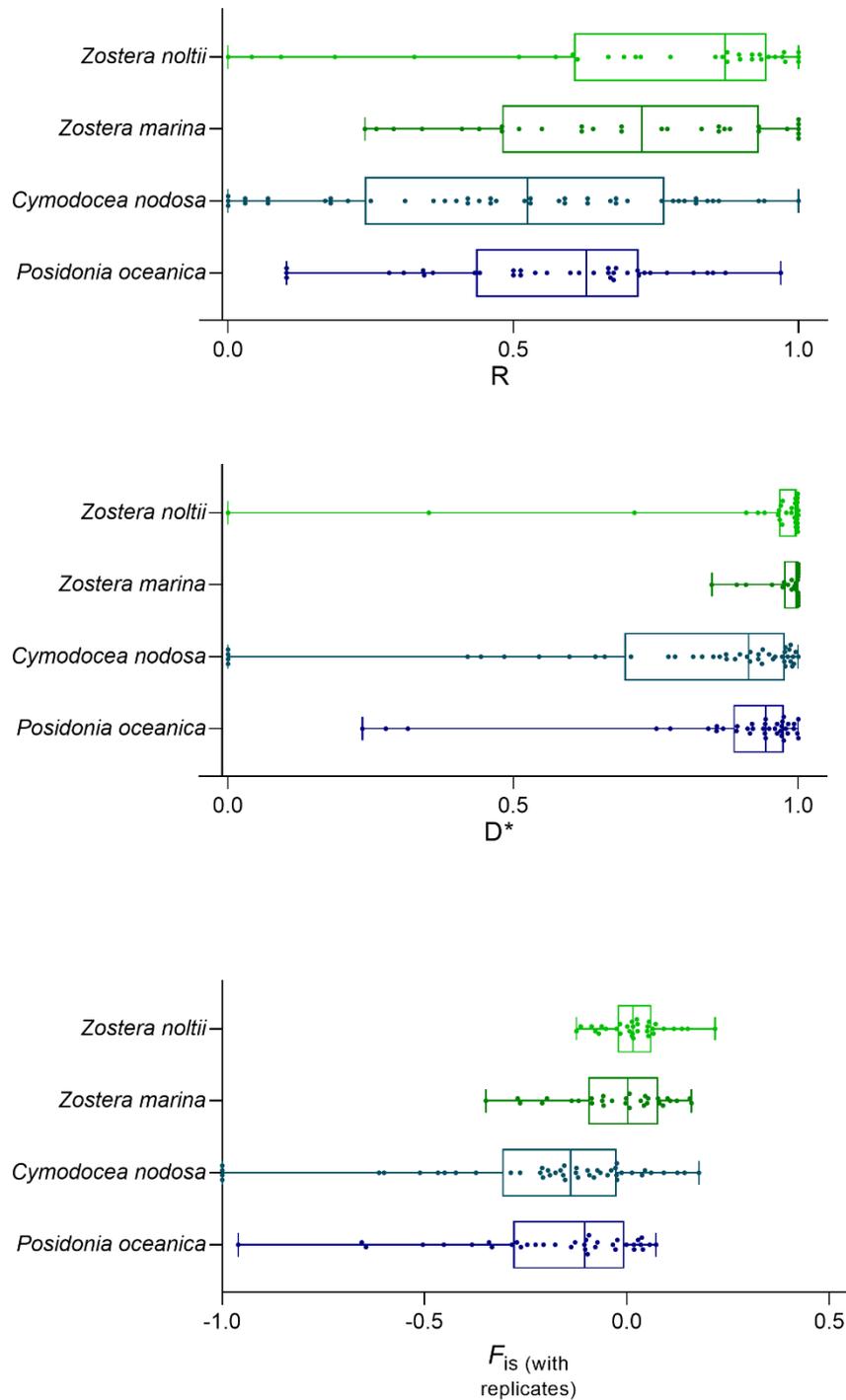
Supplementary material

Figure S1: Boxplot showing the average departure from Hardy-Weinberg equilibrium (F_{is}, with replicates) and genotypic richness (R) and heterogeneity (D*) values over all studied meadows for each of the four seagrass species: *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera marina* and *Z. noltii*
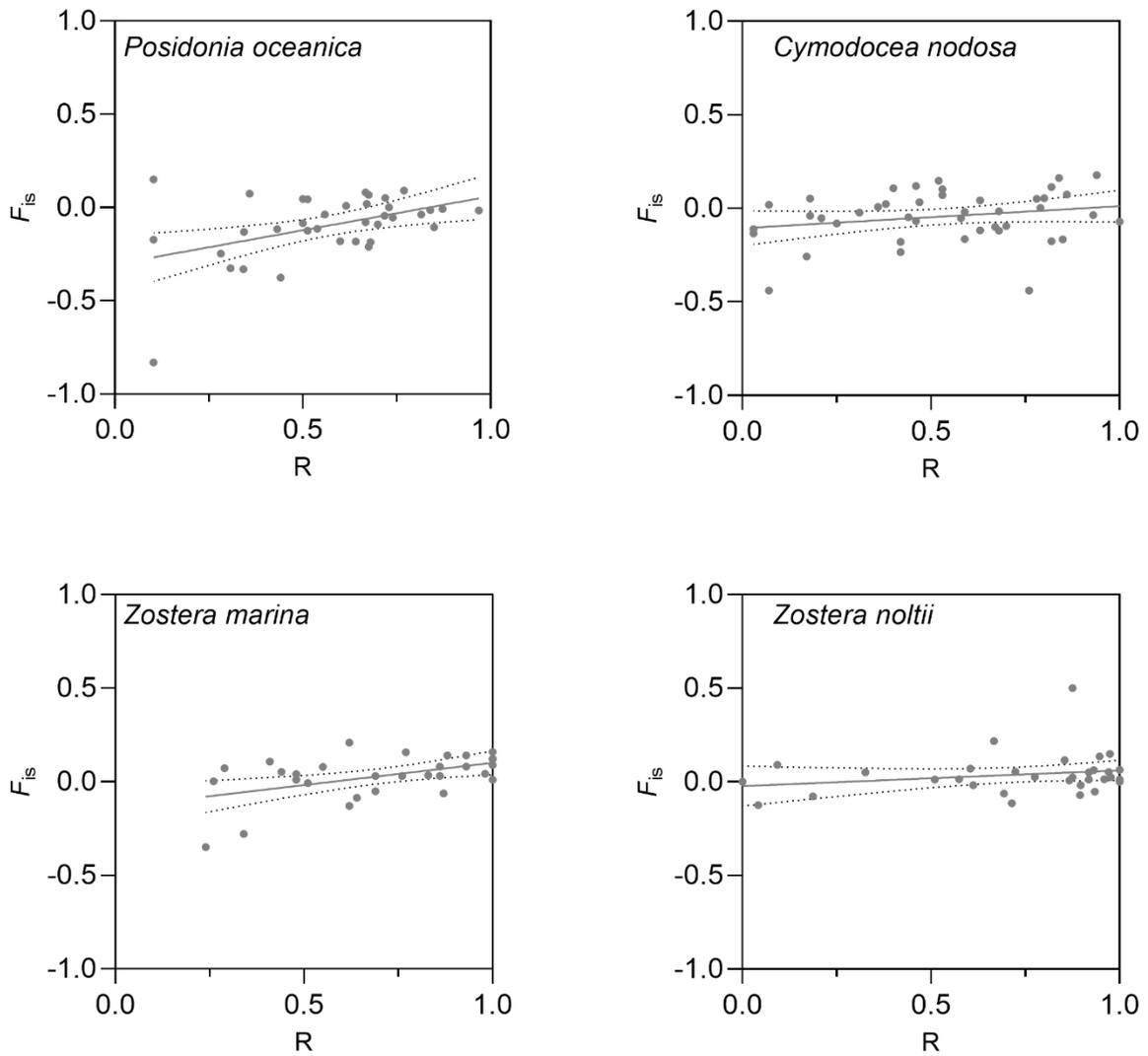
Figure S2 : Relationships between the level of (A) genotypic richness (R) and (B) complement Simpson index of heterogeneity (D*) departure from Hardy-Weinberg equilibrium (F_{is}, at the ramet level, i.e., with replicates) at the meadow scale for each of the four seagrass species
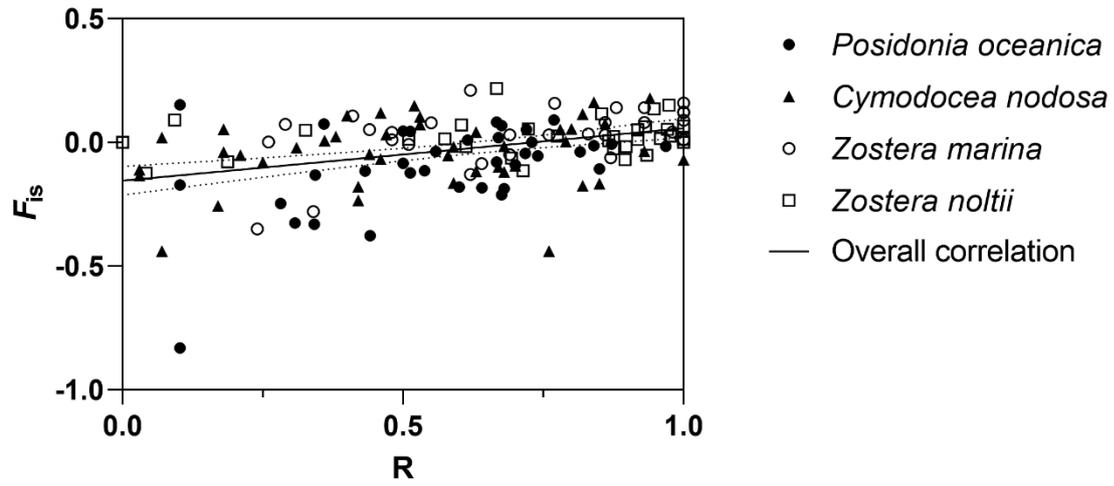
Figure S3: Overall relationships between the level of genotypic richness (R) and departure from Hardy-Weinberg equilibrium (F_{is}, at the ramet level, i.e., with replicates) at the meadow scale for each of the four seagrass species.