

Clonal architecture in an intertidal bed of the dwarf eelgrass *Zostera noltii* in the Northern Wadden Sea: persistence through extreme physical perturbation and the importance of a seed bank

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Abstract Genotypic structure and temporal dynamics of the dwarf seagrass, *Zostera noltii*, were studied in an intertidal meadow that has persisted since prior to 1936 near the Wadden Sea island of Sylt. Samples were collected from two 10 × 10 m plots separated by 250 m from May 2002 to June 2005 and from four 1 × 1 m plots from June 2003 to September 2004. All the samples were genotyped with nine microsatellite loci. No genotypes were shared between the plots separated by 250 m. Genetic diversity was higher in the Wadden Sea than in the other regions of its geographic range. The average clone size (genets) (SD) in the two plots was 1.38 (0.26) and 1.46 (0.4) m², respectively, with a range up to 9 m² and <20% persisted for >4 years. A high genetic and genotypic diversity was maintained by annual recruitment of seedlings despite a dramatic decrease in ramet density that coincided with the severe heat stress event of 2003. Fine-scale (1 m²) analysis suggested that extensive loss of seagrass cover precluded space competition among the genets, while a persistent seed bank prevented local extinction. Long-term persistence of *Z. noltii* meadows in the intertidal Wadden Sea was achieved by high genet turnover and frequent seedling recruitment from a seed bank, in contrast

to the low diversity observed in large and long-living clones of *Z. noltii* and other seagrasses in subtidal habitats.

Introduction

The dwarf eelgrass, *Zostera noltii*, is the most important seagrass in the sandy to muddy intertidal of the NE Atlantic, where it forms meadows spanning tens to thousands of square meters in size (Fig. 1). Some meadows consist of several distinct genets (genetic individuals or clones), whereas others are comprised primarily of a dominant genet with high numbers of ramets (morphological individuals arising from vegetative reproduction *sensu* Harper 1977). Clone size and number are important measures for reproductive efficiency of seagrasses in general, as limited pollen dispersal, combined with large clone sizes and few neighboring clones, may reduce the outcrossing potential within meadows (Barrett and Harder 1996; Charpentier 2001; Reusch 2001a). In the self-compatible genus *Zostera*, limited outcrossing increases the rate of inbreeding and may lead to inbreeding depression and degradation of population fitness (Ellstrand and Elam 1993; Reusch 2001a; Hämmerli and Reusch 2003). Clone size and density also directly affect recruitment success from seeds through space and resource competition, and hence genetic and genotypic diversity (Eriksson 1993). Consequently, persistence of extensive and locally adapted genets can lead to a reduction of evolutionary potential to changing environmental conditions (Silvertown and Charlesworth 2001; Hämmerli and Reusch 2002).

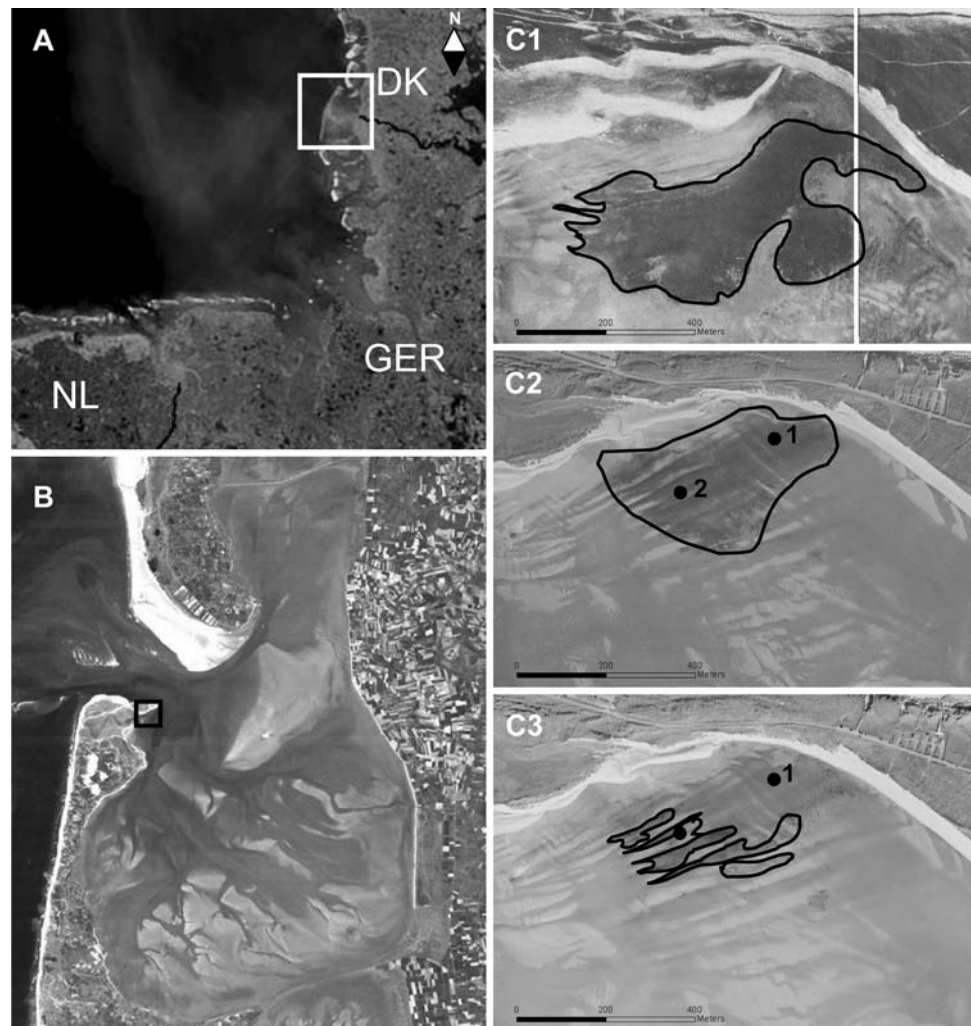
Determination of clonal or patch architecture requires variable molecular markers such as microsatellites, which provide the only reliable means of distinguishing genets (clones) among the sea of ramets (shoots) (Reusch 2001b;

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Fig. 1 Study site. **a** Island of Sylt (*white square*) in the Northern Wadden Sea. **b** List tidal basin with study site (*black square*) in Königshafen off the northern tip of the island. **c** *Z. noltii* meadow in Königshafen: **c1** 1936, **c2** 2003 and **c3** 2004 after the large decline. Seagrass bed boundaries are outlined. Locations of Plots 1 and 2 are indicated in **c2** and **c3** (*black dots*). (Aerial photographs **c1–3**: AWI-Bremerhaven, Germany)



Arnaud-Haond et al. 2005). Molecular population genetic studies on several seagrass species have not only uncovered clonal structure, but revealed patterns of gene flow and population connectivity, neighborhood size, local adaptation, and the mating system (reviewed in Waycott et al. 2006; Procaccini et al. 2007), thus providing a snapshot of pattern and process at the landscape scale.

Temporal studies of seagrass clonal architecture, however, are not common, as the natural genet longevity of the most intensively studied large seagrass species (some *Zostera marina*, most *Posidonia australis*, and all *Posidonia oceanica* populations) ranges from decades to centuries or even millennia (Waycott 1995; Reusch et al. 1999; Ruggiero et al. 2002). To our knowledge, no study has examined temporal architectural changes of seagrass genets in a fixed area for more than 1 year.

An important component of temporal stability of plant populations is the presence/absence of a seed bank. Seed banks constitute reservoirs of genetic and genotypic diversity in established populations and may enhance

recovery after population disturbance. Furthermore, recruitment from a seed bank can strongly influence population structure and genotypic diversity (Koch et al. 2003; Barrett et al. 2005; Greve et al. 2005). Seeds of *Z. noltii* are produced annually through subaquatic pollination of protogynous, hermaphroditic flowers. The four to six male and four to six female flowers are combined in a single spathe and release four to six negatively buoyant seeds (den Hartog 1970). Seeds enter the seed bank either directly upon release or after dispersal. While seeds typically are dispersed on the scale of a few meters (Orth et al. 1994; Ruckelshaus 1996), seeds may be more widely dispersed via floating spathes or rafting shoots of *Z. noltii*, thereby ensuring gene flow among distant populations (Coyer et al. 2004a; Erfemeijer et al. 2008) (Reusch et al. 2002; Ferber et al. 2008 for *Z. marina*).

Seed banks of *Z. noltii* persist for at least 3 years near the island of Sylt in the northern Wadden Sea and contribute up to 20% of the annual new recruitment (Zipperle et al. 2009). Therefore, an established meadow or large

patch of *Z. noltii* could persist more or less indefinitely and indeed, decades-old meadows are known (Nienburg 1927; Wohlenberg 1935; Reise and Kohlus 2008). However, even large meadows wax and wane over the course of years (Philippart and Dijkema 1995; Zipperle, personal observation). Meadow persistence depends in part on large-scale stochastic physical factors such as the direct effects of increased hydrodynamics due to storms (Schanz and Asmus 2003), or indirectly by sediment mobility (burial, erosion) (Hemminga and Duarte 2000; Cabaço and Santos 2007; Reise and Kohlus 2008), as well as fine-scale biological disturbances associated with grazing by waterfowl (Jacobs et al. 1981; Nacken and Reise 2000). Intrinsic instability of *Z. noltii* patches also may be caused by a demographic factor. For example, once a few large clones have achieved dominance (the closely spaced ramets exhibit phalanx growth, Brun et al. 2006), the low genotypic/clonal diversity may render them more susceptible to new stress events.

In order to gain insight into the ecological and evolutionary implications of temporal changes in the large- and fine-scale genetic and genotypic composition of seagrass meadows, an intertidal meadow of *Z. noltii* was repeatedly mapped at two permanent plots at two spatial scales over a 4-year period. We examined: (1) spatial and temporal stability of meadow composition, (2) the relative importance of sexual/clonal input, and (3) the importance of a seed bank in maintaining genotypic diversity through physical perturbations.

Materials and methods

Study site and sampling

The study was conducted within an intertidal meadow of *Z. noltii* in the shallow bay of Königshafen on the island of Sylt, Germany (55°2'N, 008°25'E) (Fig. 1). The semidiurnal tides (mean tidal range is 1.8 m) leave the meadow exposed for 6–8 h during the low-tide period and covered with a mean water depth of 0.5 m during high tide. Large-scale mapping of clonal architecture was determined in two permanent 10 × 10 m plots, separated by 250 m (Fig. 1: Plots 1 and 2). Samples were collected at the start of growing season (May/June) and at the end of growing season (September) over seven time points from May 2002 through June 2005.

Sampling for genotyping analysis followed the method of Coyer et al. (2004a) and entailed removal of leaf tissue (adult shoot or whole seedling) from plants at the center of each 1-m² subplot for a maximum of 100 samples at each time point. If no plant was present within a 10-cm radius around the center of the subplot, no sample was collected.

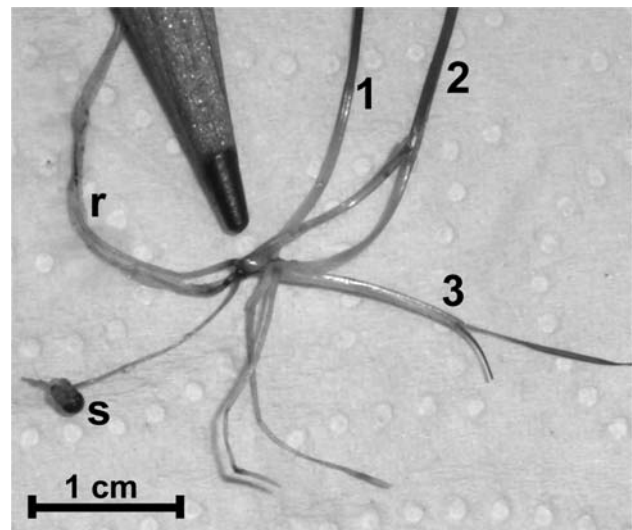


Fig. 2 Vegetative propagating *Zostera noltii* seedling in June. Attached seed, three vegetative shoots (1–3), and roots (r) are visible. Elongated rhizome internodes are lacking

Fine-scale mapping was determined in four 1-m² subplots (quad 1–4) selected randomly within Plot 1. Samples were collected at four time points in May and September of 2003 and 2004. Each of the four 1-m² subplots was further subdivided into 0.2 m × 0.2 m quadrats, and one sample was taken from the center of each quadrat for a maximum of 25 samples. Again, if no plant was present in a 10-cm radius around the center of a quadrat then no sample was taken. All plant material was stored in silica crystals. Seagrass density was estimated as percent-cover at low tide at the time of plant collection. Seedlings, identified by the emerging caulicle and/or the still attached seed as described by Zipperle et al. (2009), often were collected from the center of a subplot/quadrat during the early growth season (June 2004, 2005). Sampled seedlings displayed only restricted vegetative propagation in June (Fig. 2).

DNA extraction, microsatellite amplification and genotyping

Five mg of plant tissue was pulverized with a Retsch Mixer Mill MM 301 (Qiagen) and DNA was extracted following the protocol of Elphinstone et al. (2003). Nine microsatellite loci (Coyer et al. 2004b) were amplified as three triplets (plex-1: ZnB1, ZnH10 and ZnB3; plex-2: ZnB8, ZnH8 and ZnD6; plex-3: ZnE7, ZnF8 and ZnF11) using multiplex PCR in 20 µl reactions (2 mM MgCl₂, 0.2 mM dNTPs, 0.5 U *Taq* (Promega), 0.01% bovine serum albumin, 1× reaction buffer (10 mM Tris HCl, 50 mM KCl, 0.1% Triton X-100,) (Zipperle et al. 2009). PCR reaction conditions were the same for each primer triplet: initial denaturation 3' at 94°C, followed by 35 cycles of 40 s at

94°C, 40 s at 57.5°C and 40 s at 72°C, with a final extension step of 10 min at 72°C. Multiplexed primer triplets were visualized by gel electrophoresis (ABI 377 gene analyzer, Applied Biosystems) and scored against an internal lane standard (ROX 350, Applied Biosystems) using the GENSCAN software (Applied Biosystems).

Data analysis

Probabilities of identity by chance ($P_{\text{sex}} (F_{\text{IS}})$) were calculated for each sample to avoid false assignment of individual shoots (ramets) sharing the same multilocus genotype (MLG) by chance (GenClone 2.0; Arnaud-Haond and Belkhir 2007) to the same genet (clone). $P_{\text{sex}} (F_{\text{IS}})$ accounts for departure from Hardy–Weinberg and provides the most conservative estimates of clonal identity (Arnaud-Haond et al. 2007). Clone maps were generated with GenClone 2.0 (Arnaud-Haond and Belkhir 2007) using *xy*-coordinates of individual samples collected within the 10 × 10 m plots and the four 1-m² subplots. GenClone 2.0 also assessed genetic and genotypic diversity (R) for each sampling. R was calculated as $R = G/N$; where G is the number of genets present and N is the number of ramets sampled.

The genets were tracked from May 2002 through June 2005 in the 10 × 10 m plots to estimate their survival time and mean size. Only the genets found on multiple occasions were used to estimate genet longevity, since they were considered the dominant genets within the quadrat. The mean genet size (per plot) of *Z. noltii* was estimated by averaging sizes of genets found multiple times for each sampling date and calculating the grand mean over all samplings. A minimum estimate of genet survival was achieved by averaging the survival times of individual genets in the study period. Genotypic diversity (R) and genetic diversity were calculated for each sampling date (GenClone 2.0) (Arnaud-Haond and Belkhir 2007). Genetic diversity was estimated as allelic richness (N_e) normalized to a sample size of 28. Fine-scale clone maps of the 1-m² subplots were used to estimate the mean number of genets m⁻² by calculating the mean over quad 1–4 for individual sampling dates. R was calculated for each quadrat individually for all sampling dates.

Results

Large-scale clonal architecture (100 m²)

Seagrass cover ranged from 50 to 100% in 2002 and 2003 and declined to <10% in 2004 and <1% by the end of the study in June 2005 (Fig. 3, 4). Ramets sampled from Plot 1 ($n = 570$) and Plot 2 ($n = 419$) revealed 392 and 272

genets, respectively; of these, 41 and 44 were found more than one time (Fig. 3). Several genets (Plot 1, $n = 39$; Plot 2, $n = 41$) found in May 2002 were tracked for growth and survival. Estimates of $P_{\text{sex}} (F_{\text{IS}})$ confirmed clonal identity for all ramets sharing the same MLG on both the large- and fine-scale. The mean genet size (SD) in Plot 1 (1.46 [0.4] m²) was equivalent to Plot 2 (1.38 [0.26] m²) (t test; $p = 0.34$). However, genets exceeding 3 m² were restricted to 2002 and 2003 in both plots (Fig. 3). The 1-m² size class was dominant; but genet sizes ranged up to 9 m².

The mean genet survival time (SD) was 2.1 (0.61) years in Plot 1, significantly less than in Plot 2 (3.14 [0.9] years) (t test; $p < 0.001$). Only one genet (2.4%) survived for 4 years in Plot 1 while 18 (41%) genets survived the study period in Plot 2 (Figs. 3, 4). Genotypic diversity (R) and allelic richness remained high throughout the study period and invariant among plots. Average R was 0.84 in both Plot 1 and Plot 2 (ranging from 0.64 to 0.96 and 0.68 to 0.95, respectively) (Figs. 3, 4). The mean allelic richness was 6.2 in Plot 1 and 6.4 in Plot 2 (ranging from 5.8 to 6.3 and 6.2 to 6.6, respectively) (Fig. 4).

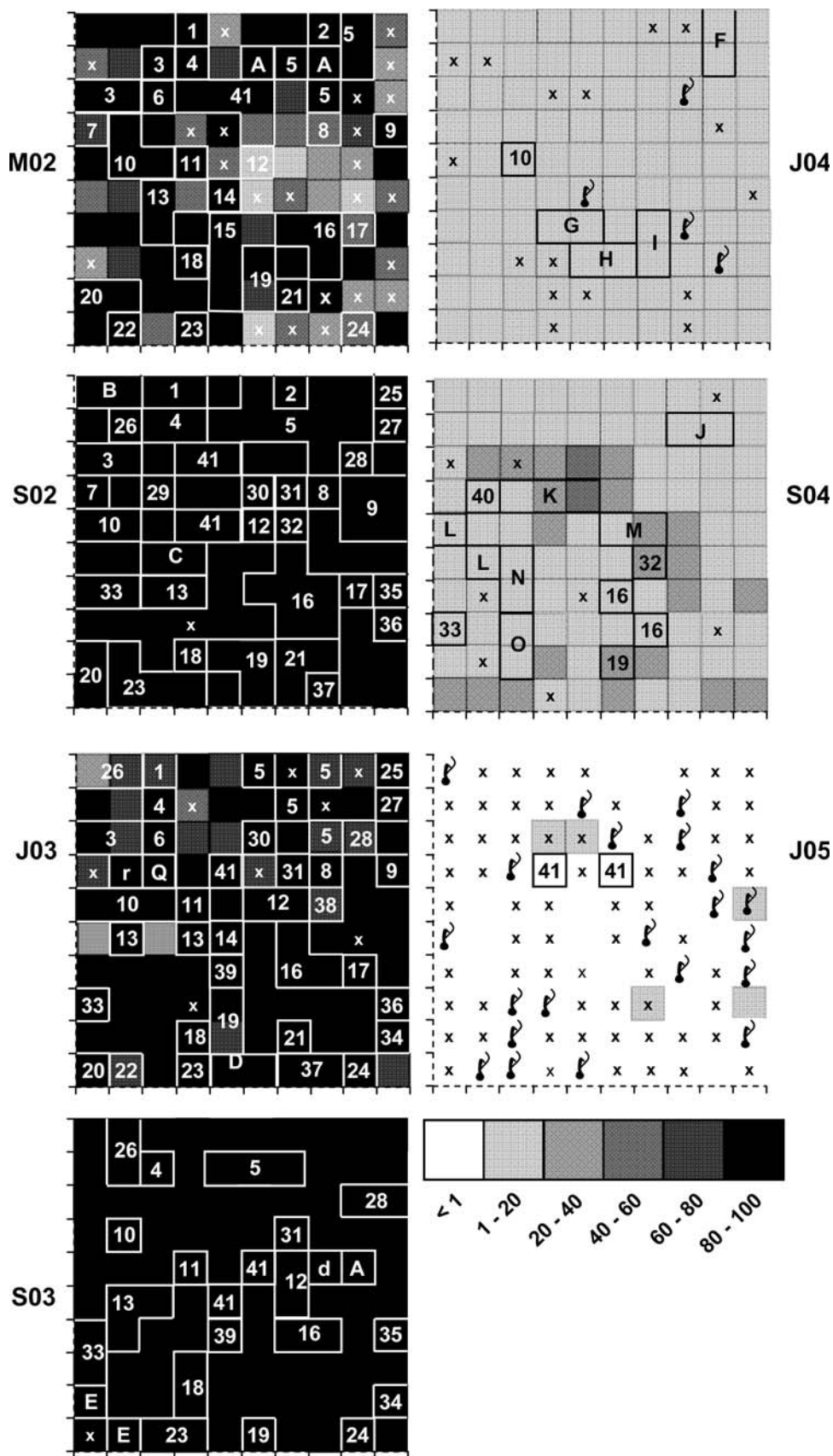
Fine-scale clonal architecture (1 m²)

Seagrass cover was 10–35% in June 2003 and increased to 100% in all four quadrats in September 2003 (Fig. 5). In June 2004 the seagrass cover was <1 to 3% and 1% in September 2004 (Table 1). A total of 106 genets were identified among the 269 ramets sampled in the four subplots over the 2 year study period, but only nine were found on multiple occasions. Genet density (grand mean m⁻² [SD]) was 7.6 [1.4] for the whole study period and did not significantly differ between sampling dates (Table 1). Genotypic diversity, however, was significantly lower in 2003 as compared to 2004 (t test; $p < 0.001$) (Table 1). In September 2003, quadrats 2 and 3 were entirely dominated by one individual clone (Fig. 5). Both of these genets were dominant by June 2003 and also survived until September 2004. In quadrats 1 and 4, two dominant genets were encountered in 2003 and September 2004 but not detected in June 2004 (Fig. 5).

Discussion

Seagrasses have two levels of genetic variation: genetic/allelic and genotypic/clonal (reviewed in Procaccini et al. 2007). Consequently, meadows may be genetically diverse but genotypically depauperate and vice versa. Understanding clonal architecture, therefore, provides a basis for understanding the dynamics of a seagrass bed in relation to habitat.

Fig. 3 Large-scale clonal architecture (10 m × 10 m) from May 2002 to June 2005 (M02 to J05). Clone maps of Plot 1 sorted by sampling date from *top to bottom* and *left to right*. Genotypes found multiple times are marked with *numbers*. Genotypes found in more than 1 m² but only at one sampling time are marked by *capital letters*. Genets are framed in white for better visualization. Blank areas represent spatially and temporally unique genotypes. *x*, no sample taken. Background coloring indicates seagrass cover in % for each 1 m² from low (*white*) to high (*black*) density (see scale)



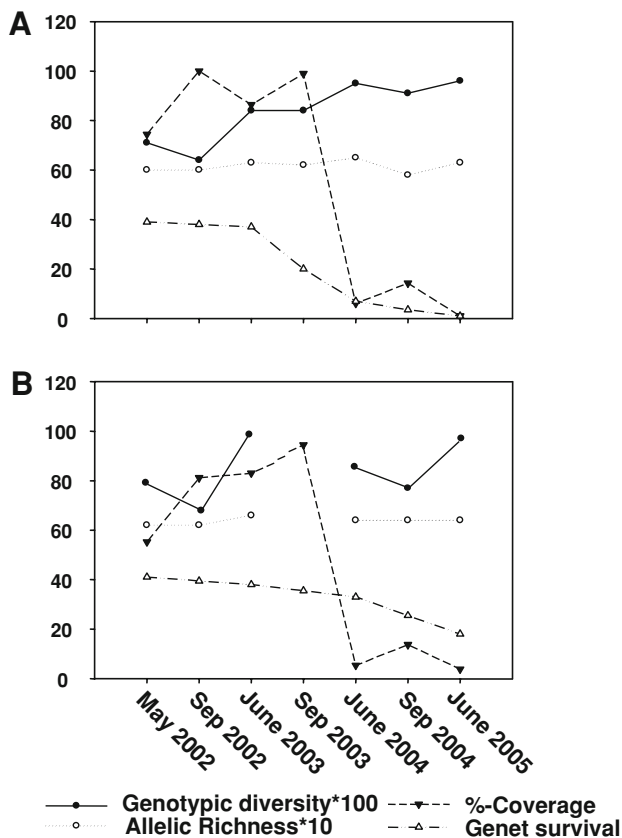


Fig. 4 Genet survival, seagrass cover, genetic and genotypic diversity in Plot 1 (a) and Plot 2 (b) for the study period (May 2002 to June 2005)

Clone size and longevity

The genet size of *Z. noltii* determined by microsatellite analysis during our 4-year study averaged 1.4 m², and ranged from 0.04 to 9.0 m². The genet size range is comparable to the range determined by physical excavation of whole genets and by digital image analysis of an intertidal population at Cadiz Bay in Spain (0.04–4.0 m²) (Brun et al. 2007). The largest clone in Brun et al. (2007) measured 5 m in length, a size attainable in 8 months based on internode production along the main axis and apical dominance, but requiring 7 years based on an average rhizome elongation rate of 0.68 m year⁻¹ (Marbà and Duarte 1998). In the present study, most clones detected on the large-scale persisted for 2–3 years, although ~20% of them were present throughout our 4-year study suggesting that genet age was not correlated with size in the intertidal. Decades-old clones of *Z. noltii*, however, have been reported from the shallow subtidal in the Black and Azov Seas, and genet size may be a better proxy for genet age under less disturbed subtidal conditions (Coyer et al. 2004a). For example, each of five 1 × 3 m subtidal plots of *Z. noltii* off Ischia (Naples, Italy) was densely covered by only two genets and a fragmented

clone extended almost 60 m² with an estimated age of 14.7 years (Ruggiero et al. 2005a).

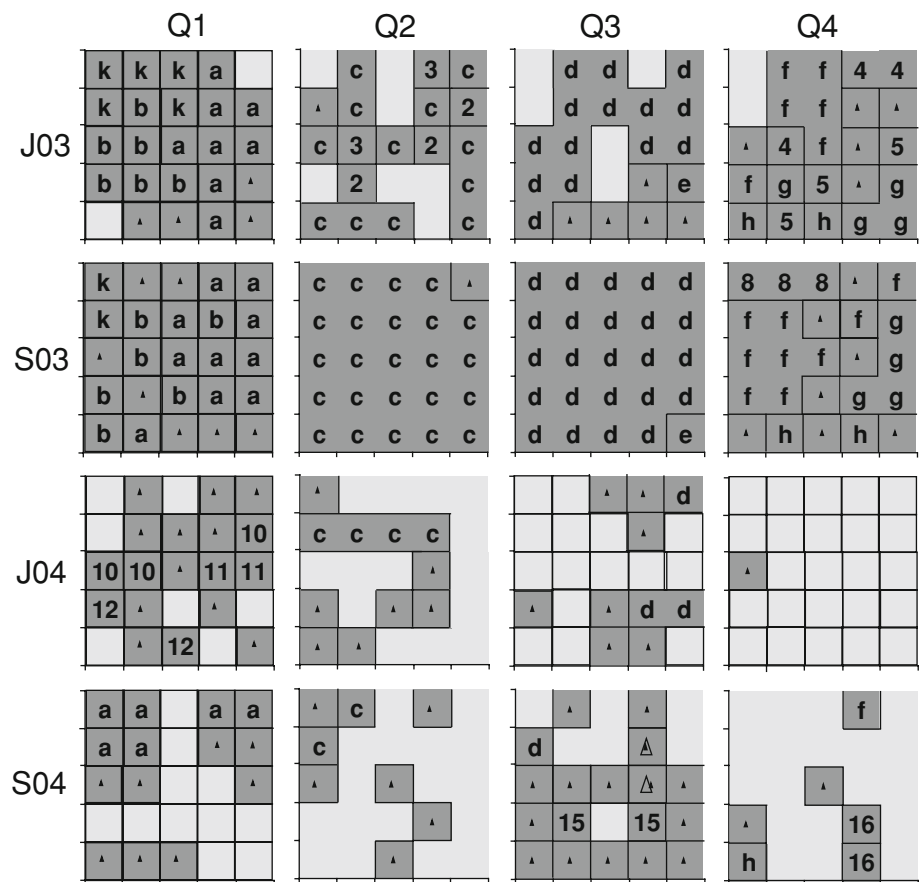
Genet persistence and size positively correlated in *Z. noltii* on the fine-scale, as clones with >50% cover at the beginning of the growing season were competitively dominant and persisted for at least 2 years (Fig. 5). The large, early-season clones undoubtedly arose from vegetative growth of overwintering rhizomes of fragmented clones. Although seedlings generally appear a month before (late April/early May) the start of vegetative propagation of the overwintering, shoot bearing rhizomes (late May/early June) (Vermaat and Verhagen 1996; Zipperle et al. 2009), subsequent vegetative growth of seedlings is not sufficient to outcompete overwintering clones by June (personal observation, Fig. 2).

Genet size of intertidal and annual populations of the sister species *Z. marina* rarely exceeded 0.01 m², while in some subtidal and perennial populations, genets can extend for hundreds of m² (Reusch et al. 1999; Olsen et al. 2004; Coyer et al. 2008) and persist for hundreds or even thousands of years (Reusch et al. 1999; Olsen et al. 2004). An emerging pattern for both *Z. noltii* and *Z. marina*, therefore, may be that clone size and longevity range from small and short-lived in the intertidal to large and long-lived in the subtidal, and are further influenced by a sliding scale of annual to perennial lifestyles. The changes of clone size and longevity from intertidal to subtidal also suggest that habitat characteristics shape the clonal architecture of *Zostera* species along a stress and disturbance gradient (e.g., from benign subtidal to harsh intertidal conditions), which ultimately cause a switch from a perennial to an annual lifestyle as an adaptation to stochastic environmental changes (Hu et al. 2003).

Genetic diversity

Allelic and genotypic (clonal) diversity remained high and stable throughout the study in spite of a 99% loss in aerial cover and number of genets in the large-scale plots from 2003 to 2004 (Fig. 4). As has been documented elsewhere (Coyer et al. 2004a), allelic and genotypic diversity of *Z. noltii* for Wadden Sea populations are among the highest recorded throughout its range and connectivity among meadows also is generally high (also for *Z. marina*; Reusch 2002; Ferber et al. 2008). At our Königshafen site, the high diversity was attributed to a combination of local and regional processes. On the larger Wadden Sea scale, entrainment and admixture of rafting, fruiting shoots and spathes are the basis for seed import, and hence population connectivity and gene flow (Coyer et al. 2004a; Erftemeijer et al. 2008; Zipperle et al., unpublished). However, fine-scale clonal diversity increased from 2003 to 2004, suggesting the scale-dependence of *R* and the necessity to

Fig. 5 Fine-scale clonal architecture of subplots (1 m²) within Plot 1 from June 2003 to September 2004. Clone maps of subplots 1–4 are shown from left to right and temporally sorted from top to bottom. Genotypes found on multiple sampling times are identified with letters. Genotypes found in more than one 0.2 × 0.2 quadrat but only on one sampling time are identified by numbers. Genets are framed in black for better visualization. Small triangles (filled triangle) indicate unique genotypes found only once in time. Light gray areas indicate bare sand



determine the appropriate scale to answer population biological questions in clonal seagrasses (Table 1). While large-scale sampling suffices to monitor population genetic parameters and stability over time in intertidal *Z. noltii*, assessment of demographical changes such as genet mortality and immigration requires fine-scale mapping.

The persistently high allelic and genotypic diversity observed in Königshafen suggests that disturbance plays an important role and sexual recruitment is frequent. For plants in general, stable habitats should favor vegetative reproduction and foster populations dominated by few large clones, whereas disturbed habitats should be dominated by more and smaller clones due to frequent seedling recruitment (de Steven 1989; Eriksson 1993; Kudoh et al. 1999). Disturbances by grazing waterfowl, lugworm bioturbation, epiphyte growth, and storm-induced sediment mobility, are common types of disturbances in the intertidal Wadden Sea (Jacobs et al. 1981; Reise 1985; Philippart 1994; Nacken and Reise 2000; Schanz et al. 2000; Zipperle et al., unpublished). All facilitate seedling recruitment of *Z. noltii* by creating variably sized gaps in the meadow into which recruitment can occur. Increased sexual recruitment under disturbed conditions is known for flowering plants and seagrasses (Peterken and Conacher

1997; Inglis 2000; Greve et al. 2005) and has been shown experimentally for *Z. marina* (Reusch 2006).

In intertidal *Z. noltii*, small clone sizes coupled with relatively large pollen dispersal distances via tidal currents, supports a predominantly outcrossing mating system, which further promotes high allelic and genotypic diversity (Zipperle, unpublished data). In contrast, large clones can lead to inbreeding depression due to pollen limitation for outcrossing, as has been shown in subtidal *Z. marina* (Hämmerli and Reusch 2003). Furthermore, as seedling recruitment substantially contributes to clonal diversity (Harada et al. 1997), it is significant that *Z. noltii* seedlings were observed in all June sampling periods (Zipperle, unpublished; see also Figs. 2, 3) and that seeds germinated annually from a short-term persistent seed bank (Zipperle et al. 2009). Thus, *Z. noltii* exhibits a repeated seedling recruitment (RSR) strategy as has been demonstrated for other seagrasses (Ruggiero et al. 2005b; Reusch 2006), as opposed to an initial seedling recruitment strategy (ISR) (sensu Eriksson 1993). Consequently, the annual losses of genets from the *Z. noltii* population in Königshafen are buffered by seedling recruitment, thereby providing the basis for the stable and high levels of genotypic diversity and allelic richness.

Table 1 Fine-scale clonal architecture of *Z. noltii*

	1	2	3	4	Mean no. of genets \times m ⁻²
June 2003					
No. of ramets (N)	23	18	20	23	
No. of genets (G)	7	4	7	10	7.0 \pm 2.45
Genotypic diversity	0.30	0.22	0.35	0.43	
Seagrass coverage (%)	10	23	35	11	
Sep 2003					
No. of ramets (N)	25	25	25	26	
No. of genets (G)	10	2	2	11	6.3 \pm 4.92
Genotypic diversity	0.4	0.08	0.08	0.43	
Seagrass coverage (%)	100	100	100	100	
June 2004					
No. of ramets (N)	17	11	10	1	
No. of genets (G)	14	8	8	1	7.8 \pm 5.32
Genotypic diversity	0.82	0.73	0.8	1	
Seagrass coverage (%)	3	1	1	<1	
Sep 2004					
No. of ramets (N)	13	8	18	6	
No. of genets (G)	9	7	17	5	9.5 \pm 5.26
Genotypic diversity	0.69	0.88	0.94	0.83	
Seagrass coverage (%)	1	1	1	1	

Number of ramets (N) and genets (G), genotypic diversity ($R = G/N$) and seagrass cover (%) for the 1 m² subplots (1–4) sorted by sampling date (top to bottom)

Persistent seagrass meadow

Although genet turnover is on the order of a few years, the intertidal *Z. noltii* meadow in Königshafen has been present since prior to 1936 (Reise et al. 1989; Dolch 2008; Fig. 1). The decades-long persistence in a harsh environment may be explained by the contribution of sexual reproduction and seed bank supply to meadow maintenance. The precipitous decline in seagrass cover from 2003 to 2004 in Königshafen may have been caused by enhanced sediment mobility, as revealed by characteristic megaripple formation (Fig. 1, C2 and 3) (Dolch, pers. comm.). The increased sediment mobility may be attributed to the loss of extensive *Z. marina* beds, anthropogenic alteration of the hydrodynamic regime in the List tidal basin, and/or an increase of mean tidal height due to variation in storm intensity over the last decades (Dolch and Hass 2008).

Alternatively, the 2003–2004 decrease may be due to the severe heat stress event (HSE) in the summer of 2003, which has been associated with biomass and density losses in European populations of *P. oceanica*, *Z. marina*, and *Z. noltii* (Mayot et al. 2005; Reusch et al. 2005; Cardoso et al. 2008). Although the decline of *Z. noltii* recorded at Königshafen was correlated with the HSE, the correlation

was not observed at the metapopulation scale (930 km²) in the North Frisian Wadden Sea, of which Königshafen is a part (Reise and Kohlus 2008). Thus, the HSE disturbance may have acted in tandem with increased sediment mobility to degrade the Königshafen meadow (local scale). Cardoso et al. (2008) invoke a comparable coupling of HSE and eutrophication stress to explain observed decreases in an intertidal *Z. noltii* bed in the Mondego river estuary (Portugal, SW Atlantic).

In spite of the biomass decline, genet survival of *Z. noltii* was 4–18 times higher in Plot 2 as compared to Plot 1. The edge location of Plot 1 relative to the more central location of Plot 2 might explain the difference. Wave impact and sediment mobility may be more severe at the edges of seagrass meadows than in the center where hydrodynamics are reduced and water retention is higher (Fonseca and Koehl 2006). Under heat stress, a center location may also be more suitable for survival because retained water prevents desiccation and hence mortality. However, it should be noted that intertidal *Zostera* populations are adapted to survive short-term desiccation (Leuschner and Rees 1993).

Percent-cover of *Z. noltii* remained low during 2004 and 2005. While sufficient sexual recruitment assures evolutionary flexibility in physically disturbed habitats, it may be insufficient to balance exceptionally high adult mortality (Forbis and Doak 2004; Weppeler et al. 2006). A patch of *Z. noltii* in the Königshafen meadow recovered only if the overwintering cover was >10% (at the beginning of the growing season). Because of the small genet size, a reduction in density is nearly equivalent to a reduction in total number of genets in the population. A reduced number of genets in spring, therefore, might not provide sufficient vegetative growth until the end of the growing season. If too few genets survive the winter to provide sufficient vegetative regrowth in the following season, then seedling recruitment may not balance this loss. Alternatively, an inhibition of regrowth in spring and early summer might also be caused by enhanced sediment mobility.

The importance of a short-term persistent seed bank to maintain allelic richness of *Z. noltii* has been demonstrated over a 3-year period (Zipperle et al. 2009). Possession of a seed bank should help *Z. noltii* overcome periods unfavorable for vegetative recovery, as has been demonstrated for seagrasses and other plants (Silvertown and Charlesworth 2001; Fenner and Thompson 2005; Greve et al. 2005). If seedling recruitment and subsequent vegetative growth can attain a density sufficient to exceed winter mortality of genets, the population is likely to recover even after exceptionally severe disturbances. The marked increase (up to 20%) of *Z. noltii* cover at the Königshafen study site in August 2008 (Reise, unpublished data), 4 years after the precipitous decline, supports the self-

preserving effects of sexual reproduction from a seed bank for *Z. noltii* in a stressful environment.

In conclusion, decadal persistence of intertidal meadows of *Z. noltii* depends on a balance of year-to-year genet survival (vegetative growth), frequent seedling recruitment (sexual reproduction), and a lasting seed bank. This is in sharp contrast to larger seagrass species, where meadow persistence over centuries involves extensive vegetative propagation (*Posidonia* species, subtidal *Z. marina*) or to subtidal *Z. noltii* meadows, where vegetative propagation dominates and recruitment from seeds is rare (Laugier et al. 1999; Ruggiero et al. 2005a, b). While seedling recruitment ensures evolutionary flexibility in face of environmental stochasticity and annual disturbances, vegetative growth of overwintering ramets promotes recovery after winter mortality. An assessment of these two factors provides a metric for predicting patch vulnerability in the intertidal.

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