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Effects of disturbance on marginal populations: human trampling on *Ascophyllum nodosum* assemblages at its southern distribution limit

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ABSTRACT: The negative effects of disturbances on the long-term persistence of populations may be more severe in marginal populations, i.e. those occurring at the boundaries of geographical and ecological distribution ranges. Human trampling is an important source of anthropogenic disturbance for intertidal assemblages. Effects of human trampling on intertidal rocky shore assemblages dominated by *Ascophyllum nodosum* (L.) Le Jol. at its southern distributional limit in Europe were tested experimentally. Four different intensities of trampling disturbances were applied to experimental areas, over a period of 5 mo. Percentage cover of *A. nodosum* and associated organisms was quantified during the disturbance period and for a recovery period of 1 yr. The 2 highest trampling intensities drastically reduced the cover of *A. nodosum*. Trampling also negatively affected *Fucus vesiculosus* and understorey species, while indirectly allowing colonisation by ephemeral green algae. One year after the end of disturbance, *A. nodosum* assemblages in the highest disturbance intensity areas were still significantly different from assemblages in the control and lowest disturbance intensity areas. These results were mainly driven by a switch in the dominant canopy species, from *A. nodosum* to *F. vesiculosus*. Results obtained in this study show that disturbance by human trampling negatively affects *A. nodosum* assemblages and that the effects are dependent on trampling intensity. These findings highlight the risk of local extinction imposed on these populations by anthropogenic disturbance, and the consequent need for protection measures for such sensitive populations.

KEY WORDS: *Ascophyllum nodosum* · Trampling disturbance · Distributional range limits · Macro-algal communities · Resilience · Portuguese biogeography

INTRODUCTION

The effects of disturbance regimes on population persistence may be particularly important in marginal populations, i.e. those occurring at geographical and ecological limits. When populations are already at the edge of the suitable range of favourable conditions for their persistence, their resistance and resilience to disturbances may be compromised by the already highly stressful local conditions and small population sizes (Guo et al. 2005). Yet, edge populations may develop unique adaptive traits for the species that confer them high conservation value and differential persistence capability when facing future environmental changes (Vucetich & Waite 2003, Hampe & Petit 2005, Johannesson & André 2006).

Disturbance, both natural and anthropogenic, plays an extremely important role in structuring natural assemblages by influencing the distribution, abundance and diversity of species (Sousa 1979, 1984, Connell et al. 1997). Disturbance events not only increase mortality, but also free resources such as light, nutri-

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ents and space that may enhance the density of local populations or the appearance of new colonists (Grime 1973, Connell 1978, Connell et al. 1997). These contrasting effects give rise to different post-disturbance assemblages, in terms of composition and dominance of species (Airoldi 1998).

In rocky intertidal areas, human activities due to recreational and harvesting uses represent one important source of disturbance that directly or indirectly affects marine organisms (Keough & Quinn 1998, Brown & Taylor 1999, Schiel & Taylor 1999, Milazzo et al. 2002a, 2004, Thompson et al. 2002). Among them, trampling has been the main focus of experimental studies showing that species composition, percentage cover, diversity and biomass of macroalgal assemblages can be significantly affected by increasing trampling intensity (Keough & Quinn 1998, Milazzo et al. 2002b).

Assemblages may develop the ability to persist under natural cyclic disturbance events, but their degree of resistance and resilience to episodic noncyclic events, such as those resulting, for example, from anthropogenic pressure, may largely depend on individual species–specific attributes (Airoldi 1998, Keough & Quinn 1998, Schiel & Taylor 1999, Benedetti-Cecchi et al. 2001). Erect macroalgae, like canopy-forming species, are particularly sensitive to perturbation events (Benedetti-Cecchi et al. 2001, Milazzo et al. 2004). Furthermore, changes affecting these species are of overwhelming importance because, as ecological engineers, they provide habitat, food and shelter for other species (Keough & Quinn 1998, Brown & Taylor 1999, Schiel & Taylor 1999).

A particularly interesting location to address hypotheses about disturbance effects on marginal rocky intertidal populations is the northern region of Portugal, a biogeographic transition zone where many intertidal species encounter their distributional limits (e.g. Ardré 1970). Among the many seaweeds that encounter their southern range limits in this zone is the brown seaweed *Ascophyllum nodosum* (L.) Le Jol., a common dominant canopy species on sheltered intertidal rocky shores in the North Atlantic. In Europe, this species extends from above the Arctic circle to Portugal, where a single border population (separated by 10s of km from the nearest neighbouring population), with an extension of about 3 km in length, is found on the Viana do Castelo shore.

Ascophyllum nodosum is a dioecious, long-lived species (Åberg 1992a,b) with a low recruitment rate (Åberg & Pavia 1997, Dudgeon & Petraitis 2005) and a low probability of long-distance dispersal of zygotes (Dudgeon et al. 2001). In terms of biomass, *A. nodosum* populations are usually dominated by large individuals (Åberg 1992a). Recruits are patchily distributed under the canopy and have an extremely slow growth rate (Åberg & Pavia 1997, Cervin et al. 2005).

Given these life history traits, *Ascophyllum nodosum* populations may be particularly susceptible to disturbance events. Field experiments have shown that removal of *A. nodosum* canopy-forming individuals (adults) causes long-term changes in the assemblage structure (Jenkins et al. 1999, 2004, Cervin et al. 2005, Ingolfsson & Hawkins 2008). To date, however, no experimental study has addressed the possible outcomes of increased trampling disturbance on *A. nodosum* assemblages or the effects of disturbance in general on range edge populations. Marginal populations are often smaller and more fragmented, and as such they are prone to suffer higher extinction risks than central populations (Guo et al. 2005).

The present paper aims at understanding whether events of disturbance due to increasing human activities can significantly increase the extinction risk of marginal populations of a major community-structuring species and associated macroalgal assemblages. To address this main goal, we experimentally imposed different levels of trampling intensities on *Ascophyllum nodosum* populations of Viana do Castelo. The specific aims of this study were to (1) experimentally assess the effects of different intensities of human trampling on *A. nodosum* assemblages at its southern limit and (2) investigate the short-term (12 mo) pattern of recovery in plots of *A. nodosum* subjected to different intensities of trampling.

MATERIALS AND METHODS

Study site. This study was conducted on the rocky shore of Praia Norte in Viana do Castelo, NW Portugal (hereafter called Viana; 41° 41′ N, 8° 51′ W; Fig. 1). The shore is granitic, gently sloping and includes semi-exposed and sheltered areas. The tidal regime is semi-diurnal, reaching 3.5 to 4.0 m during spring tides. Sea surface temperature varies between 13 and 20°C during the year. The shore is highly frequented during summer months for recreational purposes, which have increased during the last decade associated with shore development for recreation. It is also visited for shellfish harvesting activities throughout the year.

In the mid-intertidal zone of the sheltered area of Viana (between roughly 5 and 7 m above Lowest Astronomical Tide [LAT]), assemblages are dominated by brown algae, varying in dominance from higher to lower heights on the shore, from *Pelvetia canaliculata* to *Fucus spiralis*, *Ascophyllum nodosum* and *F. vesiculosus*. In some areas, patches of these species are mixed at the same height on the shore. This study was conducted on the assemblages dominated by *A. nodosum* at the southernmost distributional limit of the species in Europe. These are low-diversity assem-

Fig. 1. Geographical distribution of *Ascophyllum nodosum* in the Iberian Peninsula showing the southern border population of Viana do Castelo (Marti et al. 2001)

blages where *F. vesiculosus* occurs in low abundance, as the second canopy species, and understorey species include some turf-forming algae such as the red algae *Gelidium pusillum* and *Rhodothamniella floridula* and a few animals such as the barnacles *Chthamalus* spp. and the limpets *Patella* spp.

Survey of human presence. Natural pressure by human trampling in Viana was estimated by counting the number of persons visiting the shore in 4 areas, each approximately 100 m long and separated from the adjacent area by at least 100 m. From October 2004 to September 2005, 2 d of low tide were randomly selected each month. On each day, between the time of emersion and submersion of *Ascophyllum nodosum*, the number of persons entering each area was counted in 2 non-consecutive periods of 30 min, during a total 4 h period. The survey started in 1 area and sequentially continued to the other areas, returning again to the first area after 2 h. The number of visitors per hour in each area was calculated by summing the counts from both 30 min periods. Results were used to choose relevant levels of intensity of disturbance in the experimental study.

Experimental procedure and collection of data. To test the effects of human trampling on *Ascophyllum nodosum* assemblages, an experimental study was performed during the period of the year when the shore is less frequented by people, over 5 mo, between December 2005 and April 2006. An area of approximately 100 × 30 m of shoreline was chosen within the *A. nodosum* population, located hundreds of metres from the most frequented parts of the shore and rarely visited by people. Ideally, this study should have been conducted in areas where uncontrolled human pressure was absent, or in areas where it could be rigidly controlled. Unfortunately, this was not possible here because the shore is unprotected from human usage. However, because of the small size of the experimental areas and the regularity of our survey action we are confident that uncontrolled human trampling pressure was minimal and did not differ between the various experimental plots, thus not affecting the relative differences between treatments (see also Results).

Twelve experimental plots of 2.5 × 2.5 m, dominated by *Ascophyllum nodosum*, were chosen and marked in the corners with screws and plastic tags. Three plots were randomly allocated to each of 4 levels of trampling intensity, chosen based on the survey conducted before the start of the experiment. Each experimental plot was divided into 4 rows of similar width that were uniformly trampled by 2 different persons, both weighing about 70 kg and wearing gumboots of European size 39. The number of 'tramples' corresponded to the number of times each entire row of the experimental plot was trampled. At each disturbance event, each of the 4 levels of trampling intensity (corresponding to different numbers of tramples) was applied to the corresponding experimental plots. In each month, 2 disturbance events were applied to experimental plots (1 in each week of spring tide).

The *Ascophyllum nodosum* area is mainly visited by people collecting shellfish, with the exception of recreational use during summer months. Some of these persons cross the *A. nodosum* population to reach more exposed areas near the sea, but many stay in the *A. nodosum* area throughout the low tide period. We considered that an approximate correspondence could be established between the number of persons observed h^{-1} in each area and the number of tramples this area was experiencing during an entire period of low tide. On this basis, we selected the following intensities of disturbance: (1) low intensity of disturbance (5 tramples, LI) corresponded approximately to the mean annual intensity of trampling estimated for all areas, (2) medium intensity of disturbance (15 tramples, MI) was estimated by averaging the 5 highest values of human frequentation observed during the survey and (3) high intensity of disturbance (30 tramples, HI) was chosen to simulate an increase in the number of people visiting this shore. On 3 sampling occasions, more than 15 persons h^{-1} were found on the *A. nodosum* area. Therefore, 30 tramples were considered to reflect an increase in trampling pressure. Trampling levels chosen were carefully reduced to the minimum necessary to test the hypothesis, in order to avoid imposing further negative impacts on these populations.

Experimental plots were sampled 6 times (before the experiment started and after every second trampling event) during the experimental trampling period and 3 times during the recovery phase (3, 6 and 12 mo after the end of experimental treatments). Experimental plots were divided into 25 quadrats of 50 × 50 cm. Three quadrats within each plot were randomly sampled on each sampling date. Abundance of *Ascophyllum nodosum* and associated organisms was visually estimated throughout the study. Percentage cover of *A. nodosum* and other sessile organisms was assessed by means of non-destructive methods (Dethier et al. 1993) using a plastic frame divided into 25 quadrats of 10 × 10 cm, assigning to each taxon a score ranging from 0 to 4 per quadrat and adding up the 25 estimates. Densities of mobile organisms were expressed as the number of individuals per quadrat. Whenever necessary, sampling was stratified in different layers, and the upper visible layer of macroalgae was distinguished from substrate cover, with estimates of percentage cover made for each layer separately (Dethier et al. 1993).

Biomass loss was estimated by collecting all detached pieces of *Ascophyllum nodosum* after every trampling event in each of the experimental plots. Dry biomass was assessed after drying the material in an oven at 50°C for 48 h.

Statistical analysis. Effects of the experimental treatments during the 5 mo period when the disturbance events were regularly applied were tested on individual variables, using analysis of variance (ANOVA), as well as on the structure of the whole assemblage using permutational multivariate ANOVA (PERMANOVA; Anderson 2001), on Bray-Curtis dissimilarity coefficients, calculated on non-transformed data. Analysis included the following factors: Time (Ti) at 6 random levels (the sampling times) and Intensity (In) at 4 fixed levels (0, 5, 15 and 30 tramples), crossed with Ti, with 3 replicates, corresponding to the average values of the 3 quadrats sampled in each plot at each time. Data were considered temporally independent as on each sampling date, only 12 % of the total area of the experimental plot was sampled, and care was taken to sample different quadrats at each sampling time. For each experimental plot, quadrats were randomly allocated to each sampling time before the start of the experiment. Before each ANOVA, the assumption of homogeneity of variances was tested using Cochran's test and, when appropriate, data transformation was performed (Underwood 1997). Student-Newman-Keuls (SNK) tests were used for *a posteriori* comparisons of means.

Effects of experimental treatments on the structure of the whole assemblage were visualised by comparing the temporal trajectories of centroids describing the 'average' assemblage. Data from the 3 replicate quadrats per plot per time were first averaged, and principal coordinates (Gower 1966) from the Bray-Curtis dissimilarity matrix were calculated. Centroids were then obtained as arithmetic averages of the principal coordinates over the 3 replicates per sampling time (McArdle & Anderson 2001). Multivariate patterns were visualised with the non-metric MDS plot based on Euclidean distance that was generated with PRIMER (Clarke & Gorley 2001).

To test the effects of experimental treatments on the recovery of assemblages, the same kind of univariate and multivariate analyses were performed. In these analyses, the factor Time was defined with 3 random levels (corresponding to the 3 times of sampling: 3, 6 and 12 mo after the end of the disturbance treatments).

To test the effects of experimental treatments on *Ascophyllum nodosum* biomass loss, ANOVA was performed. Analysis included the factors Time (8 random levels, corresponding to the times of trampling, after the second trampling event) and Intensity (3 fixed levels: 5, 15 and 30 tramples).

RESULTS

Human usage

The survey to assess levels of human usage of the *Ascophyllum nodosum* area showed that the number of visitors was variable among areas and seasons, with values fluctuating from 0 to 21 persons h^{-1} (Fig. 2). The experimental plots were located in Area 2 which, together with Area 4, was least frequented, with an average of 1.1 ± 0.4 (mean ± SE) persons h^{-1} entering it. These data offer a view of the number of visitors present in the study area and show that experimental levels were chosen in the range of natural values for LI and MI of experimental trampling, and simulated a realistic increase of trampling for the HI level. Furthermore, they show that although not protected, the area in which we located our experimental plots was poorly frequented, and consequently uncontrolled human interference with our experimental treatments can be considered minimal.

Disturbance period

Multivariate analysis showed a significant interaction between In and Ti on the structure of the whole assemblage (Table 1). Pairwise *a posteriori* comparisons detected no differences among plots allocated to different trampling intensities before the start of the experiment (Ti0), but after 2 episodes of disturbance (Ti1), the 2 lowest intensities of disturbance (control [C] and LI) were already different from the highest one (HI; Table 1). These differences were maintained throughout the study period. After 4 episodes of disturbance (Ti2), MI plots became different from C plots,

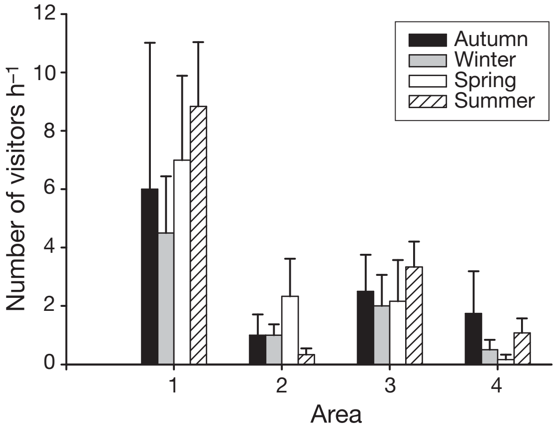

Fig. 2. Human frequentation (mean number of visitors per hour + SE, n = 6) in different areas and different seasons of the year, within the *Ascophyllum nodosum* population of Viana do Castelo

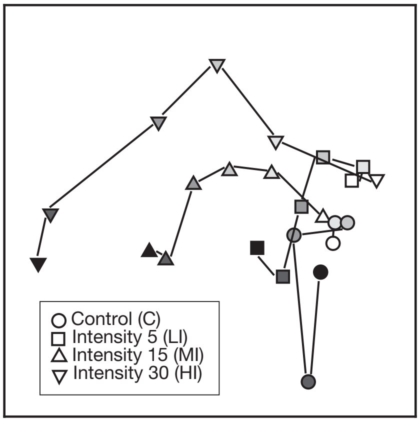

Fig. 3. Temporal trajectories of *Ascophyllum nodosum* assemblages exposed to different trampling intensities. Symbols represent centroids of the 'average' community in each plot and were calculated using principal coordinates (see 'Materials and methods' for details). Shading gradient (from white to black) indicates different times of sampling (from 0 to 5, respectively)

and at the end of the study (T5), they also differed from HI plots (Table 1). Multi-dimensional scaling confirmed the results of the analysis showing a clear pattern of progressive separation among the 3 levels of experimental disturbance, with C plots not separating from LI plots (Fig. 3).

Effects of the intensity of disturbance on the percentage cover of *Ascophyllum nodosum* were variable with time (Table 2). At T0, the cover of *A. nodosum* was similar among all plots allocated to different treatments (62.77 ± 3.96 %, mean ± SE) but after 1 mo, the abundance of *A. nodosum* in HI plots was already significantly lower than in C and LI plots (Table 2, Fig. 4a). From the second time of sampling *A. nodosum* cover was significantly lower in MI and HI (where it decreased by more than 50 % during the experiment) than in C and LI treatments (Table 2, Fig. 4a). At the end of the experiment, the abundance of *A. nodosum* was higher in C than in all other treatments, which did not differ from each other (Table 2).

Table 1. PERMANOVA testing the effects of intensity of disturbance and sampling times on the structure of the whole *Ascophyllum nodosum* community. C: control, LI: low intensity, MI: medium intensity, HI: high intensity; Ti 0: before start of experiment, Ti 1, 2, 3, 4, 5: 1 to 5 mo after start of experiment; $^{*}p < 0.05$, $^{**}p < 0.01$

Source of variation	df	MS	Pseudo-*F*	Permutable units and denominator for *F*
Time (Ti)	5	3002.21	7.73**	72 replicate plots
Intensity (In)	3	4974.95	7.74**	24 Ti × In cells
Ti × In	15	642.71	1.66*	72 replicate plots
Residual	48	388.15		

Ti × In pair-wise *a posteriori* comparisons (*t*-test)

Ti 0	C = LI = MI = HI
Ti 1	C ≠HI; LI ≠HI
Ti 2	C ≠MI; C ≠HI; LI ≠HI
Ti 3	LI ≠HI
Ti 4	C ≠HI; LI ≠HI
Ti 5	C ≠MI; C ≠HI; LI ≠HI; MI ≠HI

The amount of *Ascophyllum nodosum* biomass removed by trampling was dependent on intensity levels. Higher trampling levels caused more algal biomass to become detached, although detached biomass decreased with time in all trampling intensities (Fig. 5, Table 3).

At the beginning of the experiment, the brown seaweed *Fucus vesiculosus* was also present in all plots (20.16 ± 2.76 %, mean ± SE), and its cover did not significantly differ among plots allocated to different trampling intensities. During the experiment, a main effect of the intensity of trampling was detected on the *F. vesiculosus* cover, the abundance of which was higher in C plots than in all other treatments (Table 2, Fig. 4b). However, a temporal increase in the abundance of the alga was detected in all experimental plots, regardless of their level of disturbance intensity (Table 2, Fig. 4b).

Before the start of the experiment, *Ulva* sp. was almost absent from all plots. *Ulva* sp. cover increased in trampled plots, starting from the third month of the experiment, and this effect was larger at higher trampling intensities (significant interaction Ti × In; Table 2, Fig. 4c). *Ulva* sp. cover increased from nearly 0 to 50 % during the experiment in the HI plots (Fig. 4c).

Understorey species significantly decreased in cover, compared to controls, when exposed to the 2 highest intensities of trampling (Table 2, Fig. 4d).

Table 2. ANOVA of the effects of trampling intensities and time on percentage cover of different macroalgal species. C: control; LI: low intensity; MI: medium intensity; HI: high intensity; Ti 0: before start of experiment, Ti 1 to 5: 1 to 5 mo after start of experiment; ns: non-significant, *p < 0.05, **p < 0.01, ***p < 0.001

Species Source of variation/test		df	MS	*F*
Ascophyllum nodosum				
Time (Ti)		5	1867.90	15.07***
Intensity (In)		3	3144.19	12.49***
Ti × In		15	251.69	2.03*
Residuals		48	123.94	
Cochran's test	*C* = 0.22; ns			
Transformation	None			
SNK test Ti × In	Ti 0: In C = LI = MI = HI			
(SE = 6.43):	Ti 1: In C > HI; In LI > HI			
	Ti 2: In C = LI > MI = HI			
	Ti 3: In C = LI > MI = HI			
	Ti 4: In C > HI; In LI > HI			
	Ti 5: In C > LI = MI = HI			
Fucus vesiculosus				
Ti		5	749.97	4.68**
In		3	1814.47	10.24***
Ti × In		15	177.27	1.11 ns
Residuals		48	160.05	
Cochran's test	*C* = 0.19; ns			
Transformation	None			
SNK test In	Ti 0: In C > LI = MI = HI			
(SE = 2.98)				
Ulva sp.				
Ti		5	7.01	19.00***
In		3	3.87	4.46*
Ti × In		15	0.87	2.35*
Residuals		48	0.37	
Cochran's test	*C* = 0.14, ns			
Transformation	$\ln(x + 1)$			
SNK test Ti × In	Ti 0: In C = LI = MI = HI			
(SE = 0.35)	Ti 1: In C = LI = MI = HI			
	Ti 2: In C = LI = MI = HI			
	Ti 3: In C = LI = MI = HI			
	Ti 4: In C < LI = MI = HI			
	Ti 5: In C < MI; In C < HI;			
In LI < HI				
Understorey species				
Ti		5	152.69	0.89 ns
In		3	617.85	4.98*
Ti × In		15	124.18	0.72 ns
Residuals		48	171.86	
Cochran's test	C = 0.14; ns			
Transformation	None			
SNK test In (SE = 3.01)	Ti 0: In C > MI; In C > HI			

Recovery period

The effect of intensity of disturbance on the patterns of recovery of *Ascophyllum nodosum* assemblages varied with sampling time (Table 4, significant interaction). One year after the end of trampling disturbance, the highly trampled plots still had a structure significantly different from C and LI plots (Table 4, pairwise comparisons). Multi-dimensional scaling confirmed these results. One year after the end of experimental trampling, C plots remained similar to their state at the beginning of the study (Fig. 6). The temporal trajectory of LI plots showed a slight, though not significant, differentiation from C plots (Table 4, Fig. 6). MI and HI plots progressively differentiated both from conditions before experimental trampling and from C and LI plots (Fig. 6). At the beginning of the recovery period, there was a separation between HI and MI plots, but 3 mo after trampling disturbance, assemblage composition in these plots became progressively similar (Table 4, Fig. 6).

The abundance of *Ascophyllum nodosum* in MI and HI plots did not increase and remained significantly lower than in C and LI plots (Table 5, Fig. 7a). The pattern of recovery of *Fucus vesiculosus* suggested a tendency for separation between the 2 highest intensities of disturbance (MI and HI) and the other 2 levels (C and LI; Table 5, Fig. 7b). Six months after the end of the trampling, *F. vesiculosus* abundance in C and LI plots returned to values found before the experiment started (Fig. 7b). In contrast, in MI and HI plots, the cover of *F. vesiculosus* greatly increased after trampling disturbance stopped, becoming the dominant species in those plots, with cover of 64.11 ± 10.55% (mean ± SE) and 80.77 ± 2.47%, respectively (Fig. 7b). The abundance of *Ulva* sp. in experimental plots progressively decreased after the end of trampling and 1 yr later it had almost disappeared from all plots (Table 5, Fig. 7c). An increase in abundance of understorey species was detected 6 mo after experimental treatment stopped (Fig. 7d). However, after 1 yr, understorey species in HI plots still had lower cover than before the experiment started (Fig. 7d).

DISCUSSION

The results obtained in this study showed that trampling negatively affected *Ascophyllum nodosum* assemblages and that these effects were dependent on

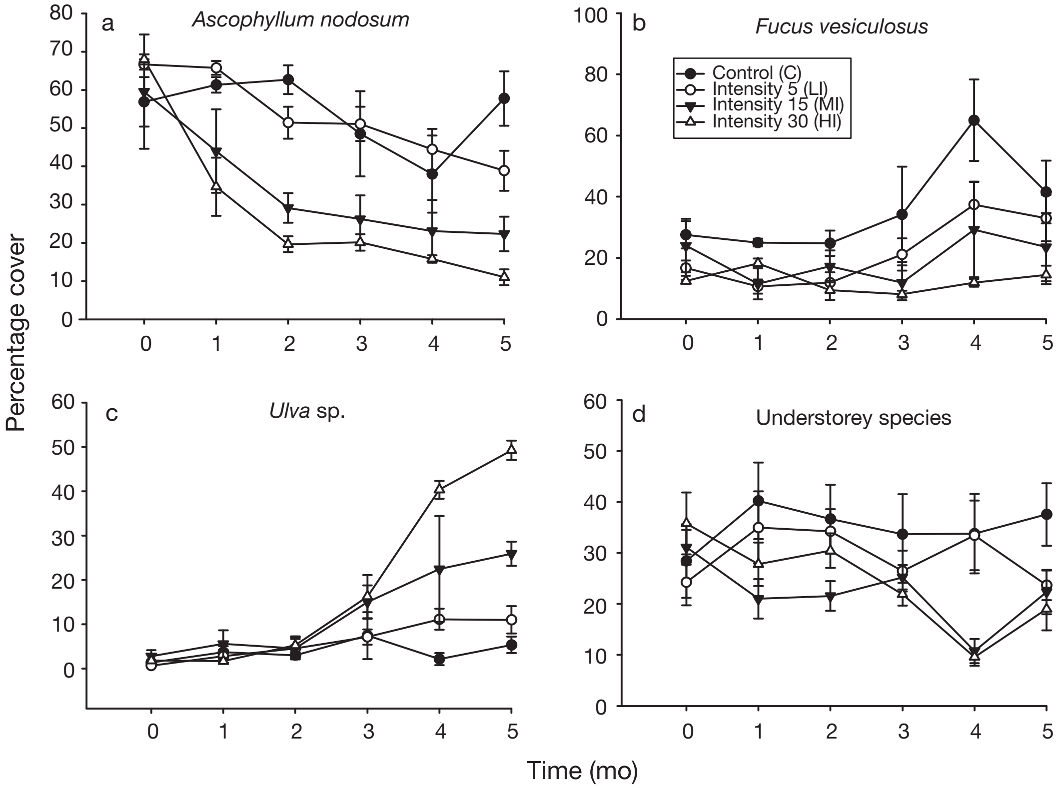

Fig. 4. Percentage cover (mean ± SE, n = 9) of (a) *Ascophyllum nodosum*, (b) *Fucus vesiculosus*, (c) *Ulva* sp. and (d) remaining macroalgae understorey species, in each plot at different times of sampling and trampling intensities; see 'Materials and methods' for details. Time is months after start of experiment

the magnitude of the disturbance. Trampling levels also affected the subsequent temporal evolution of assemblages. One year after removal of trampling disturbance, assemblages under the highest trampling intensities were unable to recover to the pre-disturbance state. The main effect recorded was a switch in the dominance of canopy species from *A. nodosum* to *Fucus vesiculosus*. *A. nodosum* drastically reduced its cover in the highest intensity plots over the disturbance period and even after 1 yr had not recovered. *F. vesiculosus* restored the canopy, reaching cover values similar to those for *A. nodosum* prior to disturbance.

When disturbances create patches of free space, the process of colonisation of available areas is influenced, among other factors, by the magnitude (duration and intensity), spatial extent and temporal variation of disturbance (Airoldi 1998, Petraitis & Latham 1999, Dudgeon & Petraitis 2001, 2005), identity of remaining species (Cervin et al. 2005), proximity of disturbed patches to potential sources of colonising species (Reed et al. 1988, Guo et al. 2005) and species-specific biological characteristics (reproductive capability, growth rate and dispersal characteristics of propagules; Airoldi 1998, Dudgeon & Petraitis 2001).

The progressive dominance of *Fucus vesiculosus* over *Ascophyllum nodosum* assemblages observed in experimental plots with increasing intensities of trampling disturbance might be influenced by life history

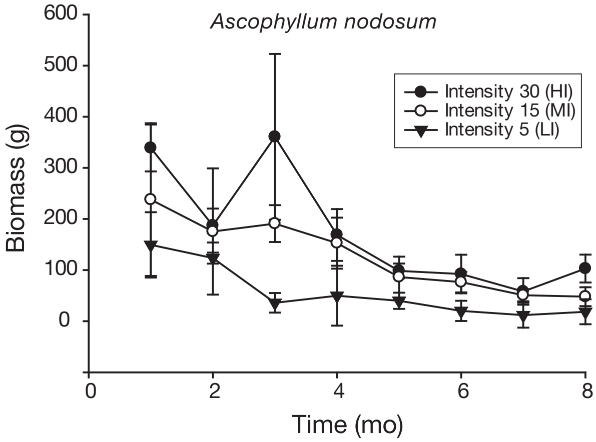

Fig. 5. *Ascophyllum nodosum* biomass detached from the substratum (mean ± SE, n = 3) at different trampling intensities (see 'Materials nad methods' for details), after the first month of the experiment. Detached biomass in control plots is not represented because it was always negligible during the experiment

Table 3. *Ascophyllum nodosum.* ANOVA testing the effects of intensity of disturbance and time on the amount of *A. nodosum* biomass removed by trampling. C: control; LI: low intensity; MI: medium intensity; HI: high intensity; ns: non-significant, $^{**}p < 0.01$, $^{***}p < 0.001$

Source of variation	df	MS	*F*
Time (Ti)	7	52.13	3.33**
Intensity (In)	2	160.17	11.78***
Ti × In	14	13.59	0.60 ns
Residuals	48	15.67	
Cochran's test		*C* = 0.17; ns	
Transformation		SQRT(x + 1)	
SNK test In (SE = 0.75)		In LI < MI = HI	

Table 4. PERMANOVA testing the effects of intensity of disturbance and time after removal of disturbance on the recovery of the whole community. C: control; LI: low intensity; MI: medium intensity; HI: high intensity; Ti 1 is 3 mo, Ti 2 is 6 mo and Ti 3 12 mo after the start of the experiment; $^{*}p < 0.05$, $^{**}p < 0.01$

Source of variation	df	MS	Pseudo-*F*	Permutable units and denominator for *F*
Time (Ti)	2	2551.70	4.69**	36 Replicate plots
Intensity (In)	3	5207.90	4.78**	12 Ti × In cells
Ti × In	6	1090.70	2.01*	36 Replicate plots
Residual	24	543.51		
Pairwise *a posteriori* comparisons (*t*-test)				
Ti 1	In C = LI = MI ≠ HI			
Ti 2	In C = LI ≠ MI = HI			
Ti 3	In C ≠ HI; In LI ≠ HI			

traits and competitive interactions between these 2 species. Dudgeon & Petraitis (2001) demonstrated that the number of *A. nodosum* recruits is exponentially reduced with distance from parental algae, probably because *A. nodosum* canopy enhances recruitment rates by providing protection against heat stress and desiccation and reducing water motion (Vadas et al. 1990, Brawley & Johnson 1991, Cervin & Åberg 1997) and at the same time by preventing the access of other species' propagules to available space (Dudgeon & Petraitis 2001). However, the removal of canopy can also positively affect the recruitment process by reducing herbivory pressure by individuals inhabiting adult algae (Viejo et al. 1999) and reducing sweeping of the substratum by fronds (Viejo et al. 1999, Dudgeon & Petraitis 2005). These are positive effects both for *A. nodosum* and *F. vesiculosus*, but the huge post-settlement mortality (Viejo et al. 1999, Dudgeon & Petraitis 2005) and the slow growth rate of *A. nodosum* recruits (Cervin et al. 2005) may determine the competitive superiority of *F. vesiculosus* individuals in occupying newly available space in the disturbed patches.

The geographical situation of the *Ascophyllum nodosum* population studied may also contribute to the observed low rate of recovery. Species abundance often declines towards marginal locations (Guo et al. 2005), and in such marginal environments the importance of interspecific competition may be magnified when patches of free space are provided (Case et al. 2005). Avoidance of competitive exclusion may require a higher colonisation rate or a lower basic extinction rate than in potentially competitive species (Case et al. 2005), which does not seem to be the case for *A. nodosum*. The absence of nearby colonising sources may also be a determinant factor in these range-edge *A. nodosum* populations, which are isolated from the nearest neighbouring populations by tens of km. In passive populations with relatively poor dispersal power (as is the case for *A. nodosum*; Dudgeon et al. 2001), migration only occurs across neighbouring habitats (Bohonak 1999, Guo et al. 2005), and geographical isolation may reduce to residual levels the contribution of neighbouring populations to the recolonisation of disturbed patches. In these cases, population size is mainly controlled by birth and death rates (Guo et al. 2005). This border *A. nodosum* population may undergo changes in its vital rates that stress its competitive inferiority in comparison to other colonisers.

Disturbance effects on *Ascophyllum nodosum*-dominated assemblages have been investigated in various contexts (Jenkins et al. 1999, Dudgeon & Petraitis 2001, Bertness et al. 2002, Cervin et al. 2004). Studies on short and long-term effects of *A. nodosum* canopy

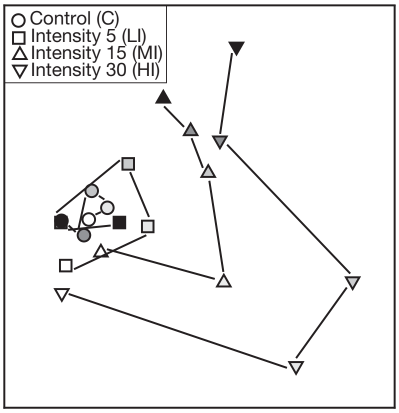

Fig. 6. Temporal trajectories of *Ascophyllum nodosum* communities at different trampling intensities (see 'Materials and methods' for details). Symbols represent centroids of the 'average' assemblage and were calculated as in Fig. 3. Colour gradient (from white to black) indicates different temporal steps of the experiment: white symbols represent assemblage composition before trampling disturbance started, light grey corresponds to the assemblages at the end of trampling disturbance experiment and increasingly darker grey symbols (to black) correspond to assemblages at 3, 6 and 12 mo after disturbance ended

Table 5. ANOVA testing the effects of intensity of disturbance and time after removal of disturbance on the recovery of different macroalgal species. C: control; LI: low intensity; MI: medium intensity; HI: high intensity; Ti 1 is 3 mo, Ti 2 is 6 mo and Ti 3 is 12 mo after the start of the experiment. ns: non-significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Species Source of variation/test		df	MS	*F*
Ascophyllum nodosum				
Time (Ti)		2	179.63	0.95 ns
Intensity (In)		3	5545.80	32.44***
Ti × In		6	170.95	0.90 ns
Residuals		24	189.54	
Cochran's test	0.31; ns			
Transformation	None			
SNK test				
Intensity (SE = 4.59)	In C = LI > MI = HI			
Fucus vesiculosus				
Ti		2	794.92	2.03 ns
In		3	1806.76	2.38 ns
Ti × In		6	758.32	1.93 ns
Residuals		24	392.34	
Cochran's test	0.28; ns			
Transformation	None			
SNK test				
Intensity (SE = 6.60)	In 1 = 2 = 3 = 4			
Ulva sp.				
Ti		2	962.00	24.09***
In		3	1096.53	3.01 ns
Ti × In		6	364.64	9.13***
Residuals		24	35.26	
Cochran's test	0.36; ns			
Transformation	None			
SNK test Ti × In (SE = 3.65)	Ti 1: In C < MI; In C < HI; In LI < HI; In MI < HI			
	Ti 2: In C = LI = MI < HI			
	Ti 3: In C = LI = MI = HI			
Understorey species				
Ti		2	261.12	1.23 ns
In		3	91.07	0.13 ns
Ti × In		6	682.28	3.22*
Residuals		24	211.83	
Cochran's test	0.36; ns			
Transformation	None			
SNK test Ti × In (SE = 8.40)	Ti 1: In C > LI = MI = HI			
	Ti 2: In C = LI = MI = HI			
	Ti 3: In C = LI = MI = HI			

removal have demonstrated that it caused a small-scale temporal change in the structure of assemblages (Jenkins et al. 1999, Cervin et al. 2004) that was not recovered over larger temporal scales (Jenkins et al. 2004, Cervin et al. 2005, Ingolfsson & Hawkins 2008). In general, canopy is restored after some years but *Fucus* sp. becomes the dominant species in mature assemblages (Jenkins et al. 2004, Cervin et al. 2005). However, these studies were all based on the experimental clearance of patches by excision of the entire plant, including the holdfast. When the disturbance source is human trampling, effects on individual plants are not uniform within plots, causing either the removal of the entire canopy (in some cases not including the removal of the holdfast) or the detachment of parts of the plant. Thus, the recolonisation of disturbed areas is expected to be dependent both on newly recruited individuals and on vegetative regrowth rates. Regeneration rates of *A. nodosum* individuals may differ depending on the cutting height of the frond, on the amount of biomass left and on the age of the plant (Lazo & Chapman 1996). In fact, harvested stands of *A. nodosum* showed a reduction in colonisation and growth with increased severity of harvesting (Keser et al. 1981, Ang et al. 1996), but these assemblages recovered to pre-harvest levels within short periods (Ang et al. 1996, Ugarte et al. 2006). Our data on short-term *A. nodosum* recovery closely resemble the results of long-term recovery recorded in total removal studies on *A. nodosum* assemblages (Jenkins et al. 2004, Cervin et al. 2005). Although total removal of algae did not occur in any of our experimental plots, the removal caused by trampling at the highest levels of intensity was severe enough to prevent rapid and effective recolonisation by *A. nodosum* individuals and to allow the establishment of *Fucus vesiculosus* as the dominant canopy species. Indeed, if a disturbance is large enough (frequency, intensity, extent or duration) it may allow a switch in assemblage dominance (Petraitis & Latham 1999).

Previous studies on the effects of total canopy removal and harvesting may provide valuable information for predicting the evolution of *Ascophyllum nodosum* assemblages exposed to trampling disturbance. However, the long-term evolution of these assemblages might differ from previous studies both because the type of perturbation (trampling) is different from those previously tested and because the population under study is a marginal southern limit population that may have differentiated traits from central populations. Nevertheless, all results available to date in this study indicate a severe negative effect of trampling on *A. nodosum* assemblages.

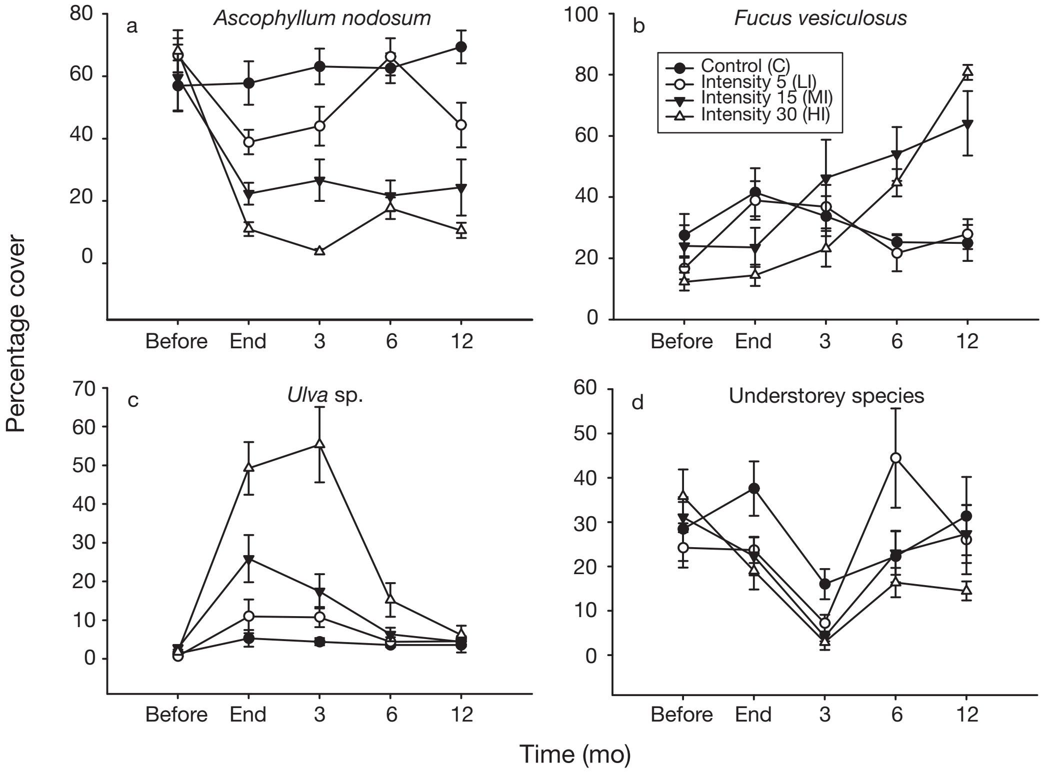

Fig. 7. Percentage cover (mean ± SE, n = 9) of (a) *Ascophyllum nodosum*, (b) *Fucus vesiculosus*, (c) *Ulva* sp. and (d) remaining macroalgae understorey species, for different trampling intensities (see 'Materials and methods' for details) before the experiment started ('Before'), at the end ('End') of the experiment and at different times of sampling (3, 6 and 12 mo) during the recovery period

These results are of concern considering natural levels of human usage estimated for the study area. The lowest level of intensity used in this study was slightly below the mean natural trampling pressure recorded during most of the year in the area more heavily visited. Even at the lowest trampling intensities, there was a small decrease in the cover of *Ascophyllum nodosum* that did not recover and a small increase in *Fucus vesiculosus*. This might be indicative of a slow but progressive process of replacement of canopy identity, occurring at continued low intensity of disturbance. Also, on some occasions, this area was subjected to visiting pressure between the intermediate and highest intensity trampling levels used in this study. The effects of trampling on *A. nodosum* were significant after 2 and 4 trampling events for the highest and intermediate trampling intensities, respectively. These results show that, at natural trampling pressure levels, this *A. nodosum* population is being negatively affected, either by continued perturbation at low trampling intensities or by episodic events of large perturbations.

The possibly high chance of extinction of the small Viana population of *Ascophyllum nodosum* is of concern given its conservation interest. From an ecological and genetic point of view, marginal populations may represent valuable systems in terms of conservation (Guo et al. 2005, Johannesson & André 2006). Populations living in geographically marginal environments often experience extreme conditions, and to deal with these conditions, they frequently develop unique ecophysiological local adaptations and genetic structure that constitute important resources for management and conservation (Bouza et al. 1999, Johannesson & André 2006). Considering its geographical isolation (and consequent isolation from neighbouring populations), its southern limit position (and consequent higher physical stress) and its vulnerability to an important source of disturbance occurring in its southern distribution area, we suggest that this border *A. nodosum* population is threatened and may become extinct in the long term.

Because the population studied here is a border population, we cannot say that the estimated effects of trampling can be generalised for all *Ascophyllum nodosum* populations. However, taking into consideration previous studies on long-term evolution of more centrally distributed *A. nodosum* assemblages subjected to canopy removal, the results of our study point to the need for protecting *A. nodosum* in areas where human trampling is increasing in order to conserve the populations of this species.

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