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Resumo

O debate diversidade-estabilidade esteve na origem de vários estudos nas ultimas décadas e uma recente aproximação a este assunto começou a dar mais atenção à variabilidade ao nível das espécies. As populações respondem de diversas formas às oscilações ambientais e podem sofrer consequentes ajustes evolutivos. O objectivo desta dissertação é utilizar dados empíricos das populações *in situ* para testar uma relação teórica entre a diversidade genética e a estabilidade das comunidades em condições naturais no intertidal rochoso. No entanto observações *in situ* não podem apontar qual a direcção da relação entre a estabilidade ambiental e a diversidade genética, este tipo de estudo teria de ser feito em ambiente controlado. A aproximação feita beneficia das condições naturais perante toda a complexidade dos ambientes naturais, incluindo a verdadeira composição das comunidades associadas e níveis de diversidade genética. Em várias localizações ao longo do norte da Europa, quadrados de duas espécies de *Fucus* sp., *Fucus serratus* e *Fucus vesiculosus*, foram observados em cinco áreas definidas ao longo do intertidal, onde a densidade da alga e a estabilidade da comunidade de invertebrados foi acompanhada ao longo de três anos. As amostras foram recolhidas em dois quadrados em cada localização, um mais estável e outro menos estável, sendo o objectivo deste trabalho testar a existência de relações positivas entre a diversidade genética de *Fucus* sp. e i) o seu nível de variância na denside ou ii) a estabilidade das comunidades associadas ao longo dos anos. Os resultados revelaram uma relação positiva entre a biodiversidade e a riqueza alélica nos respectivos quadrados onde se encontrava a espécies estrutural (*Fucus* sp.), e a estabilidade da comunidade foi obtida através de vários anos de observação. A estimativa da variância temporal na composição das comunidades foi sistematicamente maior nos ecossistemas onde existia a menor diversidade genética. Tendo em conta a prevista relação entre a diversidade genética e as tendências demográficas nas relações *in situ,* é possível observar uma relação entre a densidade de *Fucus* sp. e a sua riqueza alélica, não entre a biodiversidade e a densidade ou estabilidade da alga. Então uma relação positiva emerge entre a riqueza alelica e a estabilidade das comunidades associadas. No entanto, globalmente os resultados não podem ser interpretados como revalados como tendo uma uma influencia positiva na densidade e genética, por outro lado, resultam numa maior estabilidade das comunidade que têm como espécie estrutural *Fucus* sp..Os resultados revelam um efeito relevante dos ambientais,

baseados na estabilidade das comunidades associadas e na diversidade genética das espécies formadores dos ecossistemas estudados. Distúrbios ambientais podem afectar a variância temporal dos grupos de comunidades e paralelamente a diversidade genética dentro da espécie mas numa escala de tempo diferente da que afecta a densidade de *Fucus* sp. Esta diferença temporal imposta pela pressão ambiental pode explicar as relações positivas entre a estabilidade da comunidade e a diversidade genética, e pode revelar também que a diversidade genética da espécie estrutural num ecossistema é um bom parâmetro para estimar a ocorrência de perturbações e a longa estabilidade das comunidades.

Palavras-chave: Diversidade genética, estabilidade dos ecossistemas, espécie estrutural, *Fucus vesiculosus*, *Fucus serratus*.

Abstract

The diversity stability-debate has been at the origin of several studies in the last decades and a more recent approach to this issue starts to give more attention to variability at the species level. The population dynamics response to environmental fluctuations and the consequent evolutionary adjustments. This thesis aims at using empirical data from real populations to test for a theoretically predicted relationship between genetic diversity and community stability on natural stands on rocky shores. Although *in situ* survey cannot point to the driver of any eventual relationship between environmental stability and genetic diversity, which would require manipulative experiments. This approach benefits from natural conditions encompassing all the complexity of the natural environment, including realistic composition of associated communities and levels of genetic diversity. In several locations in Northern Europe stands from two species of *Fucus* sp., *Fucus vesiculosus* and *Fucus serratus*, have been surveyed for five areas defined along the shore, where the density of algae and the stability of invertebrate assemblages were followed across three years. Samples were collected in the two extreme quadrate of each location, the most stable and the less stable, and this thesis aims to test for the existence of a positive relationship between genetic diversity in stands of *Fucus* sp. and i) their levels and variance of density or ii) the stability of associated communities across years.

The results revealed a positive relationship between biodiversity and allelic richness in the stands of the structural species of brown algae, and the stability of community assemblages assessed through several years of field survey. The *estimates* of temporal variance in the composition of communities were systematically higher in ecosystems exhibiting lower genetic diversity. Concerning the predicted relationship between genetic diversity and demographic trends, on *in situ* relationship is observed between density of *Fucus sp.* and their allelic richness, no between species richness and density or stability of algal stands. While a positive relationship emerges between allelic richness and the stability of associated community assemblages. Although globally the results cannot be interpreted as revealing a positive influence of density and genetics on each other, resulting in a higher stability of assemblages based on *Fucus* beds, they do support a concomitant effect of the external, i.e. environmental, factors on both the stability of community assemblages and the

genetic diversity of the basal species of the ecosystem studied. Environmental disturbances can affect the temporal variance of assemblages and in parallel genetic diversity within species, in a different time scale than the density of *Fucus* sp. is affected. Such temporal difference in the answer to and imprint of environmental pressure may explain the positive relationship between community stability and genetic diversity, and supports genetic diversity of the structural species of the ecosystem as a good parameter or *proxy* to estimate the occurrence and extent of perturbations and the long term stability of communities.

Keywords: Genetic diversity, ecosystems stability, structural specie, *Fucus vesiculosus*, *Fucus serratus*.

1. Introduction

1.1. Global Ecology, Climate Change and Human Impact

Ecology is the science that studies relationships of organisms (plants, animals and prokaryotes) and their environment. We can approach the study of ecology from three points of view: descriptive, functional and evolutionary. The descriptive approach is the foundation of all ecological science and is consolidated mainly by natural history like the description of nature, animals and plants and their interactions within ecosystems they live. It delivers maps of qualitative and quantitative distributions of different species and the biotic and abiotic characteristics of the environment in which they live, in that are essential to the accurate functional or evolutionary studies. The functional approach is guided by the dynamics and relationships, it seeks to study the dynamics of species interactions, as well as the dynamic responses of populations and communities to immediate factors of the environment. Finally the evolutionary approach studies organisms and relationships among organisms, and the way evolution shaped the dynamics of population and of species interactions through drift, migration and the selection of polymorphism generated by mutations (Krebs, 2001).

Climate change is expected to be one of the greatest environmental challenges of the 21st century, and its impact is starting to be noticeable in the marine environment (Heip *et al.*, 2009). Climate models show that even if the concentration of greenhouse gases could be stabilized the temperatures may continue to increase and sea level may rise 320% more than its current level, in the end of the 21st century (Meehl *et al.*, 2005), thermal and sea level rises will be accompanied by abiotic changes, like acidification, eutrophication, increase ultraviolet radiation (Naeem, 2002), oxygen concentrations and salinity changes (Heip *et al.*, 2009). Also some changes in ocean currents, important for marine organisms in a part of their life cycle, are predicted (Heip *et al.*, 2009; Meehl *et al.*, 2005). Yet climate change is not the only threat to the marine environment, human activities and pollution are such that there is a wreck of habitats, ecosystems and communities being degraded and destroyed, and species driven to extinction (Primack, 2002; Chapin *et al.*, 2000). Biotic interactions may change over time, through a process of extinction, invasion of other species (exotic species) or changes in abundance (Carlton *et al.*, 1999; Chapin *et al.*, 2000;

Yodzis (1981) revised those findings showing that models based on biologically plausible interactions rather than random ones, tended to support the existence of a positive effect of an ecosystem diversity on its stability; 4) Recent work about this debate follow two different lines: one is statistical line, exploring general trends between diversity and stability (Tilman, 1996; Yachi & Loreau, 1999) and the other is more mechanistic, where relations between the nature and structure of assemblages such as food-web structure and stability are seeked (Naeem & Li, 1997; Loreau & Behera, 1999).

In the 1990s, a new blink emerged and the focus slightly moved from populations, communities and food-webs to target ecosystems as a whole, and the relationships across the associated communities (Loreau *et al.*, 2002). This new approach shows explicitly the link between the variability of individual species and ecosystem properties, the population dynamic response to environmental fluctuations and the ongoing evolutionary adjustments (Doak *et al.*, 1998; Loreau & Behera, 1999; Yachi & Loreau, 1999; Hillebrand *et al.*, 2008).

Resilience and stability properties are usually responsible for the persistence of an ecosystem in an unpredictable environment (Loreau & Behera, 1999). The ability to adapt to unpredictable environments, which is the basis for stability in ecosystems (Booy *et al.*, 2000) tends to be higher in ecosystems characterized by higher diversity (Loreau & Behera, 1999; Tilman, 1999; Yachi & Loreau, 1999; McCann, 2000).

1.4. Genetic Diversity-Stability

The diversity-stability debate has thus far been largely dominated by the exploration of the effect of species diversity, but there are other important and less studied aspects influencing ecosystem functioning, such as genotypic and genetic diversity. Environmental variations can modify the expression of a genotype on a phenotypic level, this is called phenotypic plasticity (Sultan, 2000). Within certain limits, plants, particularly clonal ones (Reusch *et al.*, 2005; Hughes & Stachowicz, 2004, 2009; Becheler, 2010), can therefore acclimate to environmental fluctuations without underlying genetic changes. Phenotypic plasticity is similar to changes in interactions between loci subjected to different environments; it is

also linked to the ability of some genes to be expressed slightly differently in different environments. Phenotypic plasticity and such acclimation capacity is therefore independent of genetic diversity as estimated by allelic richness or heterozygosity (see authors in Booy *et al.*, 2000).

Genotypic diversity reflects the number of identical genetic individuals descendants from distinct clonal lineages that can be found in a set of samples of a population (Arnaud-Haond *et al.*, 2010). There are contradicting evidence from experimental and small scale field studies showing a positive relationship between genotypic diversity and resistance/resilience of simplified experimental assemblages (Hughes & Stachowicz, 2009; Reusch *et al.*, 2005; Ehlers *et al.*, 2008), and large scale *in situ* surveys showing either an inverse or no effect of genotypic diversity and supporting an effect of genetic diversity sensu stricto (heterozygosity or allelic richness; Arnaud-Haond *et al.*, 2007; Becheler *et al.*, 2010). Those field results are supported by recent experimental data manipulating explicitly allelic richness in parallel of genotypic diversity (Massa *et al.*, submitted), also questioning the interpretation of previous experiments or at last their extrapolation to natural systems.

To which extent phenotypic plasticity can compensate low levels of genetic variability in different environments (Booy *et al.*, 2000) is unknown, but it has been shown to reach extremes in the seagrass *Zostera marina* (Becheler *et al.*, 2010), and genotypic diversity can differ dramatically across all spatial scales (Reusch *et al.*, 2000; Olsen *et al.*, 2004; Coyer *et al.*, 2006; Arnaud-Haond *et al.*, 2007b; Alberto *et al.*, 2008). On the other hand, phenotypic plasticity when existing cannot compensate for all extremes in environmental fluctuations (Foster Hünneke, 1991), and species rely at some point on the adaptive potential conferred by the existence of distinct genotypes and alleles in their genetic pools, allowing response to different environmental conditions (Dobzhansky *et al.*, 1977; Hartl & Jones, 2006).

Genetic diversity can therefore affect a wide range of population, communities and ecological processes. The level of genetic diversity and its distribution in a system of populations depends strongly on the history of species (accumulation of mutations, drift) and size of populations (drift), on the mating system in a given population as well as on the movement of individuals (migration) and the heterogeneity of environments they are and

have been exposed to (selection). A population is a group of individuals where individuals mate and produce offspring (Dobzhansky *et al.*, 1977); individuals of a population are usually genetically different from one another (Dobzhansky *et al.*, 1977; Primack, 2002; Hartl & Jones, 2006) except in clonal organisms (Arnaud-Haond *et al.*, 2007a). Genetic variation is the amount of differences among individual genomes, where different alleles occur at different loci (Primack, 2002; Hartl & Jones, 2006). The differences between alleles at the same locus commonly happens through mutation (a change in the sequence of nucleotides in DNA), and may be a simple nucleotidic substitution or an insertion or deletion or addition of several nucleotides, or recombination with unequal crossing over during the meiosis (Hartl & Jones, 2006). The migration of individuals from a different population may also be a source of input of new alleles and of increase of genetic diversity. Natural selection (certain alleles that improve survival and increase the "fitness" will be selected instead of others) may either act for (in cases of balancing selection for example) or against (in cases of directional selection) genetic diversity at the population level (Dobzhansky *et al.*, 1977; Primack, 2002).

Genetic diversity is an important element for conservation as genetic diversity is expected to reflect the adaptive potential of species to environmental variations (Thompson & Starzomski, 2007). Besides experimental studies that showed the importance of genotypic diversity for population persistence, two ranges of studies are now needed: 1) Studies focusing on genetic richness rather than genotypic richness, as this is the actual parameter that will influence the fate of populations and species whether they are clonal or sexual and; 2) *in situ* surveys to test for the validity of experimental observations on synthetic, simplified populations, on real and likely more complex populations and communities (Arnaud-Haond *et al.*, 2010).

1.5. Description, Habitat and Importance of *Fucus vesiculosus* and *Fucus serratus*

The approach to studying the relationship between diversity and stability in this thesis is by surveying natural population of purely sexual (i.e., non clonal) brown algae supporting communities exhibiting contrasting levels of stability.

Within a community there may be species or groups of species with certain characteristics that can influence the persistence of other species within the community or even the organization of the entire community, such species can for example be structural species, or keystone species (Mills *et al.*, 1993). Macroalgae *Fucus* sp. have a key structuring role on habitat forming processes, providing a refuge for several species of macrobiota. They are primary producers in costal environments (Thompson *et al.*, 1996), and represent a dominant biomass species on rocky shores (Lüning, 1990). The *Fucaceae* are a family of closely related seaweeds that underwent recent radiation. In the genus *Fucus* two dioecious species, *Fucus vesiculosus* (Linnaeus, 1753) and *Fucus serratus* (Linnaeus, 1753) (Serrão *et al.*, 1999) have overlapping distributions. *F. serratus* seems to be a better colonizer than *F. vesiculosus*, and grows at a higher rate. Eventually in a situation of co-occurrence *F. serratus* usually monopolises the space despite its bad dispersing potential (Arrontes *et al.*, 2002).

Brown algae *Fucus* play an important role in the habitat forming component of the ecosystem (Davison & Pearson, 1996; Thompson *et al.*, 1996). Fucoid algae exist mainly at the rocky interface between marine and terrestrial habitats (Davison & Pearson, 1996) where they usually form extensive belts (Lüning, 1990). Typically these habitats are open severe ecosystems, with steep environmental gradients, where species are subjected to periods of immersion alternate with aerial exposure as tides rise and fall. Besides this high variability, rocky shores are also very resilient habitats that were shown to recover rather rapidly due to recruitment from unaffected areas (Thompson *et al.*, 2002). These habitats therefore provide an accurate "natural laboratory" to study *in situ* the relationship between genetic diversity and community stability.

BOX 1: Brief description and distribution of *Fucus vesiculosus* L. and *Fucus serratus* L.

The seaweed genus *Fucus* dominates biomass on the intertidal shores of many warm and cold temperate regions in the Northern Hemisphere (Lüning, 1990) and presents its greatest diversity in the Atlantic Ocean (Garreta, 2001). There are thirteen species of the genus *Fucus* (Fucaceae, Phaeophyta) recognized taxonomically, two of them are *Fucus vesiculosus* (Linnaeus, 1753) and *Fucus serratus* (Linaeus, 1753) (ALGAEBASE, www.algaebase.org). These two species are dioecious, have separate sexes (Serrão *et al.*, 1999). Fucoid seaweeds are perennial algae and in general individuals have about 3-4 years of live (Lüning, 1990). The genus *Fucus* is a group of taxa with morphological plasticity, so is difficult to make a description (Garreta, 2001). Below there are images of both *F. vesiculosus* (Figure B1) and *F. serratus* (Figure B2) with a brief description and distribution, respectively (Figure B3 and Figure B4).

Figure B1. Image of *Fucus vesiculosus*. Size between 1-100 cm in height; attached to the substratum by a basal disc; frond strap-shaped and wavy margins **with paired air-bladders**; receptacles apical variable in shape (Garreta, 2001).

Figure B2. Image of *Fucus serratus*. Size between 33-60 cm height; attached to the substratum by a conical disc; frond between the holdfast and the first dichotomy 2-7 mm wide and 1-4 cm long **with serrate margins**; receptacles apical slightly swollen compressed and with toothed margins (Garreta, 2001).

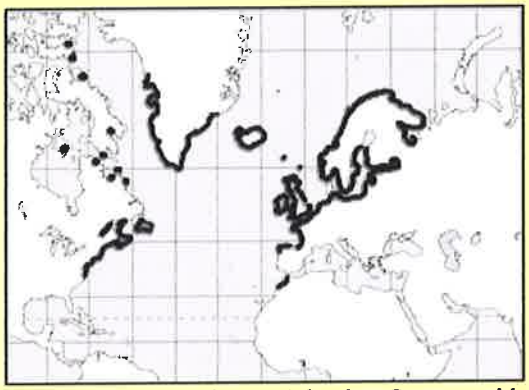


Figure B3. Northern Atlantic Ocean with distribution of *Fucus vesiculosus* by Lüning, 1990.

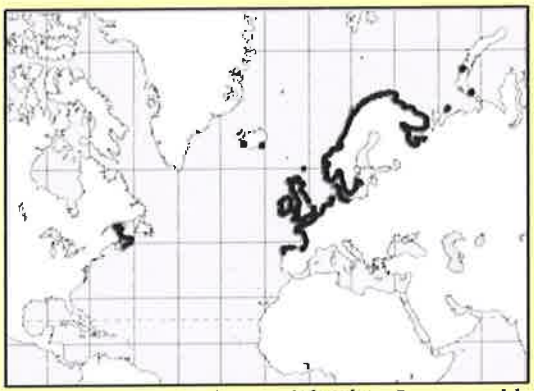


Figure B4. Northern Atlantic Ocean with distribution of *Fucus serratus* by Lüning, 1990.

1.6. Aims

In several locations in Northern Europe beds from two species of *Fucus* sp., *Fucus vesiculosus* and *Fucus serratus*, were surveyed for five areas defined along the shore, where the density of algae and the stability of invertebrate assemblages were followed across year for three years. Samples were collected in the two extreme quadrate of each location, the most stable and the less stable, in order to test for the existence of a positive relationship between genetic diversity in stands of *Fucus* sp. and i) their levels and variance of density or ii) the stability of associated communities across years.

We aim at testing for the existence of a relationship between genetic diversity and community stability on natural stands on rocky shores. This work is planned being aware that, as theory predicts both the positive influence of environmental stability on genetic diversity through demography of populations, and the positive influence of genetic diversity on the demographic stability populations when facing environmental challenges, the motor of such relationship will be hard to establish in such *in situ* survey. On the other hand, by performing an *in situ* survey instead of a manipulative experiments we benefit from natural conditions encompassing all the complexity of the natural environment, including the real composition of associated communities and a realistic level of genetic diversity at the scale we study the populations. Here we therefore simply aim at adding more information on the importance of the genetic component of diversity, by testing for the existence of a theoretically predicted relationship with stability.

2. Material and Methods

This study was integrated in the Project BIOFUSE: Effects of biodiversity on the functioning and stability of marine ecosystems – European scale comparisons (available at www.marbef.org/projects/biofuse).

2.1. Sampling sites and procedure

Several sites were selected in the Northern Atlantic (Europe): United Kingdom (Heybrook and Looe in Plymouth), Estonia (Hiiumaa), Ireland (Rush and Raghly), Germany (Langen),

from the apical tissue) and stored in silica crystals, identified with the date, name of the site, species and level of stability until DNA extraction.

Table 1 – Number of individuals (n) of *Fucus serratus* and *Fucus vesiculosus* sampled per quadrat with less stable (LS) and more stable (MS) assemblages in different locations in the North Europe. Data analysis was performed using the same number of individuals on LS assemblages and MS assemblages for each site.

			Sampling (n)		**Data Analysis (n)**	
			Stability			
	Country	**Location**	**LS**	**MS**	**LS**	**MS**
F. serratus	United Kingdom	Looe/Heybrook	25	25	25	25
	The Netherlands/Germany	Voorhaven/Langen	24	25	24	24
	France	Roscoff	27	27	27	27
F. vesiculosus	Estonia	Hiiumaa	30	30	30	30
	Ireland	Raghly/Rush	17	25	17	17
	The Netherlands/Germany	Voorhaven/Langen	26	26	26	26
	France	Roscoff	12	12	12	12

2.2. DNA extraction, PCR reaction and genotyping

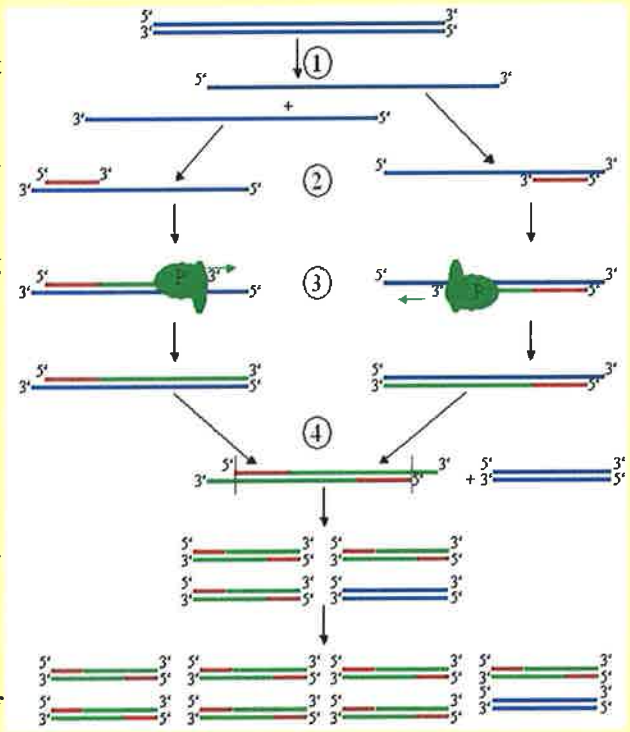
DNA extraction. Before extraction of DNA we lyophilize the algal material for 24 hours to ensure that the tissue stay dry (Thermo, Electron Corporation: Modulyo D, Freeze Dryer). DNA for genotyping was extracted from approximately 5-10 mg of dried tissue using the protocol in Hoarau *et al.* (2007), modified in the following ways: **1)** The time and frequency was increased in the pulverization step to 2 min and 30g, respectively (Mixer Mill MM300, Retsch); **2)** We chose to use a 96-well microtitre filtration plate Millipore MultiScreen HTS, FB Cat. # MSFBN6B10 instead of a 96-well microtitre filtration plate Millipore HVPP MAHV S45-10; **3)**The filtration plate with supernatant and binding buffer was centrifuged at 1000 rpm for 15 min, followed by 2000 rpm for 10 min; **4)** After wash buffer was added to each sample the plate was centrifuged at 3000 rpm for 10 min and dried at room temperature for 30 min; **5)** DNA was eluted with 100 µL of warm elution buffer (55°C), incubated for 5 min, and centrifuged at 1500 rpm for 10 min. After the DNA extraction was finished we diluted the DNA 100 times to facilitate its use in other steps of the work. The adapted protocol of Hoarau *et al.* (2007) didn't work for the population of

Ireland (*F. vesiculosus*), so we tried to extract DNA with NucleoSpin® 96 plant kit (Macherey-Nagel, April 2004) and then DNA was diluted 100 times. Eluted and diluted DNA was stored at -20ºC.

BOX 2: Microsatellites (EST-SSRs) and Polymerase chain reaction (PCR)

Expressed sequence tags of simple sequence repeats (EST-SSRs) are markers or microsatellites with simple repeats motifs (about 2 or 3 nucleotide repetitions) that are typically found to be polymorphic, EST-SSRs can be used in related taxa and produce data easy to analyse (Ellis & Burke, 2007). SSRs within untranslated regions are very polymorphic and EST of these regions provide genes potentially under selection (Li *et al.*, 2004).

SSRs are frequently species-specific markers developed to a taxon that cannot be easily transferred to another. On the contrary, EST-SSRs are gene-based SSRs markers that can be significantly more transferable among taxa (see authors in Ellis & Burke, 2007). So EST-SSRs allow us to standardize data as much as possible among taxa by using the same markers (Ellis & Burke, 2007). Polymerase chain reaction (PCR) is very useful for generating large quantities of specific DNA sequence. PCR amplifications uses DNA polymerase and a pair of short nucleotide sequences (primers) flanking the DNA sequence that is intended to amplify, called microsatellite (Hartl & Jones, 2001, Hartl & Jones, 2006). An overview of PCR is shown in Figure B5Figure .



http://serc.carleton.edu/images/microbelife/research_methods/polymerase_chain_reaction_v2.jpg

Figure B5. Schematic image of the PCR steps. (1) DNA denaturation. (2) Annealing of primers to DNA strands. (3) Extension of each template strand of DNA by DNA polymerase (P). (4) The 1st cycle is complete. The two new DNA strand resulting will be used on the next cycle and so on. The amount of DNA will double at each new cycle.

PCR reaction. From the locations referred in Table 1 we amplify DNA of 153 and 178 individuals of *F. serratus* and *F. vesiculosus*, respectively, using ten polymorphic EST-

SSTRs loci from Coyer *et al.*, 2009 (F9, F12, F17, F19, F21, F22, F42, F49, F60 and F72) (Table 2). PCR amplifications were performed in simplex in 10 µL of reaction mixture containing 1 µL of template DNA (1:100), 1×GoTaq polymerase buffer (Promega), 2 mM $MgCl_2$, 0.05 mM of each dNTP, 0.3 µM of each primer (forward primer of each primer pair 5'→3' fluorescently labelled - Table 2) and 0.5 U GoTaq polymerase (Promega). All PCR reactions were carried out in a thermocycler ABI 2720 (Applied Biosystems) with the following cycling parameters: Initial denaturation step of 2 min at 94°C, "touchdown" consisting in 25 cycles of 20 s at 94°C, 10 s at 60°C reduced by 0.2°C each subsequent cycle (or 58°C reduced by 0.2°C each subsequent cycle only for F72), and 35 s at 72°C, then the following step are 10 cycles of 20 s at 94°C, 10 s at the annealing temperature (Table 2), and 35 s at 72°C, to finish exists a final extension of 10 min at 72°C. The amplification results were loaded onto a 1% agars gel to see if there are bands around 200 bp (≈ microsatellites range).

Table 2 – Primer sequences, motif and annealing temperatures (T_a) of ten polymorphic loci from Coyer *et al.*, 2009 used to amplify DNA sequences of *Fucus serratus* and *Fucus vesiculosus*.

Locus	Motif	Primer sequences (5'→3')	T_a (°C)
F9	$(GT)_{16}$	**F:** GGCGGAAGTCGATTTGAATA • **R:** ACTTGGCTGACGTCCAGAAT	55
F12	$(GT)_{15}$	**F:** TATGTGTCCGACGACCTGAG ▼ **R:** TGAAGTCAAATGCTTGTTCG	55
F17	$(AT)_7$	**F:** GCAGACAGAGAGGGCAGAAG ▼ **R:** CCCCTCTCTCCCAGGTATTT	55
F19	$(GT)_{15}$	**F:** AGGTTTCAACCTGCTTCTGG • **R:** TGCTACATCCAAGAATTGCAG	55
F21	$(TG)_{15}$	**F:** CATGTAGCGTGAAGCGTTTG • **R:** CACGCAAACAAAACGTCAAC	55
F22	$(AT)_6$	**F:** CCGTCTACGTTTCGTTTCGT • **R:** ATCCGAGAGACGGATAGCAA	55
F42	$(AGC)_5$	**F:** AGTGTGACTGCCCATTAGGG * **R:** AGACGTAACCCAGTGCTGCT	55
F49	$(AT)_6$	**F:** TGCTGTAGAAGGCCGAAGTT ■ **R:** AACGAGTTCGTCGAGTGTCC	55
F60	$(CA)_8$	**F:** GGGGTTGTTTTCGATAAAAGG ■ **R:** GCAATCGACCTCGAGAAATC	55

F72	$(AG)_6$	**F:** ATCTCCGCCTTAACCCAGTC ** **R:** CAGCTGGATACGGATGGAGT	53

•: primer labelled with FAM; ▼: primer labelled with HEX; ■: primer labelled with NED; *: primer labelled with FAM in some PCR and labelled with HEX in others PCR to facilitate the genotyping; **: primer labelled with FAM in some PCR and labelled with NED in others PCR to facilitate the genotyping.

Genotyping. To determine alleles size for each locus a mixture with ≈ 1 µL amplified DNA, 9.25 µL of formamide and 0.25 µL of ROX350 size standard (Applied Biosystems) was denatured at 95°C for 5 min and loaded in an ABI 3130 XL automated DNA sequencer (Applied Biosystems).

2.3. Data analysis

Genetic analysis. The genotyping chromatogram was analysed by two different persons and the readings compared after to minimize reading errors (BOX 3). To analyse the chromatogram we used two opensource programs, STRand Analysis Software 2.3.79 (Davis, 2007) and R package (R Development Core team, 2008) MsatAllele (Alberto, 2009).

In total 152 of the 153 and 170 of the 178 individuals sampled of *F. serratus* and *F. vesiculosus*, respectively, were used for data analysis (Table 1). All the genetic analysis were performed in separate for *F. serratus* and *F. vesiculosus*. For each location, number of alleles for all ten loci and the mean number of alleles or allelic richness (Â) overall loci were estimated for populations of LS and MS quadrates. Genetic diversity within populations was estimated by the allelic richness (Â) referred above, observed heterozygosity (H_O) and unbiased expected heterozygosity (H_E) (Nei, 1978) (Table 3). Deviation from Hardy-Weinberg equilibrium or inbreeding coefficient (F_{is}) were estimated for each locus and overall 10 loci for all the population and its significance was tested using 1000 permutations to test whether F_{is} was significantly different from 0 (Table 4). Genetic divergence (F_{st}) was estimated between pairs of populations with θ estimator (Weir & Cockerham, 1984), being it significance tested with 1000 random permutations of the individuals between samples (Pairwise Fst or differentiation values were all significantly different from zero among sites, and most quadrats where differentiated within site, except

in Roscoff for both species, Langen/Voorhaven and Hiumaa in *F. serratus* and *F. vesiculosus,* respectively (Table 5 and Table 6).

Table 5 and Table 6). All tests and estimations were performed in GENETIX 4.05.2 (Belkhir *et al.*, 2004).

Field survey, demography and community stability. The community existing in both quadrats with different levels of stability was characterized each year from 2006 to 2009 and estimates of temporal variance in total abundance of species richness were obtained for each level of stability using a generalized linear mixed-effect model fitted by restricted maximum likelihood (REML). Estimates of temporal variances were homogeneous after logarithmic transformation (Figure 2). The year of collection being 2006-2007, the more and less stability quadrates were determined on the basis of the first year of survey, and the mean density and variance in density of *Fucus* sp. present in these MS and LS assemblages for each site were estimated. As the experimental sites were monitored until 2009, mean density and variance in density were also estimated overall experiment as well (2006-2009), in order to check for the evolution of trends observed on the first year (Figure 3 and Figure 4) on both sites in each location. The Coefficient of Variability (CV) (Underwood & Chapman, 2000) for the population of *F. serratus* and *F. vesiculosus* was determined for the first year of experiment and overall (Figure 5).

Statistical analysis. In statistical analysis we want to evaluate if there exists a relationship between genetic diversity within the local population of the structural species (*F. serratus* and *F. vesiculosus*) and several parameters: estimated community stability; population density in a year and overall experiment, variance in density over time; and estimates temporal variance of species richness. For this several paired t-Test with significance level of 0.05 (α= 0.05) were made. The null hypothesis (H0) was the lack of correlation between genetic diversity of *Fucus* sp. and ecosystem stability as characterized through REML and used to define MS and LS quadrates; and the alternative hypothesis (H1) was that genetic diversity of structural species would exhibit a positive relationship with community stability, either due to the positive effect of overall stability on demography and population sizes, and consequently on the population genetic diversity, or due to the positive effect of genetic diversity on the resistance and resilience of structural

population, and consequently on their demographic stability and on the stability of the ecosystem based on those brown algae. A correlation test was also performed to know if there exists some relation between genetic diversity and both density and variance in density of the population.

3. Results

Genetic Analysis

Allelic richness (Â) in *F. serratus* varied from 1.9 to 6.5 and from 2.2 to 7.1 alleles in LS and MS assemblages, respectively. Voorhaven and Langen had the lowest allelic richness (1.9-2.2) and Roscoff had the highest allelic richness (6.5-7.1). In *F. vesiculosus*, allelic richness (Â) was smaller than in *F. serratus* and its variability ranged from 3.0 to 4.0 alleles in LS assemblages and 3.1 to 3.8 in MS assemblages. In opposition to *F. serratus* the populations of *F. vesiculosus* in Voorhaven and Langen had the largest number of alleles (4.0-3.9) and the smallest number was observed in Raghly (3.0-3.1) (Table 3). In the comparison of the values of allelic richness (Â) in LS versus MS populations of both species, the p-value of a paired t-Test with significance level of 0.05 (α= 0.05) was 0.036 (p-value of one-tailed test; p-value $\leq \alpha$). This statistical analysis allows rejecting the null hypothesis of absence of relationship between genetic diversity and community stability. Expected heterozygosity, H_E, for the populations of *F. serratus* is higher in MS assemblages than in LS ones but in the population of *F. vesiculosus* this only happens in Hiumaa, in the other studied populations for this species it is the opposite, H_E is higher in LS assemblages. In all population for both species an heterozygote deficiency was observed (H_O) except in Langen (MS) for *F. serratus* which has an heterozygote excess (Table 3). Statistical t-tests showed no significant relationship between heterozygosity and the stability of community assemblages.

BOX 3: Briefly how to read chromatograms of *Fucus serratus* and *Fucus vesiculosus* in STRand and examples of peak pattern.

At least two different persons should read the chromatograms independently to minimize reading errors. Methods: Do a reading test for each locus to know the peak pattern of an homozygous or a heterozygous separately and choose the better peak to square (usually is the peak more consistent, in our case is the rightmost peak). Usually in a heterozygous the peak pattern is the same in both peaks (see images of heterozygous below). Note that heterozygous with 1 bp (base pair) can exist (Figure B6 and Figure B8), in this case read all individuals of the same population from this locus and if there exist both homozygous with 1 bp difference, we have to consider the heterozygous with 1 bp difference too. If only one of the homozygous exists it is better to repeat the genotyping to confirm the result. Usually in a heterozygous the 1st peak is bigger than the 2nd but this rule is not always true (Figure B8 and Figure B9), read the chromatogram carefully.

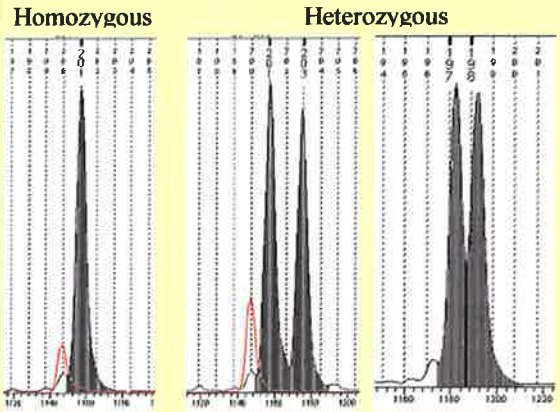


Figure B6. Example of peak pattern for an homozygous and two heterozygous for locus F49

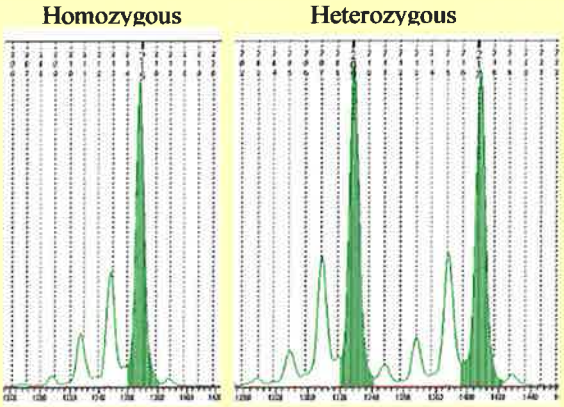


Figure B7. Example of peak pattern for an homozygous and an heterozygous for locus F12

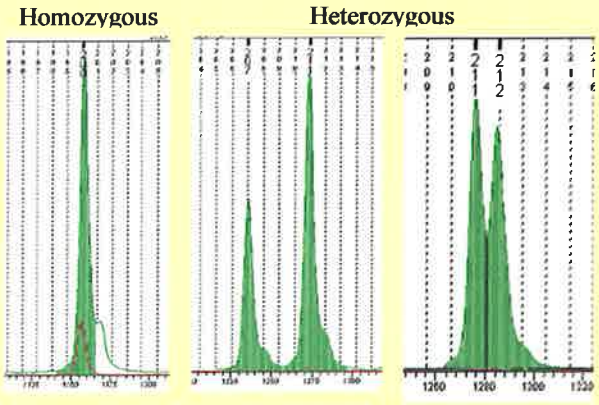


Figure B8. Example of peak pattern for an homozygous and two heterozygous for locus F17

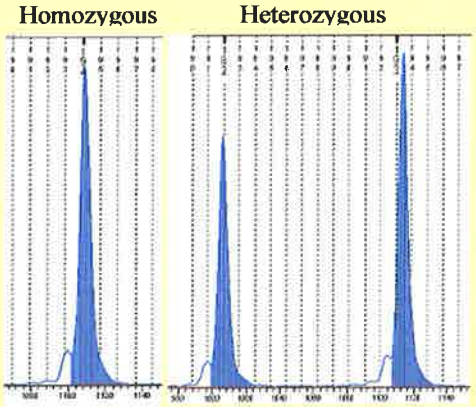


Figure B9. Example of peak pattern for an homozygous and two heterozygous for locus F72

Table 3 – For each species (*F. serratus* and *F. vesiculosus*) and location with LS quadrat and MS quadrat total number of alleles over all ten loci were calculated. Genetic descriptors like allelic richness (Â), observed heterozygosity (H_O) and unbiased expected heterozygosity (H_E) were calculated for each level of stability in the sites studied.

	F. serratus						***F. vesiculosus***							
	Looe	**Heybrook**	**Voorhaven**	**Langen**	**Roscoff**		**Hiumaa**		**Raghly**	**Rush**	**Voorhaven**	**Langen**	**Roscoff**	
Locus	**LS**	**MS**	**LS**	**MS**	**LS**	**MS**	**LS**	**MS**	**LS**	**MS**	**LS**	**MS**	**LS**	**MS**
Total number of alleles per quadrat and Allellic richness														
F9	9	10	1	3	12	11	1	3	2	3	3	4	3	2
F12	8	7	3	3	10	15	8	11	8	7	9	8	9	12
F17	2	3	1	1	3	4	3	2	1	1	2	2	1	1
F19	15	12	3	3	17	16	5	3	3	3	7	5	4	4
F21	4	5	4	5	4	7	3	3	2	4	2	4	2	4
F22	2	1	1	1	2	3	1	1	3	2	2	2	2	3
F42	2	3	2	2	3	3	4	5	3	2	4	4	4	3
F49	3	4	1	1	2	3	2	3	2	3	3	3	3	2
F60	4	4	2	2	9	6	3	5	4	4	4	4	6	5
F72	2	3	1	1	3	3	2	1	2	2	4	3	4	2
Â	5.1	5.2	1.9	2.2	6.5	7.1	3.2	3.7	3.0	3.1	4.0	3.9	3.8	3.8
Heterozygosity per quadrat														
H_O	0.44	0.46	0.18	0.22	0.39	0.43	0.32	0.26	0.39	0.32	0.40	0.34	0.33	0.38
H_E	0.51	0.53	0.19	0.20	0.48	0.55	0.37	0.40	0.43	0.42	0.48	0.42	0.52	0.46

For each locus and over all ten loci used in this study for both species, deviation from Hardy-Weinberg equilibrium (HWE) was estimated for the different levels of stability in all locations. Estimated values of F_{is} or inbreeding coefficient significantly different from zero show deviation from HWE. From the observation on Table 4 of the F_{is} almost all populations and quadrats showed significant departures to HWE, only two population of *F. serratus* and one of *F. vesiculosus* were in HWE except Vorhaven (LS), Langen (MS) for *F. serratus* and Raghly (LS) for *F. vesiculosus*.

Table 4 - - For each species (*F. serratus* and *F. vesiculosus*) and location with less stable (LS) quadrat and more stable (MS) quadrat deviation from Hardy-Weinberg equilibrium or inbreeding coefficient were estimated for each locus and over all 10 loci. F_{is}, * significant different from 0 (P<0.05) after a 1000 permutation test.

F_{is}	*F. serratus*						*F. vesiculosus*							
	Looe	Heybrook	Vorhaven	Langen	Roscoff		Hiumaa		Raghly	Rush	Vorhaven	Langen	Roscoff	
Locus	LS	MS	LS	MS	LS	MS	LS	MS	LS	MS	LS	MS	LS	MS
F9	0.07	-0.03	-	-0.03	-0.02	0.18*	-	0.79*	-0.14	0.23	1.00*	0.65*	0.40	-0.08
F12	0.14	0.42*	-0.03	0.48	0.49*	0.32*	0.28*	0.25*	0.26*	-0.02	0.23*	0.22*	0.54*	0.29*
F17	-0.22	0.30	-	-	0.10	0.01	0.50*	1.00*	-	-	1.00*	1.00*	-	-
F19	0.22*	0.19*	0.12	-0.06	0.09	0.21*	0.52*	0.85*	0.27	0.53*	0.29*	0.53*	0.69*	0.68*
F21	0.14	-0.07	-0.01	-0.03	0.17	0.08	-0.94	-0.66	-1.00	-0.42	-0.84	-0.73	-0.83	-0.73
F22	0.00	-	-	-	-0.02	-0.05	-	-	0.39	-0.05	-0.11	0.02	0.37	0.48*
F42	0.34	0.18	0.00	0.00	-0.10	0.46*	0.22	0.39*	0.20	0.16	0.07	0.06	0.19	0.08
F49	0.29*	-0.08	-	-	0.84*	0.37*	-0.13	0.33*	-0.11	0.36	0.16	0.50*	0.42	-0.05
F60	0.12	0.19	-0.05	-0.35	0.15	0.32*	0.49*	0.73*	0.35*	0.65*	0.76*	0.63*	0.56*	0.33
F72	0.47	0.02	-	-	0.28	0.22	0.79*	-	0.00	1.00*	0.09	0.37	0.84*	0.65
Total	0.15*	0.13*	0.02	-0.07	0.19*	0.22*	0.14*	0.36*	0.08	0.24*	0.16*	0.20*	0.38*	0.18*

Pairwise F_{st} or differentiation values were all significantly different from zero among sites, and most quadrats where differentiated within site, except in Roscoff for both species, Langen/Voorhaven and Hiumaa in *F. serratus* and *F. vesiculosus*, respectively (Table 5 and Table 6).

Table 5 – Pairwise comparison of population differentiation in *Fucus serratus* among different locations with two populations each (LS – population in less stable quadrat; MS – population in more stable quadrat). F_{st} (Weir & Cockerham, 1984), * significantly different from zero (P<0.05) after 1000 random permutations.

F_{st}		*F. serratus*					
		Looe	Heybrook	Voorhaven	Langen	Roscoff	
		LS	MS	LS	MS	LS	MS
Looe	LS	-	0.047*	0.292*	0.284*	0.045*	0.039*
Heybrook	MS	-	-	0.282*	0.273*	0.059*	0.043*
Voorhaven	LS	-	-	-	0.008	0.276*	0.232*
Langen	MS	-	-	-	-	0.264*	0.217*
Roscoff	LS	-	-	-	-	-	0.008
	MS	-	-	-	-	-	-

Table 6 - Pairwise comparison of population differentiation in *Fucus vesiculosus* among different locations with two populations each (LS – population in less stable quadrat; MS – population in more stable quadrat).). F_{st} (Weir & Cockerham, 1984), * significantly different from zero (P<0.05) after 1000 random permutations.

F_{st}		*F. vesiculosus*							
		Hiumaa		**Raghly**	**Rush**	**Voorhaven**	**Langen**	**Roscoff**	
		LS	**MS**	**LS**	**MS**	**LS**	**MS**	**LS**	**MS**
Hiumaa	**LS**	-	0.010	0.209*	0.208*	0.123*	0.164*	0.157*	0.199*
	MS	-	-	0.192*	0.180*	0.107*	0.147*	0.125*	0.174*
Raghly	**LS**	-	-	-	0.123*	0.061*	0.048*	0.072*	0.052*
Rush	**MS**	-	-	-	-	0.113*	0.148*	0.146*	0.160*
Voorhaven	**LS**	-	-	-	-	-	0.018*	0.056*	0.086*
Langen	**MS**	-	-	-	-	-	-	0.065*	0.061*
Roscoff	**LS**	-	-	-	-	-	-	-	0.032
	MS	-	-	-	-	-	-	-	-

Field survey, demography and community stability

After field surveying of the several sites studied with different levels of stability with the algae *F. serratus* and *F. vesiculosus*, REML for species richness, mean density and variance in density of canopy algae were arranged for a better visualization in Figure 2, Figure 3 andFigure 4, respectively.

Estimated temporal variance in total abundance of species richness over all surveying shows that abundance in species richness were always higher in quadrats exhibiting low stability (Figure 2). From the observation of Figure 3 is possible to see that mean density of *Fucus* sp. in both quadrats of the several locations are similar over one year and over all, where in almost locations LS assemblages had a greater density cover than in MS ones. The same pattern is visible in both species for variance density (Figure 4), with exception of one population, *F. vesiculosus* in Ireland. The result of p-value for bilateral t-Test with significance level of 0.05 (p-value of two-tail; p-value $\geq \alpha$) between both, mean density and variance in density, and species richness were 0.09, 0.52 and 0.08, 0.29 for one year of surveying and over all experiment, respectively. These parameters seem to be inversely related but statistical analysis shows non significant results.

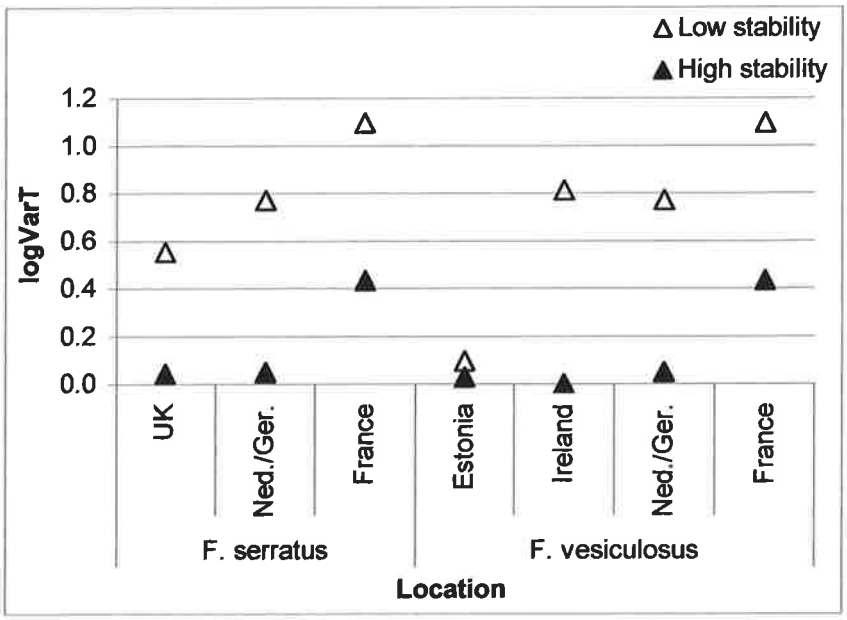


Figure 2. Logarithmic transformation of temporal variance in species richness along 2006-2009 for the two quadrats with different levels of stability per location where the structural algae *Fucus* sp. were present.

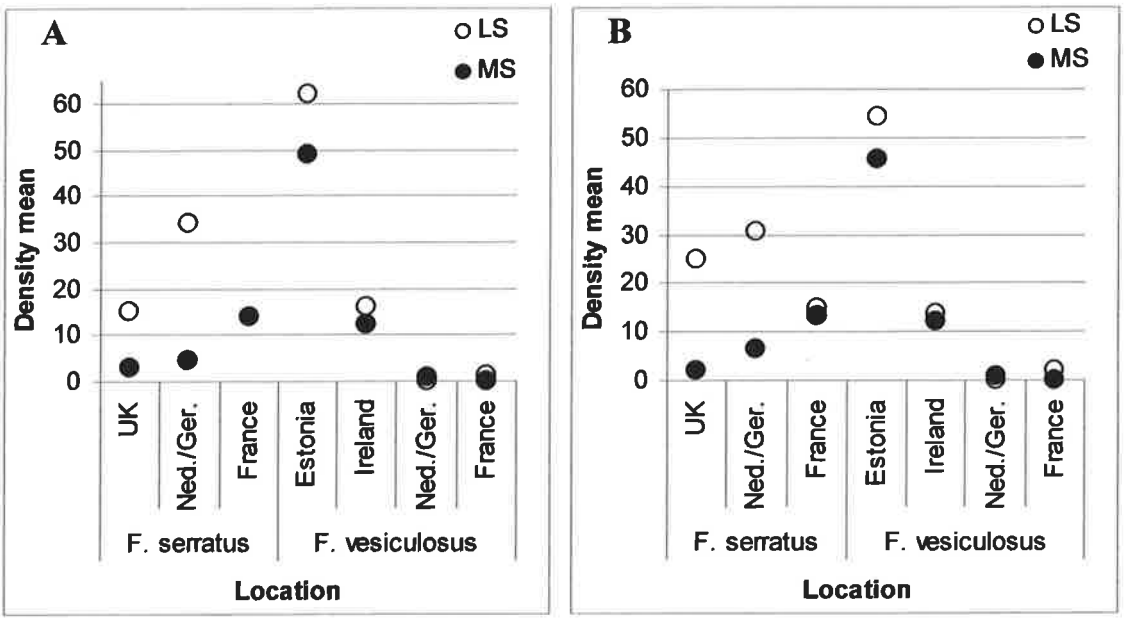


Figure 3. . Mean density over one year (2006-2007 – **A**) and over all experiment (2006-2009 – **B**) of the population of *Fucus* sp. in the two quadrats with different levels of stability (LS – less stable assemblage; MS – more stable assemblage) per location.

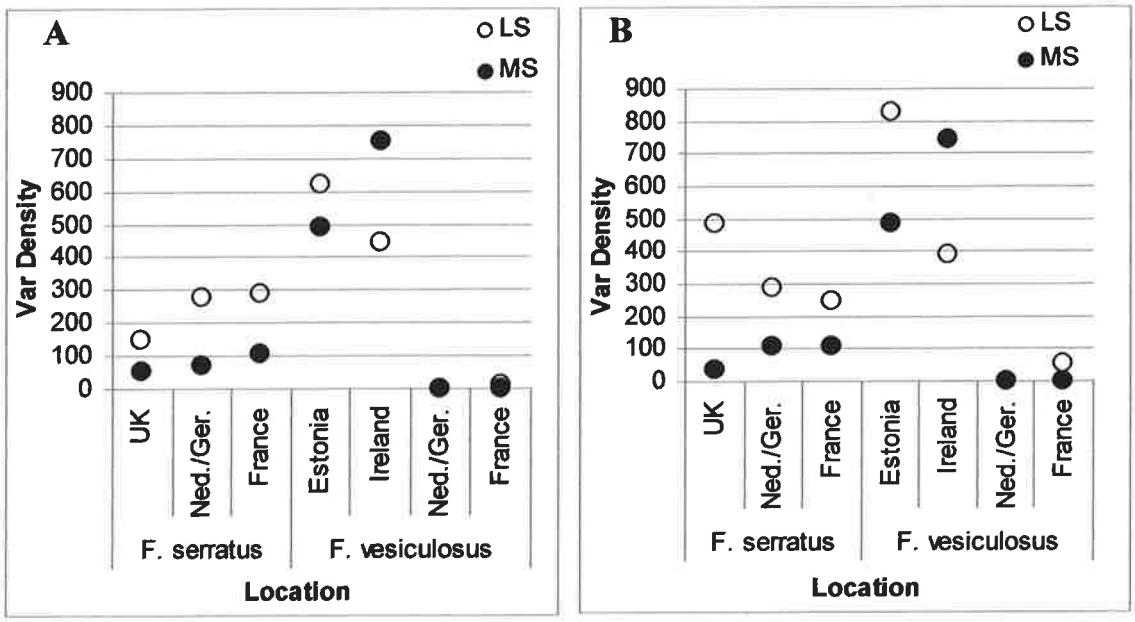


Figure 4. Variance in density over one year (2006-2007 – **A**) and over all experiment (2006-2009 – **B**) of the population of *Fucus* sp. in the two quadrats with different levels of stability (LS – less stable assemblage; MS – more stable assemblage) per location.

In the correlation analysis relationships between variables were non significant. So from the data obtained it seems that there does not exist a relationship between REML estimates of variance in species richness and density of *Fucus* sp., which means that maybe there are no correlation with density enhancing allelic richness and so enhancing community stability too.

Table 5 illustrates the coefficient of variation (CV) or temporal variance of species, *F. serratus* and *F. vesiculosus*, across one year and over all the experiment we can see that results were similar over the three years of surveying in LS and MS assemblages per location.

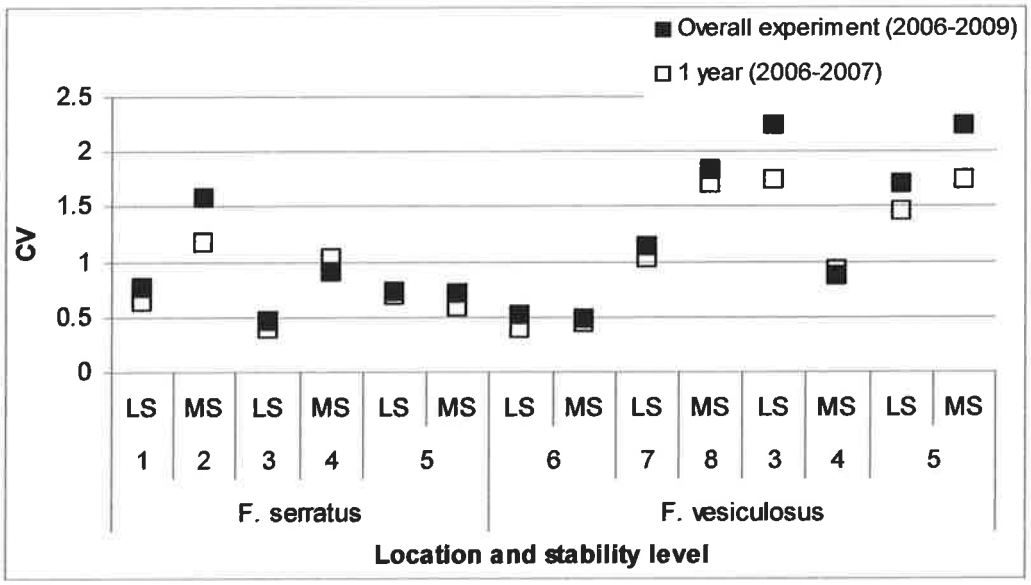


Figure 5.Coeficient of Variation (CV) over one year (2006-2007) and over all experiment (2006-2009) of the population of *Fucus* sp. for each location and quadrat with different levels of stability (LS – less stable assemblage; MS – more stable assemblage). 1 – Looe; 2 – Heybrook; 3 – Voorhaven; 4 – Langen; 5 – Roscoff; 6 – Hiumaa; 7 – Raghly; 8 – Rush.

4. Discussion

Diversity stability-debate has been at the origin of several studies in the last decades (Tilman, 1999; McCann, 2000; Loreau *et al.*, 2002) and a more recent approach to this issue starts to give more attention to variability at the species level, the population dynamic response to environmental fluctuations and the consequent evolutionary adjustments (Doak *et al.*, 1998; Loreau & Behera, 1999; Yachi & Loreau, 1999; Hillebrand *et al.*, 2008). Resilience of population can be enhanced by higher within species diversity such as clonal diversity (Reusch *et al.*, 2005; Hillebrand *et al.*, 2008). Yet the genetic components of diversity, i.e. allelic richness and heterozygosity, and their relationships with demographic stability and higher levels of biodiversity such as community composition or its variance in time have been poorly explored up to now.

Here we established a positive relationship between a genetic component of biodiversity, allelic richness in the stands of the structural species of brown algae, and the stability of community assemblages through several years of field survey (Figure 2 where LS assemblages are more variable in time in terms of species richness and MS assemblages

had a lower variability in species). The estimates of temporal variance in the composition of communities assemblages (REML) were systematically higher in ecosystems exhibiting lower genetic diversity, estimated through allelic richness, for all locations.

The theoretical relationship between genetic diversity and demographic trends in populations is supported by some observations (refs) yet *in situ* observations did not allow discriminating the possible driver of this relationship as both components influence each other positively in a feed-back relationship. Here however, no *in situ* relationship is observed between density of *Fucus* sp. and their allelic richness, nor between species richness and density or stability of algal stands, while a positive relationship emerges between allelic richness and the stability of associated community assemblages. Field manipulation of seagrasses showed that higher genotypic diversity, i.e. clonal diversity, enhanced the demographic resistance to disturbances (Hughes & Stachowicz 2004); similar stabilizing effects of clonal diversity against temperatures extremes were demonstrated in Baltic seagrasses (Reusch *et al.*, 2005), and a positive relationship was established between genotypic and community diversities, likely due to an increase of seagrass density (and consequent resources and carrying capacity) with genotypic diversity. This kind of stabilizing effect is similar to effect in species diversity in terrestrial grasslands (Tilman, 1996) and aquatic protist communities (Naeem & Li, 1997). In these systems generally when species diversity increases the overall stability of community increases, yet the stability of individual species abundance decreases. These observations support the theory predicting the existence of negative covariance's among species in their reaction to environmental oscillations should produce a stabilizing effect of diversity on total community (Tilman, 1999). Results obtained in Valone & Barber (2008) are not supported by the theory of "insurance hypothesis" (Yachi & Loreau, 1999), in their results between species correlations in several assemblages shows that positive correlations are more abundant than negative ones, therefore "insurance hypothesis" or covariance may not be a strong stabilizer of the fluctuations in the community. Instead of that positive correlations among pair of species destabilize community oscillations (Tilman, 1999; McCann, 2000). The coefficient of variation (CV) in the abundance of *F. serratus* and *F. vesiculosus* (Figure 5) across three years shows that when species diversity increase the density of *Fucus* sp. is more variable/instable, while the stability overall community increases (MS assemblages) .

clear that populations more distinct genetically are the most geographically distant as we can see between populations of UK and Nederland or Germany. The opposite happens when population are geographically closer as we can observe between Looe and Heybrook (more panmitic populations) or between Roscoff and the two populations referred before. For the other fucoids species the same pattern of distance/genetic differentiation is verified. In these cases can occur gene flow and genetic drift, in *F. vesiculosus* drift is an acceptable explanation because this algae had bladders that helps it to drafting and if exists mate between individual coming from distant sites the genetic structure maybe affected without present patterns of isolation by distance (Muhlin & Brawley, 2009). Hiumaa in Estonia is the farthest population from the others and had highest values of genetic differentiation between locations. Comparing Rush and the rest of the populations, we can see that F_{st} is a little bit higher related with the geographical distance. This fact maybe is due to the population location which can be in a remote site, inducing a higher isolation of this population of *F. vesiculosus*. Genetic structure between populations seems influenced by distance, in agreement with the hypothesis of a progressive expansion of these two species toward northern Europe (Baltic Sea and White Sea) from refugee in the Iberian Peninsula, throughout SW Iceland, the Faeroe Islands and Nova Scotia (Coyer *et al.*, 2003; Coyer *et al.*, 2010).

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