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A meta-analysis reveals a positive correlation between genetic diversity metrics and environmental status in the long-lived seagrass *Posidonia oceanica*

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Abstract

The seagrass *Posidonia oceanica* is a key engineering species structuring coastal marine systems throughout much of the Mediterranean basin. Its decline is of concern, leading to the search for short- and long-term indicators of seagrass health. Using ArcGIS maps from a recent, high-resolution (1–4 km) modelling study of 18 disturbance factors affecting coastal marine systems across the Mediterranean (Micheli *et al.* 2013, <http://globalmarine.nceas.ucsb.edu/mediterranean/>), we tested for correlations with genetic diversity metrics (allelic diversity, genotypic/clonal diversity and heterozygosity) in a meta-analysis of 56 meadows. Contrary to initial predictions, weak but significantly positive correlations were found for commercial shipping, organic pollution (pesticides) and cumulative impact. This counterintuitive finding suggests greater resistance and resilience of individuals with higher genetic and genotypic diversity under disturbance (at least for a time) and/or increased sexual reproduction under an intermediate disturbance model. We interpret the absence of low and medium levels of genetic variation at impacted locations as probable local extinctions of individuals that already exceeded their resistance capacity. Alternatively, high diversity at high-impact sites is likely a temporal artefact, reflecting the mismatch with pre-environmental impact conditions, especially because flowering and sexual recruitment are seldom observed. While genetic diversity metrics are a valuable tool for restoration and mitigation, caution must be exercised in the interpretation of correlative patterns as found in this study, because the exceptional longevity of individuals creates a temporal mismatch that may falsely suggest good meadow health status, while gradual deterioration of allelic diversity might go unnoticed.

Keywords: anthropogenic disturbance, genetic diversity, local extinction, meadow resistance, *Posidonia oceanica*, seagrass, temporal mismatch

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Introduction

Recent biodiversity conservation actions and mitigation measures are aimed at reducing the impact of anthropogenic activities on the natural environment, for example the Aichi targets of the Convention for Biological Diversity (CBD), the European Marine Strategy

Framework Directive (MSFD) and Water Framework Directive (WFD) and the Ecosystem Approach of the Mediterranean Action Programme (UNEP-MAP). A major concern is the high rate of species-level extinctions, often equated with loss of biodiversity, as well as extinctions of local populations (Allendorf *et al.* 2013). Especially in the marine environment, where connectivity is generally higher, actual species extinctions appear to be relatively rare, whereas local population declines or local extinctions of species are more

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commonly reported (Carlton *et al.* 1991; Almany *et al.* 2007; Occhipinti-Ambrogi 2007; Short *et al.* 2011; Cardinale *et al.* 2012).

Intraspecific genetic diversity is the most fundamental level of biodiversity, and the conservation of genetic diversity is an explicit goal of the CBD (Laikre *et al.* 2010). Assessment of genetic diversity metrics, population structure and connectivity provides insights about the demographic history under different types of anthropogenic disturbance, which in turn provides both a retrospective view and a glimpse ahead to the future evolutionary potential of a population (Procaccini *et al.* 2007). Seagrasses often form mono-specific meadows, which are—despite the low species diversity of the habitat-providing species—extremely productive and show a high species richness of associated fauna and flora. Field and mesocosm experiments designed to test for relationship between genetic diversity and ecosystem function (BEF) in the seagrass genus *Zostera* have shown that high genotypic diversity (i.e. the number of genetically different clones in a given sample) is associated with increased resistance (Hughes & Stachowicz 2004; Massa *et al.* 2013), resilience (Reusch *et al.* 2005; Ehlers *et al.* 2008) and productivity (Hughes *et al.* 2008). Likewise, genetic diversity expressed as allelic richness and/or heterozygosity has been shown to be important for positive population dynamics and ecosystem services in both *Zostera* and *Posidonia* (Procaccini & Piazzini 2001; Williams 2001; Reynolds *et al.* 2012; Massa *et al.* 2013).

Seagrasses are important ecosystem engineers of coastal regions worldwide, but are increasingly threatened (Waycott *et al.* 2009). Anthropogenic disturbances are the main cause for population decline in seagrasses (Waycott *et al.* 2009; Short *et al.* 2011; Grech *et al.* 2012), and the potential effect of disturbances (natural and anthropogenic) on genetic diversity can be summarized in four main scenarios: (i) extinction of populations under severe disturbance; (ii) a decline in allelic diversity, that is the standing variation and genetic potential on which selection for adaptation operates (and in the long term mainly affected by small effective population size (N_e), which magnifies the effects of genetic drift and loss of variation); (iii) the decline in genotypic diversity through the survival of large, long-lived clones as a result of selection, clonal integration and general resistance; and/or (iv) an increase in both allelic and genotypic diversity as a consequence of increased sexual reproduction and recruitment in sparse or gap areas within large meadows. All of the aforementioned scenarios can be considered in the context of the intermediate disturbance hypothesis (IDH; Connell 1978), in which regular disturbances of intermediate strength are predicted to lead to maximal diversity, with low and

high disturbances leading to lower diversity levels. Although the IDH has been recently criticized (Fox 2013), it remains a conceptually useful model and has been experimentally shown to play a role in the eelgrass, *Zostera marina* (Reusch 2006). The relationship between disturbance and genetic diversity is, however, not straightforward: disturbance can affect genetic diversity of a population, while genetic diversity can also affect the response of a population to disturbance. This reciprocal causality (Hughes *et al.* 2007) renders it difficult to gauge the relative contribution of disturbance strength and frequency in relation to its short-term vs. long-term effects on genetic components on diversity.

Posidonia oceanica (L.) Delile is an iconic habitat-forming seagrass species endemic to the Mediterranean, with essential ecological functions including wave attenuation, carbon storage and oxygenation of the water column (Diaz-Almela & Duarte 2008). It is extremely long-lived (decades to centuries; Ruggiero *et al.* 2002; Arnaud-Haond *et al.* 2012) and has a very low growth rate, and sexual reproduction is rare (Diaz-Almela *et al.* 2006). Some meadows consist of large clones extending for hundreds of metres and can even be completely uniclinal (Ruggiero *et al.* 2002; Arnaud-Haond *et al.* 2007). Available estimates of *P. oceanica* areal extent indicate that up to 50% might have been lost in the last 50 years in the western Mediterranean and that a considerable proportion of the remaining meadows have become more fragmented and less dense (Marbà *et al.* 2014).

Only two studies in *P. oceanica* have specifically investigated relationships of genetic diversity components in relation to population growth, mortality and disturbance. Diaz-Almela *et al.* (2007a) examined the impact of fish farms on meadow regression at four locations in the Mediterranean. The authors found that the mean number of alleles and the presence of rare alleles were decreased at impacted locations, but that particular genotypes/clones were larger as compared to the less impacted control sites. They concluded that allelic richness would likely continue to be lost in subsequent generations and therefore negatively affect the population in the future. They further suggested that survival of the larger clones under disturbed conditions was facilitated by clonal integration and foraging capacity, while the loss of small clones was in response to high sedimentation rates close to the fish farms. In a subsequent study, Arnaud-Haond *et al.* (2010) surveyed 30 meadows ranging from severely impacted fish-farm locations to meadows with stable population dynamics. Contrary to theoretical expectations, they found increased mortality with increasing genotypic diversity, and decreased population growth rates with increasing

allelic diversity. They also attributed their observations to dominance of large clones (lower genotypic diversity) arising through past selection and higher fitness, and/or competitive exclusion and clonal integration. Both of these processes would confer resistance advantages to short-term environmental impacts. Interestingly, when the extreme outlier cases from the fish-farm meadows were removed, there was no correlation between diversity and mortality indicating a threshold of disturbance beyond which clonality no longer played a decisive role. Both studies concluded that, while meadows may have reduced genotypic diversity (i.e. dominance of a few large clones) and superior resistance to disturbances, long-term loss of allelic diversity would continue. For these reasons, it is important that studies utilizing genetic diversity metrics carefully separate the roles of allelic and genotypic/clonal diversity from environmental impact effects on ecosystem function.

From a management and conservation perspective, the search for indicator metrics of contemporary seagrass health is ongoing (Marbà *et al.* 2013b). In this study, we posed the question: How well do genetic diversity metrics correlate with environmental impact categories? To this end, we performed a meta-analysis using previously published population genetic data on *P. oceanica* covering a substantial portion of the Mediterranean basin, and using ArcGIS maps (Micheli *et al.* 2011) that modelled 18 disturbance factors as well as cumulative impact across the Mediterranean (Halpern *et al.* 2008; Micheli *et al.* 2013). Specifically, we (i) test for correlations between 18 specific anthropogenic threats and genetic and genotypic diversity; (ii) qualitatively evaluate pairwise combinations of low, medium and high allelic richness with low, medium and high impacts; (iii) assess how well the combination of threat mapping and genetic diversity corresponds to perceived levels of impact at specific sites based on local appraisal questionnaires by scientists who conducted the original research; and (iv) evaluate the limitations of using genetic metrics as an indicator in assessing ecosystem health status.

Material and methods

Genetic data

Genetic diversity data from a total of 74 meadows were reviewed from published studies. Meadows for which no human impact data were available (mainly in very shallow water or lagoons) and duplicate meadows were excluded. This resulted in 56 meadows being selected for the meta-analysis (Fig. 1 and Table S1, Supporting information). Sampling scales were roughly similar among studies, ranging from 5- to 10-m intervals

between shoots, which is appropriate for *Posidonia oceanica* (Table S1, Supporting information). Sample sizes per meadow ranged from 16 to 50 and were subsequently standardized into two data sets. The first data set was standardized on an equal number of ramets, while the second was standardized on an equal number of unique genotypes (see below). In all cases, only results obtained with the same set of 13 microsatellite loci were compared [Po 15, Po 5, Po 5–40, Po 5–49, Po 5–10, Po 4–3, Po 5–39, Poc trn, Poc35, Poc 45, Poc42, Poc26, Poc5 (Procaccini & Waycott 1998; Alberto *et al.* 2003)]. Samples with missing genotypes were excluded from the analyses.

Because *P. oceanica* is characterized by extensive clonal growth, the number of shoots sampled in a given area may represent distinct genotypes or they may represent duplicate genotypes collected from the same clone. To account and correct for this feature, identification of duplicate multilocus genotypes (MLGs) was performed in GenClone (Arnaud-Haond & Belkhir 2007) through the calculation of P_{sex} , the probability that identical MLGs arose by chance vs. those that arose from sexual reproduction. Genotypic richness was then calculated for the standardized data set ($N = 14$) according to Dorken & Eckert (2001): $R = (MLG - 1) / (N - 1)$, where N is the number of individuals.

Allelic richness and heterozygosity were calculated in two ways: (i) based on a standardized number of samples ($N = 14$) from all 56 meadows with GENALEX 6.5 (Peakall & Smouse 2012) and (ii) based on a smaller data set of 31 meadows (Table S1, Supporting information) in which duplicate genotypes were removed, and for which a standardized set of at least 20 genotypes were present. For the smaller data set ($MLG \geq 20$), allelic richness was calculated using the STANDARICH package (<http://www.ccmr.ualg.pt/maree/software.php?soft=sarich>) and R software (R Development Core Team 2012) and observed heterozygosity was calculated with R software (R Development Core Team 2012), based on individual observed heterozygosity as implemented in the GENHET program (Coulon 2010).

Disturbance data

Mapped threat categories across the Mediterranean basin were downloaded from <http://globalmarine.nceas.ucsb.edu/mediterranean/> in which 19 different threats (Table 1) were identified based on publicly available, *in situ* and satellite data (Micheli *et al.* 2011).

Threat categories included (for a detailed description of threat categories included in the analysis, see Halpern *et al.* 2008) (i) nondestructive demersal fishing with low bycatch; (ii) destructive demersal fishing; (iii) non-destructive demersal fishing with high bycatch; (iv)

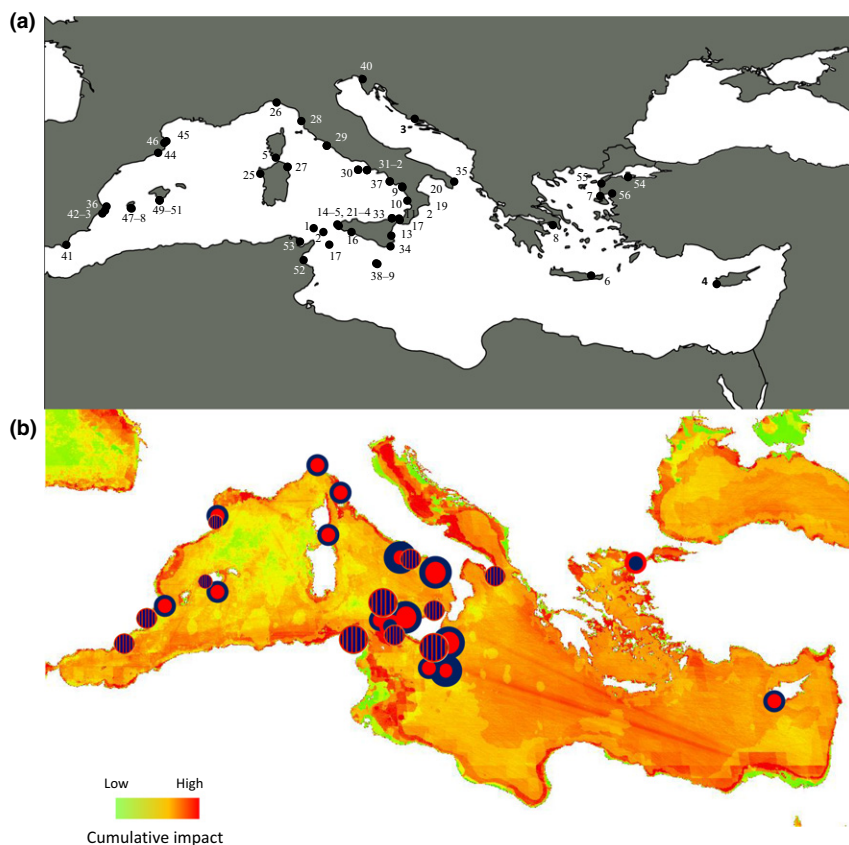


Fig. 1 Sampling locations and impact map. (a) *Posidonia oceanica* meadows included in the correlation analysis. Details of the meadow names and their geolocation can be found in Table S1 (Supporting information). (b) Categorized values of genetic diversity as allelic richness (blue) of the smaller data set (MLG = 20) and cumulative impact (red) superimposed on the heat map of cumulative impact from Halpern *et al.* (2008), modified. Dot sizes (small, medium and large) are proportional to the level of allelic richness and cumulative impact. Striped dots indicate that diversity and impact were of the same category level. Only 27/31 locations are shown as four of the locations were very close by and with the same values.

pelagic fishing with low bycatch; (v) pelagic fishing with high bycatch; (vi) artisanal fishing; (vii) nutrient input (fertilizers); (viii) commercial shipping; (ix) organic pollution (pesticides); (x) UV radiation; (xi) urban run-off; (xii) benthic structures (oil rigs); (xiii) ocean acidification; (xiv) sea surface temperature increase; (xv) invasive species; (xvi) coastal population density; (xvii) oil spills; (xviii) risk of hypoxia; and (xix) cumulative threat. Cumulative threat was based on all disturbances, combined with the sensitivity of the habitat/per pixel (1 km²), assessed through expert opinion. Map resolution for most threats was 1 km, but ranged between 1 and 4 km. Threat categories were matched as closely as possible with the geolocations of the *P. oceanica* meadows used in the genetic analyses. In some cases, the threat data matched within a few hundred metres of the genetic sampling coordinates, but more typically the accuracy of the meadow overlay coordinates was 1 km (exceptionally up to 10 km).

Statistical analysis

Correlations between anthropogenic disturbance and genetic diversity measurements were performed in R (R Development Core Team 2012) using the parametric

Pearson's product-moment correlation r . Regression lines were also calculated and visualized in the plots generated in R (R Development Core Team 2012). Allelic richness, observed heterozygosity and genotypic diversity were separately correlated with each of the 18 threats plus the cumulative threat. Possible confounding correlations with latitude or longitude were checked and accounted for following the method suggested by O'Brien *et al.* (2011). Specifically, when a co-variation of both tested parameters with latitude or longitude was observed, an analysis of the deviations from the common independent variable was performed. Results were only reported if a significant correlation between the two tested traits persisted after factoring out the effect of latitude (as only latitude showed a co-variation). Correlations between each of the genetic metrics and each of the disturbances were performed on the full data set and confirmed on the reduced data set, following the removal of duplicate genotypes. For assessment of the chance of committing a Type 1 error, in which a spurious correlation might occur, we show the level of significance and the strength of correlations in Table 1. All correlation analyses were performed on actual impact values for each of the 19 disturbances taken from <http://globalmarine.nceas.ucsb.edu/mediterranean/>. For

Table 1 Correlation coefficient r of the correlation analyses between genetic metrics and the 18 disturbance factors modelled in Micheli *et al.* (2011)

Disturbance	R ($N = 14$)	H_O ($N = 14$)	iH_O (MLG = 20)	N_a ($N = 14$)	A_{20} (MLG = 20)
Nondestructive demersal fishing with low bycatch	0.016	-0.019	nt	0.050	nt
Destructive demersal fishing	0.064	0.011	nt	0.155	nt
Nondestructive demersal fishing with high bycatch	-0.264	-0.197	nt	-0.243	nt
Pelagic fishing with low bycatch	0.103	0.130	nt	0.020	nt
Pelagic fishing with high bycatch	-0.245	0.122	nt	0.078	nt
Artisanal fishing	-0.108	0.143	nt	-0.056	nt
Nutrient input (fertilizers)	0.134	0.118	nt	0.174	nt
Commercial shipping	0.132	0.310* [†]	0.023 [†]	0.282* [†]	0.249 [†]
Organic pollution (pesticides)	0.311*	0.290*	0.174**	0.337*	ns
UV radiation	0.117	0.024	nt	0.037	nt
Urban runoff	-0.105	0.085	nt	-0.020	nt
Benthic structures (oil rigs)	0.025	-0.008	nt	0.132	nt
Ocean acidification	0.198	0.420	nt	0.214	nt
Sea surface temperature increase	-0.112	-0.219	nt	-0.036	nt
Invasive species	0.159	0.122	nt	0.016	nt
Coastal population density	0.172	0.056	nt	0.027	nt
Oil spills	-0.379*	0.227	nt	-0.150	nt
Risk of hypoxia	-0.046	-0.007	nt	0.039	nt
Cumulative impact	0.076	0.224 [†]	nt	0.501** [†]	0.466** [†]

R (genotypic richness, $N = 14$); H_O (observed heterozygosity, $N = 14$); iH_O (individual heterozygosity, $MLG \geq 20$); N_a (number of alleles/locus, $N = 14$); A_{20} (allelic richness, $MLG = 20$); * $P < 0.05$; ** $P < 0.01$). Nonsignificant correlations (ns, $P > 0.05$) and not tested correlations (nt). [†] P -values that were obtained after factoring out the effect of latitude.

the expert questionnaire only (described below), we binned disturbances into six impact categories as defined in Halpern *et al.* (2008; 1 = very low impact, 2 = low impact, 3 = medium impact, 4 = medium high impact, 5 = high impact and 6 = very high impact, which does not exist in our data set). For better visualization of correlations between cumulative impact and genetic descriptors, we further merged the five categories into just three categories, as suggested by Halpern *et al.* (2008; i.e. the two categories of 'very low' and 'low impact' were summarized as 'low impact'; the 'medium' and 'medium high' impact categories were summarized as 'medium impact', and the 'high' and 'very high' impact, the latter being absent from our data set, were summarized as 'high impact'). Allelic richness was also classified in three categories, that is low (<2.75), medium (2.75–3.5) and high (>3.5). Genotypic richness was classified in three categories to equally cover the whole range between the value zero to one in low (<0.33), medium (0.33–0.66) and high (0.66–1). Maps of the genetic data and the sampling locations were created using QGIS (Quantum GIS Development Team 2013) and ARCMAP 10.2.2. Plotted cumulative impact values were taken from <http://globalmarine.nceas.ucsb.edu/mediterranean/>. Additional details on data generation and map development can be found in Halpern *et al.* (2008) and Micheli *et al.* (2013).

Expert opinion questionnaire

To empirically ground-truth results of our study, a questionnaire listing the 19 threats was sent to colleagues involved in the original sampling of the assessed meadows used in our meta-analysis (Table S2, Supporting information). They were asked to assess each threat with a score from 1 to 5 (i.e. 1 = very low impact, 2 = low impact, 3 = medium impact, 4 = medium high impact, 5 = high impact; Halpern *et al.* 2008). Results from the questionnaire were tallied and compared to the results of Micheli *et al.* (2011).

Results

Correlation among genetic diversity metrics

A significant positive correlation was found between the number of alleles per locus and genotypic richness, as expected (Fig. 2a). As the maximum possible observed number of alleles per locus in a population also depends on whether any given locus is heterozygous, it is also expected that allelic richness positively correlates with heterozygosity (Fig. 2b). In contrast, but according to theoretical expectations (Hämmerli & Reusch 2003; Allendorf *et al.* 2013), our data set did not show a significantly positive correlation between

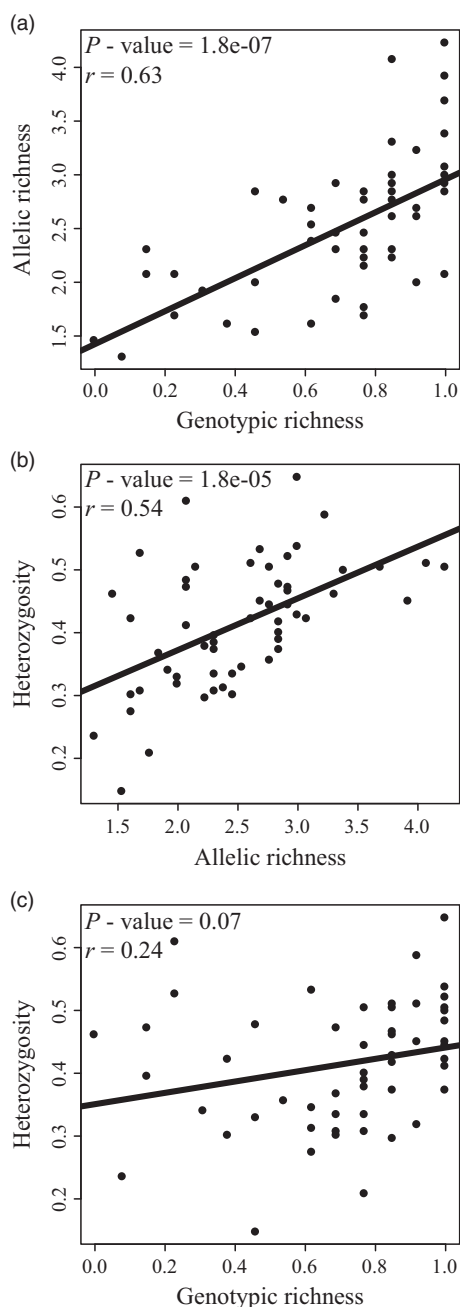


Fig. 2 Correlations between standardized genetic diversity measurements: (a) genotypic richness with allelic richness (number of alleles per locus); (b) allelic richness with observed heterozygosity; (c) genotypic richness with observed heterozygosity. All measurements are based on an equal sample size of $N = 14$.

heterozygosity and genotypic richness, although a trend was present ($P = 0.07$, $r = 0.24$; Fig. 2c).

Correlations of genotypic diversity with threats

Genotypic richness (R ; $N = 14$) did not correlate with latitude or longitude, which made corrections

unnecessary. R was positively correlated with the level of organic pollution, which is based on pesticides ($P = 0.020$, $r = 0.311$, Table 1, Fig. 3a). A negative correlation was also present with oil spills ($P = 0.004$, $r = -0.379$; not shown), although only based on 11 meadows, as most meadows had values of 0 (Table 1). All other correlations were nonsignificant.

Correlations of heterozygosity and allelic diversity with threats

Observed heterozygosity (H_O) based on the standardized number of samples ($N = 14$) was positively correlated with organic pollution ($P = 0.030$, $r = 0.290$, Table 1, Fig. 3a) and with commercial shipping ($P = 0.020$ after correction for latitude, $r = 0.282$, Table 1, Fig. 3b). Observed individual heterozygosity calculated for the smaller data set (31 meadows; $MLG \geq 20$) remained significantly correlated with organic pollution ($P = 1.054e-07$, $r = 0.174$, Table 1), but not commercial shipping.

The number of alleles per locus (N_a ; $N = 14$) was positively correlated also with organic pollution ($P = 0.011$, $r = 0.337$, Table 1, Fig. 3a) and with commercial shipping ($P = 0.035$ after correction for latitude, $r = 0.386$, Table 1, Fig. 3b), as well as with cumulative impact ($P = 8.4e-05$ after accounting for latitude, $r = 0.260$, Table 1, Fig. 3c). As with heterozygosity, we recalculated allelic richness for the smaller data set of 31 meadows. Allelic richness standardized for 20 MLGs (A_{20}) remained correlated with cumulative impact ($P = 0.008$ after accounting for latitude, $r = 0.466$, Table 1).

Relation between cumulative impact and genetic diversity metrics

Allelic richness (A_{20} ; $MLG = 20$) ranged from 1.185 to 4.423 alleles/locus across the 31 meadows included in the analysis. The categorized A_{20} values (low, medium and high) were compared to low, medium and high values for cumulative impact. The relationship between cumulative impacts (red dots) and allelic diversity (blue dots) was then superimposed on the heat map of cumulative impact generated by Halpern *et al.* (2008; Fig. 1, bottom). In all but two cases (6.5% of the 31 meadows), allelic diversity was equal to or higher than the impact category (blue circle around red dot), rather than our original expectation of reduced allelic diversity in areas of increasing impact (red circle around blue dot). The low-diversity locations at medium impact were Dardanos, Dardanelles Strait, Turkey; and Atolls, located in the enclosed Marsal Lagoon, Sicily.

Dissecting the relationship between impact and genetic diversity metrics further, we plotted low,

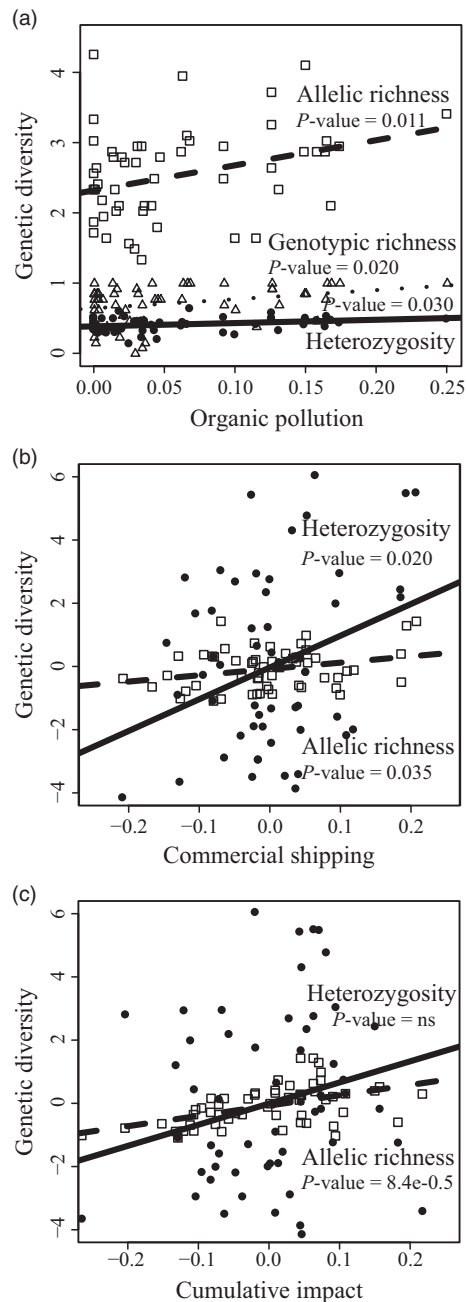


Fig. 3 Plots of significant correlations between genetic diversity metrics (standardized to $N = 14$) and (a) organic pollution (based on pesticides), (b) commercial shipping and (c) cumulative impact as modelled in Micheli *et al.* (2011). The amount of organic pollution is represented by a value between 0 (no impact) and 1 [highest level observed in the entire original Halpern *et al.* (2008) data set]. Correlations with commercial shipping and cumulative impact are shown after an analysis of the deviations from the common independent variable (latitude), and neither the x -axis nor the y -axis represents actual values of genetic diversity or the level of impact, but is representative of the range of values. Refer to Table 1 for r values.

medium and high cumulative impact against low, medium and high allelic and genotypic richness (Fig. 4), and calculated the percentage of each combination. Percentage was used because we did not have an equal number of meadows for each comparison. No meadows with low or medium allelic richness values were found at locations with a high impact. For genotypic richness, there was no significant positive correlation with cumulative impact (Table 1). We did, however, observe a meadow with medium genotypic richness at a highly impacted location (Location 16: Sciacca, Italy). No meadows with low genotypic richness were observed at a high-impact location (Fig. 4).

Corroboration of modelled threats

Questionnaires compiled from colleagues and one of the authors (GP) ranked cumulative impact as the most important threat (rank 1), followed by nutrient inputs, fishing and boat anchoring (2), and by commercial shipping, organic pollution, urban run-off, sea surface temperature increase, invasive species and population density (3). Colleagues were also asked to provide values of cumulative impact per meadow (ranging from a score of 1: low to 5: high) based on their professional opinions. This information was provided for 17 of the 56 meadows. In general, values given by scientists corresponded well with those obtained from the Mediterranean threat-distribution map (Micheli *et al.* 2011), that is 53% of meadows were given the same score of cumulative impact and an additional 35% differed by only one score (Fig. 5). A plus-minus difference of one score was judged as minimal as, for example, discriminating between 'very light impact' and 'light impact' or 'medium' and 'medium high' impact is highly subjective. Differences of two or more category scores occurred in 12% of the responses, at Sciacca and Porto Conte, Italy. On average, the impact score given by experts was lower (corresponding to an average of '2', 'light impact') for the assessed meadows than the impact score suggested by the model (corresponding to an average of '3', 'medium impact'). Direct evaluation by experts never reached the value of 5 (high impact; Fig. 5).

Discussion

Anthropogenic impacts are chronic, often strong and thus rapidly changing the selection regime, which may extend beyond the resistance capacity of *Posidonia oceanica*. While there is no question as to the importance of standing genetic variation for long-term adaptive potential, observed levels of genetic diversity in long-lived

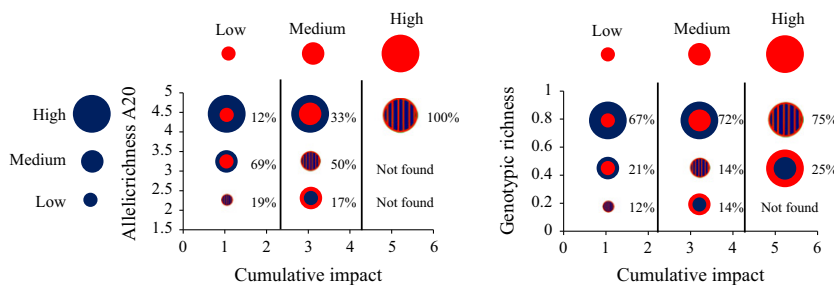


Fig. 4 Low, medium and high cumulative impact (red) vs. low, medium and high allelic richness (A_{20} ; MLG = 20) and genotypic richness (R ; $N = 14$; blue). Striped dots indicate that the genetic diversity measurement and impact were of the same category level. Percentages reflect average combinations per quadrant for each impact category. For example, 33% of the surveyed locations with a medium level of cumulative impact exhibit high allelic diversity, while 100% of the surveyed locations with a high impact also exhibit high allelic diversity. Note the absence of data points of low allelic richness at medium and high impact and low genotypic richness at high impact.

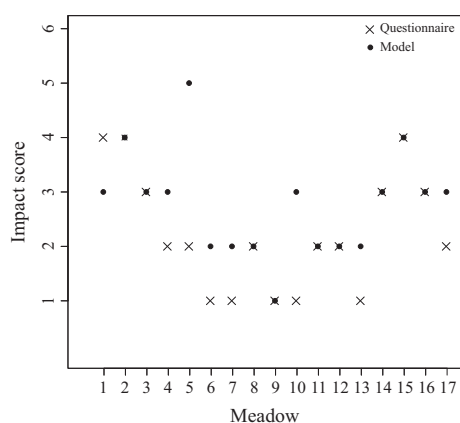


Fig. 5 Comparison of the values of cumulative threat given by experts (crosses) and the Micheli *et al.* (2011) model (points) for the seventeen meadows for which information by both sources was available. Discordance between the model and expert opinion differed most often by one score of the five (1 = very light impact, 2 = light impact, 3 = medium impact, 4 = medium high impact and 5 = high impact), but scores given by experts were generally lower or the same as the model. Refer to doi:10.5061/dryad.744fb for references to the individual meadows.

species often have a much deeper history than the relatively recent (decadal) anthropogenic disturbances mapped in Micheli *et al.* (2011, 2013). Past climate change and life history features enhance persistence and resistance, but also create complex phylogeographic patterns that may generate particular vulnerabilities under rapidly changing environmental stress.

First, monospecific stands of *P. oceanica* often represent ancient colonization events that occurred millennia ago following the last glacial maximum. The underlying phylogeographic imprint (Arnaud-Haond *et al.* 2007, 2014) is still visible in the distribution of allelic diversity consistent with recolonization patterns at the basin scale. The highest allelic diversities are found in the

central Mediterranean (Fig. 1b, blue dots), where the Tunisian–Sicilian break represents a secondary contact zone after the end of the glacial period, and a transition zone between western and eastern Mediterranean basins (Serra *et al.* 2010). Distinguishing historical diversity signatures from contemporary disturbance effects and endangerment has not yet been investigated in *P. oceanica* even though its phylogeography (Arnaud-Haond *et al.* 2007, 2014) and geological evolution of contemporary patterns of Mediterranean oceanographic circulation are well known (Serra *et al.* 2010). In *Posidonia australis*, an endemic to Australia, Evans *et al.* (2014) attempted to assess whether endangered southeastern coastal populations had lower genetic diversity levels as compared with more stable, but naturally fragmented populations in the north (the biogeographic range edge). Northern areas were characterized by lower genetic diversity and more shared genotypes as compared with southern populations. They concluded that the patterns of low diversity were due to palaeoclimatic bottlenecks and range-edge effects, as opposed to recent environmental stress. An important point in their study was the observation that the current endangerment of meadows did not *cause* lower genetic diversity, but the presence of naturally low allelic diversity and added environmental stress together likely exacerbated the recovery of those populations. In this study, the absence of populations with low genetic variation at highly impacted locations suggests probable local extinctions that have already occurred as individuals and populations exceeded their resistance capacity. In fact, between 50% and 75% of regularly monitored and recently reviewed *P. oceanica* meadows show declining trends in area, cover and density (Marbà *et al.* 2014). A number of *P. oceanica* meadows in the North Adriatic have been lost, presumably due to increased turbidity (Zavodnik & Jaklin 1990) in combination with low genetic diversity and low connectivity with other

Mediterranean populations. In contrast, meadows that are reported as extinct in the western Mediterranean in Marbà *et al.* (2013a,b) were located in areas close to major harbours (e.g. Genoa and La Spezia). In this high genetic diversity centre of the species' distribution, disturbance of 'commercial shipping'-related activities (Balduzzi *et al.* 1984; Bianchi & Peirano 1995) is likely the sole cause of loss.

Second, reduced sexual reproduction and a 35-year generation time for *P. oceanica* (Short *et al.* 2011) translate to a very slow production of new genetic variation by sexual reproduction. On average, only 0.7% of shoots sexually reproduce in a given year (Diaz-Almela *et al.* 2006), and seedling success rates are extremely low (Diaz-Almela *et al.* 2008). The consequence is that recent disturbances will not necessarily be reflected in the genetic metrics.

Third, a peculiarity and vulnerability of foundational species is that initial colonization often leads to a 'founder takes all' domination of space (Winters *et al.* 2013) through early selection of competitively superior genotypes/clones that may persist for extremely long periods of time and in densities that restrict new recruitment into the meadow. In the case of *P. oceanica*, carbon dating of cores taken from the rhizomal *matte* indicates that some areas are at least 4000 years old (Mateo *et al.* 1997). As long as conditions remain relatively stable, these well-adapted clones will persist and potentially become more resistant through clonal integration (Migliaccio *et al.* 2005; Arnaud-Haond *et al.* 2012). Persistence and resistance are general features of large clones, as theorized in the general-purpose genotype model (Lynch 1984). In the Baltic, for example, ancient clones of *Zostera marina* (Reusch *et al.* 1999) are characterized by relatively low allelic richness, especially in marginal/edge locations with low salinity stress; yet they persist for hundreds to thousands of years in these relatively undisturbed regions. Likewise, in the California Channel Islands, large clones of *Z. marina*, typically characterized by lower allelic richness, are consistently located in areas of low impact (Coyer *et al.* 2008). Although case studies in *P. oceanica* have generally revealed low genotypic/clonal richness at the range edges and at the physiological tolerance limits (Ruggiero *et al.* 2002; Meinesz *et al.* 2009; Tomasello *et al.* 2009), we did not find any evidence for a range-edge scenario in our meta-analysis. Meadows with large clones were consistently found at locations with low cumulative impact (Fig. 4).

Disturbance

The point at which disturbance becomes destructive is difficult to quantify because, within limits, disturbance

can also have a positive effect on, for example, the reproductive rate and recruitment success, leading to an increase in genetic and genotypic diversity, as hypothesized in the IDH (Connell 1978) and as observed in some southern Californian populations of *Z. marina* that have undergone repeated disturbance mitigation in a particular area (Olsen *et al.* 2014). Higher flowering rates under disturbance stress are well documented in both terrestrial plant species (Wada & Takeno 2010 and references therein) and seagrasses (Ferber *et al.* 2008 in *Z. marina*; Hämmerli & Reusch 2003 in *Z. marina*; Alexandre *et al.* 2005 in *Z. noltii*; Diaz-Almela *et al.* 2007b in *P. oceanica*). Quantification of the amount of reproductive effort has even been suggested as a useful indicator for disturbance in several seagrass species (but not including *P. oceanica*; Cabaço & Santos 2012). It has also been suggested that even if flowering rates remain constant under anthropogenic pressure, dispersal and recruitment might be altered as a response to habitat changes (see Diaz-Almela *et al.* 2008 for *P. oceanica*). On the more negative side and considering the reciprocal relationship between disturbance and diversity that has been revealed in many biodiversity–ecosystem functioning studies (Reusch & Hughes 2006; Worm *et al.* 2006; Hughes *et al.* 2008; Hughes and Stachowicz 2011), it is also possible that stress and sexual reproduction could actually reinforce the decline of *P. oceanica*: first, the mortality of less genetically (genotypic and genetic) diverse and therefore less resistant and resilient (Hughes & Stachowicz 2004 in *Z. marina*; Massa *et al.* 2013 in *Z. noltii*; Reusch *et al.* 2005 in *Z. marina*) individuals might be occurring in meadows that receive high levels of anthropogenic disturbance. Second, and simultaneously, the remaining individuals and populations might increase their sexual reproductive effort (Cabaço & Santos 2012 for several seagrass species, but excluding *P. oceanica*), relocating resources away from the main mechanism of spread through rhizome expansion, and therefore also contribute to meadow decline.

The projection of low, medium and high disturbance and low, medium and high genetic diversity metrics onto a hypothetical IDH curve (Fig. S1, Supporting information and Fig. 4) provides a number of insights: high allelic richness was found in 12% of meadows with low impact, while this increases to 33% of meadows at medium impact. However, 100% of meadows experiencing high impact also exhibited high allelic richness. In contrast, genotypic richness showed relatively stable levels in 67–75% of the meadows with high genotypic richness values for all three impact categories (Fig. 4). Low genotypic richness values were only found at locations with low or medium cumulative impact (Fig. 4, right panel), indicating that populations with low genetic diversity levels (i.e. both genotypic

diversity and allelic diversity) may have surpassed their resistance levels at high-impact locations. We note, however, that only a few meadows with high-impact values were available and the absence of meadows with low genetic diversity at high-impact locations could reflect a sampling error.

The two cases of low allelic diversity and medium impact (Dardanos-55, Dardanelles Strait, Turkey; and Atolls-21, Marsala Lagoon, Sicily, Fig. 1) may well be attributable to the effects of disturbance and isolation (rather than history; Meinesz *et al.* 2009; Tomasello *et al.* 2009). For the Atolls site, both the model and the questionnaire generated an impact value of 4 (medium high impact), which was in both cases mainly based on stress due to the harsh physical and chemical properties. In contrast, the Dardanos site was only assessed by the model and received a cumulative impact value of 3 (medium impact). In this case, small effective population size and isolation from other meadows might be a better explanation for low allelic richness values despite relatively high genotypic richness ($R = 0.62$).

The cumulative threat model and expert opinion

Among the 19 threats mapped by Micheli *et al.* (2011), three showed a correlation with genetic diversity metrics (Table 1) at a significance level of $P = 0.05$. Even when a more stringent P -value of 0.01 was considered (to account for the multiple independent tests conducted), two of those disturbances remain significantly correlated with at least one genetic diversity metric (Table 1). First, 'organic pollution (pesticides)' greatly affects coastal bays and harbours by increasing eutrophication via agricultural run-off in combination with increased sedimentation and turbidity (Halpern *et al.* 2008). Seagrasses are extremely sensitive to poor water quality and particularly turbidity as it affects photosynthesis (Diaz-Almela *et al.* 2007a; Apostolaki *et al.* 2010). Second, 'commercial shipping' provides a mainly indirect effect that comes from coastal development of harbours, dredging and use of shallow shipping routes (reviewed in Boudouresque *et al.* 2009). Third, 'cumulative impact' was judged as the most important (Table 1 and results of questionnaire). Although Micheli *et al.* (2011) did not provide a separate category for 'anchor drops', the hundreds of harbour-associated marinas and thousands of pleasure boat anchor drops are also acknowledged to be among the most detrimental impacts to *P. oceanica* meadows in many parts of the Mediterranean (Francour *et al.* 1999; Montefalcone *et al.* 2006, 2008; Ceccherelli *et al.* 2007; Okudan *et al.* 2011).

The top threats ranked in our questionnaire were also among the top nine threats reported in an expert workshop on anthropogenic impacts in the Mediterranean

basin (Grech *et al.* 2012) with the exception of 'sea surface temperature'. Increasing sea surface temperature is now widely considered to be a significant factor in the mortality of *P. oceanica* and may possibly lead to its extinction in parts of the western Mediterranean by mid-century (Jordà *et al.* 2012 and references therein). High impact, assessed as 'cumulative impact', also follows trends observed in quantitative comparisons between declining and stable meadows in the western Mediterranean, where increasing disturbance and genetic diversity were also found to be positively associated in declining meadows (not shown). Overall, we conclude that modelled threats agree well with expert opinion, especially assessed at the level of 'cumulative threat'.

The utility of genetic diversity metrics

Population genetic characterization of meadows at the local (and increasingly the landscape scale) provides valuable information that goes beyond the simple genetic diversity metrics reported here. Genetic surveys provide direct insights into demographic status with respect to stability (clonal spread) and new recruitment (sexual reproduction), which link to the diversity metrics and the generation of new genetic variation important for the long-term evolutionary potential and adaptation. Population genetic data can also provide insights about isolation and meta-population connectivity, which can directly contribute to establishing management units and design of marine protected areas (Procaccini *et al.* 2007; Olsen *et al.* 2014). Finally, baseline genetic status data also provide practical information relevant to mitigation, and sourcing material for transplants and restoration (see Olsen *et al.* 2014 and references therein for *Z. marina*; Procaccini & Piazzini 2001 for *P. oceanica*).

The search for both 'status' and 'early-warning' indicators of seagrass health (reviewed in Marbà *et al.* 2013b) is making steady progress as both evolutionary and ecological processes are considered together (Spielmann *et al.* 2004). However, the distinction between short-term (annual or even decadal) estimates of demographic change related to current environmental impacts has to be distinguished from long-term meadow history. So far, this double aspect has not been given adequate consideration in the management context. Likewise, basin-scale oceanographic and niche models provide a good estimate of large-scale trends, and cumulative threat mapping remains an important tool. However, neither provides the needed detail for population-level considerations.

In conclusion, genetic diversity metrics are an important component of 'status', but caution must be

exercised in the interpretation of broad-scale correlative patterns as found in the present study. This is because the exceptional longevity of *P. oceanica* can create a temporal mismatch that may be falsely suggesting good meadow health status, while gradual deterioration of allelic diversity continues unnoticed. It is increasingly recognized that genetic estimates at the single meadow need to be placed in a meta-population context of relative connectivity or isolation in relation to local oceanographic models. So far, this remains challenging because of the need for fine-scale geographic resolution. However, detailed GIS-based maps of population diversity in combination with connectivity matrices are the way forward towards understanding the causes behind and evolutionary meaning of genetic diversity metrics for application in conservation management.

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M.J. and G.P. conceived and designed the research project. G.P. provided the microsatellite data set. M.J. analysed the data. M.J., J.L.O. and G.P. interpreted the data and wrote the article. All coauthors participated in the conception of the article, revised it critically and approved the final version to be published.

Data accessibility

Sampling locations, genetic diversity data and level of disturbance, as well as the results of the questionnaire, are publicly available on Dryad doi:10.5061/dryad.744fb.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 List of sampling locations included in the correlation analysis of genetic diversity of *Posidonia oceanica* with human impact.

Table S2 List of questions asked in the questionnaire.

Fig. S1 Allelic and genotypic richness vs. cumulative impact (as described in Fig. 4) superimposed on a hypothetical intermediate disturbance hypothesis (IDH) curve (blue).