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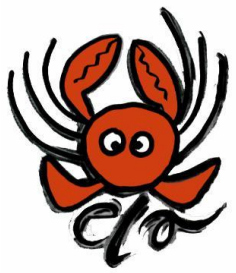
EXPERIMENTAL TESTS OF THE EFFECTS OF COMPOUNDED ANTHROPOGENIC PERTURBATIONS ON INTERTIDAL ASSEMBLAGES ON PORTUGUESE ROCKY SHORES

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Author's declaration

The author states to have afforded a major contribution to the conceptual design, technical execution of the work, interpretation of the results and manuscript preparation of the published or under publication articles included in this thesis.

Publications

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List of Abbreviations

CO2 – Carbon dioxide

DDT - Dichlorodiphenyltrichloroethane

IDH – Intermediate disturbance hypothesis

IPCC - Intergovernmental Panel on Climate Change

MSc - Magister Scientiae (Master of Science)

N – Nitrates

NW –North West

P – Phosphates

PAH - Polycyclic Aromatic Hydrocarbons)

PCB - Polychlorinated Biphenyls)

pH - potential of hydrogen

PhD - Doctor of Philosophy

POP - Persistent Organic Pollutants

Ppm – Parts per million

Resumo

As populações humanas sempre se concentraram perto de áreas costeiras e, de acordo com as previsões mais recentes, este comportamento deve aumentar nas próximas décadas. Este fenómeno está associado a uma série de perturbações, que incluem modificações da paisagem (por exemplo, a drenagem de zonas húmidas com respetiva conversão para o meio terrestre, a construção de estruturas de defesa costeira nas zonas intertidais ou a construção de estradas e edifícios), sobre-exploração (com colheita e pesca excessiva das populações marinhas com valor comercial), poluição ou atividades recreativas (por exemplo o impacto causado pelo pisoteio em costas altamente frequentadas). Todos esses fatores podem operar individualmente ou interagir entre si, coexistindo ainda com outros distúrbios antropogénicos (por exemplo, as alterações climáticas e a acidificação dos oceanos) ou naturais (por exemplo, tempestades mais intensas e frequentes e a ação das ondas), com consequências ecológicas que são, na maioria dos casos, imprevisíveis.

Esta tese de doutoramento teve como objetivo testar hipóteses relativas a uma série de possíveis impactos antrópicos sobre os sistemas intertidais rochosos do norte de Portugal, concentrando-se sobre as respostas das populações e comunidades de algas e invertebrados a perturbações combinadas já existentes ou previstas para o futuro. Foram utilizadas quatro abordagens, com procedimentos experimentais distintos, com o objectivo de analisar quer como os organismos intertidais lidam com os distúrbios que ocorrem numa escala de tempo definida, quer a forma como conseguem recuperar assim que estes terminam. Estas abordagens consistiram em (1) avaliar os efeitos das alterações da intensidade (induzidas pela utilização de técnicas diferentes para simular os distúrbios naturais) e da época da aplicação dos distúrbios; (2) avaliar a capacidade de organismos "*habitat-formers"* (como mexilhões) de amortecer distúrbios quer antrópicos, quer físicos semelhantes aos causados por eventos climáticos extremos, que

ocorram simultaneamente; (3) comparar escalas de variação espaciais e temporais das comunidades naturais entre áreas altamente urbanizadas e outras de referência; (4) examinar a capacidade de recuperação dos organismos bentónicos após o fim dos distúrbios experimentais.
Os resultados que cada projeto desta tese obteve estão, evidentemente, relacionados com os objetivos específicos de cada trabalho que foi realizado, no entanto, foi possível extrair algumas conclusões gerais que são transversais na ecologia intertidal rochosa moderna. Entre estas inclui-se o número total de espécies (um indicador da riqueza de espécies) que se manteve relativamente constante em todos os locais avaliados, ao longo do tempo; a relação directa documentada, pelo menos dentro dos limites de tolerância da maioria dos organismos, entre a intensidade do distúrbio e a heterogeneidade da comunidade, de forma provavelmente compatível com algumas possíveis previsões de teorias ecológicas gerais (por exemplo, a Hipótese do Distúrbio Intermédio); a necessidade da inclusão de escalas temporais e espaciais adequadas quer como variável preditiva quer de resposta em estudos ambientais; e a confirmação da capacidade de adaptação dos organismos intertidais a grandes variações das suas condições ambientais e à ocorrência recorrente de distúrbios, conseguindo resistir-lhes ou recuperando deles rapidamente, sendo introduzidas apenas alterações na sua abundância mas mantendo-se a sua identidade.

Embora o aumento da intensidade e variedade de influências antropogénicas em sistemas costeiros seja susceptível de determinar efeitos ecológicos nocivos, adequados para posterior pesquisa experimental, as conclusões obtidas no decurso desta tese podem contribuir de uma forma relevante para o desenvolvimento do conhecimento científico que é, em geral, essencial para uma integração sustentável entre a utilização antrópica do litoral e o bem-estar ecológico, procurando dotar a comunidade decisória de ferramentas que possam facilitar a gestão ambiental desta zona de interface.

Abstract

Human populations have always gathered near coastal areas and, according to recent evidence and predictions, this behavior is expected to increase on the next decades. This phenomenon is associated to a range of perturbations, including landscape modifications (e.g. wetlands drained and converted to upland areas, intertidal areas transformed by the construction of breakwaters, construction of roads and buildings), overexploitation (harvesting and overfishing of commercially valuable populations of marine organisms), pollution and recreational activities (e.g. trampling disturbance on highly frequented shores). All these factors can operate individually or interact between themselves and with natural (e.g. storms, wave and action) and/or other human-induced (e.g. climate change and ocean acidification) disturbances, with ecological consequences that are in most cases unpredictable.

This PhD project was aimed to test various hypotheses on a range of possible anthropogenic impacts on rocky intertidal systems of north Portugal, focusing on responses of algal and invertebrate populations and assemblages to present and expected compounded disturbances. Four different contexts and experimental procedures were taken into account to examine how intertidal organisms cope with disturbances occurring over a defined time scale and how they can recover once disturbances are over. These consisted in (1) evaluating the effects of changes in the intensity (modulated through different types of events) and time of application of disturbances; (2) assessing the capacity of habitat-forming organisms (i.e. mussel beds) to buffering concomitant physical storm-like impacts; (3) comparing spatial and temporal scales of variation of assemblages between highly urbanized and reference areas; (4) examining the ability of benthic organisms recover after the end the experimental disturbances.

Abstract

Each component of the project provided results inherent to its own primary objectives. Nevertheless, transversal conclusions could be drawn with respect to general issues of rocky intertidal ecology. These included: the relatively constant total number of taxa (a proxy for species richness) that were detected at all study sites; the documented direct relationship, at least within the limits of tolerance of most organisms, between the intensity of disturbance and the heterogeneity of whole assemblage, consistently with some of the predictions from general theories (e.g. the Intermediate Disturbance Hypothesis); the highlighted need for including proper temporal and spatial scales as both predictive and response variables in environmental studies; the confirmed adaptation of intertidal organisms to large variations of environmental conditions, including the recurrent occurrence of disturbances, through either resisting them or rapidly recovering afterwards in order to maintaining their identity in spite of even drastic alterations of relative abundances.

Though the increasing intensity and variety of anthropogenic influences is likely to determine ecological effects on coastal systems that are suitable for further experimental research, present findings can add a relevant contribution to a piece of accurate scientific knowledge that is, in general, essential for a sustainable integration between the human land use and the environmental wellbeing.

Introduction

The relationship between coastal areas and human populations is as old as our species and has been increasing through time. Populations living in littoral areas drastically increased during both 19th and 20th centuries and this trend is expected to continue (IPCC 2014 report (Wong *et al.*, 2014)). At present, low elevated coastal areas contribute with 2 % of the world's land, but contain 10 % of the whole world's population, with about 65 % of world's cities with more than 5 million people located in these areas (McGranahan *et al.,* 2007). Human activities are expected to increase in the near future, with rapid urbanization and growth of megacities and consequent threats and pressures to coastal resources (IPCC 2014 report (Wong *et* al., 2014)). It is, therefore, evident that assessing and predicting the effects of anthropogenic perturbations is a major concern for ecologists, policy-makers and the general public (Jackson and Burd, 2001; Lotze *et al.,* 2006).

A main difficulty in achieving this objective is given by the fact that anthropogenic disturbances, intended as any potential source of stress to natural populations due to human activities (e.g. Bampton, 1999), normally interact, directly or indirectly, with biological processes varying over a large range of spatial and temporal scales to modify patterns of distribution, abundance and diversity of natural populations and assemblages (Addessi, 1994; Keough and Quinn, 1998; Lindberg *et al.*, 1998). Under such circumstances, carefully designed ecological studies are needed to tease apart the human effects specifically under examination from the potentially confounding influence of the variability of co-occurring natural factors (Underwood and Chapman, 1996; Benedetti-Cecchi *et al.,* 2003; García-Charton *et al.,* 2000; Fraschetti *et al.,* 2005a).

Natural disturbances are key forces affecting the organization of communities (Sousa, 1984; Pickett and White, 1985). Although their direct effects are normally considered deleterious, they can indirectly increase both the

abundance of populations and the overall diversity of assemblages, depending on complex interactions between traits of the disturbance itself and life-traits of organisms (Dayton and Hessler, 1972; Sousa, 1984). The loss or increase of diversity will be dependent on the balance between the larger mortality caused by the disturbance and the capacity to utilize the resources made available (e.g. free space) (e.g. Bertocci *et al.,* 2005). This latter ability is governed by species-specific characteristics, including variations in settlement or recruitment (also in respect with the timing of the disturbance), biotic interactions and their links with abiotic features of the habitat (Fraschetti *et al.,* 2001; Incera *et al.,* 2010).

Variability in the intensity and the spatial and temporal patterns of disturbances are also drastically relevant, as natural catastrophes (e.g. hurricanes and earthquakes), with short but very intense interventions, and chronic disturbances, with larger temporal duration but smaller intensity, will usually be responsible for different consequences (e.g. Sousa, 2001). In fact, several classic studies have related the effects of different types and traits of disturbance with patchy distributions of natural assemblages due to the elimination of more sensitive species and the concomitant use of released resources by more resistant species (Grime, 1973; Connell, 1978; Huston, 1979, 1994; Benedetti-Cecchi and Cinelli, 1993; Connell *et al.,* 1997).

Of particular ecological importance is the concept of recovery after natural or anthropogenic disturbances. This is traditionally intended as the process by which, after the end of disturbance, natural communities return to their pre-disturbance condition or one indistinguishable from it (Underwood, 1989; Dethier *et al*., 2003). Nevertheless, the return to the previous condition might not indicate, per se, a true recovery as even undisturbed systems are naturally variable over time. Therefore, recovery should be properly assessed in comparison to the present state of systems as much as possible similar to the disturbed one, but for the lack of past disturbances whose effects are under examination.

Depending on their multiple traits and on life-traits of exposed organisms, disturbances can create mosaics of free and used resources and affect patterns of post-disturbance recovery. Nevertheless, such traits normally affect drastically the initial stages of recovery (e.g. recolonization is more likely to occur through vegetative propagation of organisms from the edges of a cleared patch or through external larval supply depending on the size and shape of the patch itself and of the life-cycles of species (Connell and Keough, 1985) and its duration (e.g. slow-growing invertebrates would need more time to recover than fast-growing ones (Vance, 1973; Watling and Norse, 1998), in the lack of new disturbances this process will usually progress to the same end-point in terms of structure of assemblages (e.g. Rosenberg *et al.,* 2002; Bevilacqua *et al.,* 2006).

Rocky shores are ideal "lab benches" to test and analyze these natural and human interactions and have been extensively used in manipulative experiments. A number of properties make them suitable particularly for small and medium-scale studies. Rocky shores are usually abundant, with easy access and visibility, with sessile or hemi-sessile taxa occurring in large abundances and diversity and with fast turn-over rates (Underwood, 2000).

Moreover, coastal habitats are at the interface between land and sea and are, therefore, subject to threats from both realms. The main classes of stress directly or indirectly caused by anthropogenic activities to coastal systems are described below.

Habitat Loss

Habitat loss is, in general, the most important threat to biodiversity (Brooks *et al.,* 2002; Hanski, 2005, 2011; Groom *et al.,* 2006). The negative effects deriving to populations and communities from drastic habitat degradation or complete loss are self-evident. Nevertheless, the ranges and rates of biological

responses to these stressors are not. For instance, ecological responses are often not linear at the landscape level and typically delayed in time as habitat loss can cause a gradual decay of the abundance of species before extinction (Hanski, 2011). This "extinction debt" (Tilman *et al.,* 1994) can be responsible for underestimations of the actual threat to biodiversity by habitat loss.

Widespread examples of habitat loss include wetlands drained and converted to upland areas (Gutzwiller and Flather, 2011), intertidal areas transformed into break-waters (Granja *et al*., 2011) (such as the Aguda study site of the present research), bottom trawling (Althaus *et al.,* 2009), cyanide (Mous *et al.,* 2000) or dynamite fishing (Pet-Soede *et al.,* 2000).

Although this threat is not new and globally increasing, only in some cases it has been accompanied by the implementation and enforcement of protection measurements specifically targeting endangered species and habitats (e.g. oyster reef exploitation in some countries (Kirby, 2004)). In most cases, concomitant priorities and resource use conflicts make habitat loss to continue almost uncontrolled, such as dramatically evident for tropical mangroves that are being heavily impacted and replaced by aquaculture, timber harvest and urbanization (Alongi, 2008) and for the loss of coral reefs due, for instance, to overexploitation, global warming and ocean acidification (Gardner *et al.,* 2003; Selig and Bruno, 2010). It is estimated that 67 % of wetlands, 65 % of seagrasses and 48 % of other submerged aquatic vegetation were lost, over the last 150 to 800 years, due to the increased pressure caused by human settlements in estuarine habitats (Lotze *et al.,* 2006).

Overexploitation

Marine animals, including fish, invertebrates and others belonging to any group, and plants, have represented important sources of food and nutrients for humans since ancient times. Though their limited harvest is not

necessarily unsustainable, the recent increase of needs by a drastically increasing human population, associated to the technological progress of harvesting means has led to the realized or risked overexploitation of valuable stocks of species and whole systems. This is particularly evident with fish, as the failed or lacking management of fisheries has resulted in a situation where 32 % of commercial stocks were overexploited to some extent, 53 % were already fully explored (Dulvy *et al.,* 2003; Kappel, 2005) and only 15 % have some potential for increased captures (Toppe *et al.,* 2011). Wild marine fish captures peaked in the 1980's and landings are diminishing, even though fishing effort has been increasing (Christensen *et al.,* 2007). Even more, the trophic level of catch has declined, suggesting that we are fishing down (Pauly *et al.,* 1998) and through (Essington *et al.,* 2006) marine food webs, depleting their lower levels. Scientists generally agree that current production from wild fish populations cannot continue much further (Toppe *et al.,* 2011). Though several factors modulate the impact of harvesting activities over coastal systems, including the identity of the target habitats and species, gear types and selectivity overexploitation is, in general, considered as a leading threat to marine taxa (Kappel, 2005; Venter *et al.,* 2006; Crain *et al.,* 2009) and whole ecosystems (Halpern *et al.,* 2007, 2008). It is predicted, for example, that most fisheries might collapse in the next 50 years if management strategies are not altered and correctly implemented (Worm *et al.,* 2006).

Besides the direct impact of overexploitation on target populations, possible indirect effects through modifications of biological relationships along food webs are of particular ecological concern. The case of the intense hunting of Alaskan sea otters that led to the replacement of kelp forests by urchin barrens as these were released from predation causing an excessive grazing is one of the best documented examples of habitat modification triggered by overexploitation of a top-predator (Estes and Duggins, 1995).

Eutrophication and Hypoxia

Originally, the term 'eutrophic' was intended to mean '*good nourishment'* and 'eutrophication' the process by which water bodies naturally increase their productivity (Thiennemann, 1918; Naumann, 1919). This perspective changed around the 1970's, as it became clear that nutrient enrichment, particularly when driven by intense anthropogenic sources, may cause considerable environmental impacts both in freshwater, such as lakes and reservoirs (Vollenweider, 1968; Carlson, 1977), and coastal systems (Bayley *et al.,* 1978; Lohrenz *et al.,* 1999). Nixon's original proposal for a new definition of eutrophication as "*an increase in the rate of supply of organic matter in an ecosystem*" (Nixon, 1995) was later altered by the OSPAR convention in 1998 to "*the enrichment of water by nutrients, causing an accelerated growth of algae and higher forms of plant life to produce an undesirable disturbance to the balance of organisms present in the water and to the quality of the water concerned*" (Urban Waste Water Treatment Directive (UWWTD), 91/271/EEC) to refer clearly to the undesirable effects of anthropogenic enrichment by nutrients.

In fact, eutrophication is currently used to indicate the process typically leading to a cycle of algal blooms, followed by algal death, decomposition and oxygen depletion, which is considered a widespread problem in coastal areas (Howarth *et al.,* 2000; Cloern, 2001; Breitburg *et al.,* 2009). This is due to the drastic increase of nutrient (Nitrates-N and Phosphates-P) inputs to almost all freshwater and coastal areas as a consequence of human activities, including the use of fertilizers for agriculture, the release of urban and industrial effluents and deforestation (Howarth *et al.,* 2011). In the 1990's, global fluxes to coastal oceans were two-fold greater for N and two- to three-fold greater for P than before the agricultural and industrial revolutions. Unfortunately, although many countries have been implementing water treatment plants, only P inputs have been sharply reduced, while N pollution has remained high (Howarth and Marino, 2006).

The direct impacts of eutrophication on aquatic systems are related with changes in species composition, as introducing limiting nutrients can modify competitive hierarchies (Emery *et al.,* 2001) and facilitate invasions by nonnative species (Williams and Smith, 2007). Nevertheless, the worst effect of this human perturbation is given by hypoxic water conditions deriving from microbial decomposition of blooming algae, most likely to occur when the circulation of water is not adequate and the renewal of oxygen is slow (e.g. Rabalais *et al.,* 2009).

Chemical Pollution

Coastal systems are exposed to a range of contaminants that reach the marine environment through terrestrial runoff or marine dumping. This raises concerns regarding their dynamics in the sea and their short- and long-term biological effects. Some substances are classified as POP's (Persistent Organic Pollutants) and include PAH's (Polycyclic Aromatic Hydrocarbons), flame-retardants, organometals and several pesticides (e.g. DDT). For example, PCB's (Polychlorinated Biphenyls) are particularly persistent and accumulate through the food chain, with negative effects particularly evident on top predators, including humans (de Wit and Muir, 2010; Mallin *et al.,* 2011). The effects of such contaminants on lower trophic levels are, instead, relatively less known as they may be very subtle, not causing direct mortality, but influencing relevant life stages such as recruitment, reproduction (Gibbs and Bryan, 1986) and larval viability (Negri and Hoogenboom, 2011; Kavanagh *et al*., 2013).

Inorganic heavy metals are also a common cause for concern, as they have a very long half-life and the ability to persist and increase in concentration in marine systems. Long-term effects of these compounds are still far from having been fully elucidated as specific lab-based toxicity tests can be

unrelated to their actual toxicity under natural conditions due to complex interactions with other stressors (Heugens *et al.,* 2001). Moreover, several monitoring programs for the concentration of these contaminants in the field usually focus of their water levels rather than on biological effects (Wu *et al.,* 2008).

Finally, since the industrial revolution starting in the 18th century, the use of fossil fuels has continuously increased. It requires their transportation from the extraction platforms to the delivery places all around the world, generally by sea routes in big tankers. Several and recurrent accidents have occurred during such process in the last decades (Aguilera *et al.,* 2010). The main ecosystem constituents affected by these spills are, usually, marine flora and fauna, such as birds and bivalve mollusks, but human health in heavily affected coastal areas can be endangered too (Aguilera *et al.,* 2010; Levy and Nasseta, 2011). Studies show a strong correlation between spill volume and wildlife fatalities, aggravated by the fact that many natural systems can take decades to recover (Aguilera *et al.,* 2010).

Invasive Species

Globalization, associated to worldwide and large movements of people and goods, has been and is responsible for the intentional and unintentional introduction and spread of organisms into novel geographic areas and habitats where they can become invaders. This process has increased steadily in the last 50 years (Ruiz *et al.,* 2000), exerting strong impacts on ecosystems and human welfare (Mooney *et al.,* 2005; Pimentel *et al.,* 2005). Coastal areas are considered among the most invaded systems, as they concentrate several activities that can promote biological invasions, including shipping, aquaculture, fisheries and aquarium trade (Williams and Grosholz, 2008).

The main ecological effects of biological invasions are related to the introduction of plant or animal species into environments lacking their natural

enemies, to the development of new associations between alien parasites and hosts, to the possible keystone role of the invader and to interactions with other past or present disturbances (Pimentel *et al.,* 2001).

Altered Salinity

Several human-related activities and processes, including tidal restrictions, inlet channelization, drainage plants, freshwater diversions and global warming (causing melting of ice caps and shifts in water circulation or precipitation patterns), can result in modification of salinity regimens in coastal systems (Jacobs *et al.,* 2002)

Salinity, and the tolerance of organisms to this variable, is a structuring factor of natural assemblages from estuaries, as indicated by documented longitudinal changes of the composition of communities across salinity gradients in such systems (Kinne, 1996; Gillanders and Kingsford, 2002).

While many coastal and estuarine species are euryhaline (i.e. they are tolerant to relatively large ranges of water salinity), altered salinity regimens can become a significant stressor when natural fluctuations associated to tidal and/or seasonal patterns are exceeded for prolonged periods of time or through intense pulses, such as due to periodic saltwater intrusions caused by groundwater pumping, navigation channels and sea water rise or dam discharges (Chang *et al*., 2011; Neubauer, 2013; Dai *et al*., 2011).

Altered Sedimentation

Rivers are major sources of terrigenous sediments to the continental-marine transition, although coastal erosion can strongly contribute to the sedimentary budget at the continental margin (Rachold *et al.,* 2000). Rivers

release sediments at their mouths, where waves, tides and oceanic currents are responsible for their dispersal. Deltas, estuaries and coasts form highly dynamic depositional systems (Hinderer, 2012) that always have been preferred regions for human settlement and activities due to the easy access to many natural resources and trading opportunities. At the same time, however, undesired consequences of this high dynamism led globally to the implementation of extensive engineering measures aimed at stabilizing coastlines and protecting urbanized coastal areas. This phenomenon, adding to a number of human activities was, on purpose or indirectly, able to affect sediment inputs to coastal areas and is responsible for alterations of sediment dynamics and of their ecological effects on a global scale. For example, damming and diverting freshwater or tidal influence can importantly reduce sediment delivery to coastal wetlands (e.g. Kuhn *et al.,* 1999; Garel and Ferreira, 2011), while deforestation and land clearing lead to terrestrial erosion and subsequent increase of coastal sediment delivery.

Intense sediment inputs can cause direct burial and suffocation of benthic organisms and alter physical and chemical characteristics of the water (Thrush *et al.,* 2004). Moreover, the interaction between sediments and wave action can negatively affect patterns of settlement and recruitment of sessile organisms through the smothering and abrasion of recently settled propagules and less tolerant species (Vaselli *et al.,* 2008). On the other hand, altered sedimentation regimens can increase the local availability of nutrients, prevent the monopolization of space by otherwise dominant species and protect from other disturbances (Taylor and Littler, 1982; Littler *et al.,* 1983; McQuaid and Dower, 1990; Gorgula and Connell, 2004; Connell, 2005).

Climate Change

The present geological era is sometimes called Anthropocene (Crutzen, 2002), because of the large and pervasive human influence over all natural systems.

Objectives

All ecosystems and life on Earth are exposed to multiple global-change stressors directly or indirectly resulting from anthropogenic activities. In such a context, a key process is the human-driven rising of the atmospheric concentration of greenhouse gases, which is considered a main responsible factor for recent past, present and predicted alterations of global patterns of climatic variables (Grimm *et al.,* 2013).

Man-made greenhouse gases have already been responsible for an increased ocean and air temperatures of 0,4 ºC to 0,8 ºC in the last century and IPCC 2014 report predicts a future increase of approximately 0,8 ºC to 6,4 ºC in the next (Fig. 1).

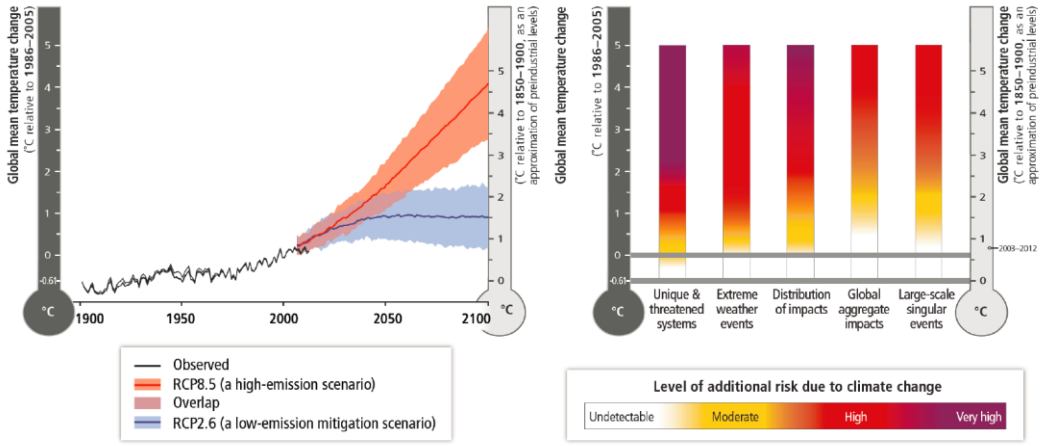


Fig. 1 - Risk of climate-related impacts results from the interaction of climate-related hazards (including hazardous events and trends) with the vulnerability and exposure of human and natural systems (IPCC, 2014).

Marine systems are impacted by temperature in several ways from the level of organisms (i.e. changes in morphology, behavior and physiology), to populations (i.e. altered transport processes affecting recruitment and dispersal) and whole assemblages and ecosystems (i.e. altered species interactions) (Harley *et al.*, 2006; O'Connor *et al.,* 2007).

All marine systems are vulnerable to climate-change, particularly if they were already impacted by other human or natural disturbances that have weakened

their ability to adapt (Pauly *et al.,* 1998; Jackson and Burd, 2001; Myers and Worm, 2003).

In fact, risks from climate change are particularly high for organisms and whole systems that are already near their tolerance limits, as any further stress can potentially lead to local extinctions and entire ecosystem loss. This is likely to occur, for instance, in tropical areas where reef-building corals live normally close to their thermal limits and a further increase in temperature might lead to drastic bleaching and extended die-off events (Scavia *et al.,* 2002).

The combination of anthropogenic and climate stressors currently threatening natural systems has been indicated able to produce negative impacts on global biodiversity as great as, or even greater than, that of the five mass extinction events ever occurred on Earth (Barnosky *et al.,* 2011).

Of particular societal and ecological concern are the recently documented and predicted alterations to the regime (mean intensity and temporal patterning) of storms and other meteorological variables, with special focus on extreme events (Easterling *et al.,* 2000; Beniston *et al.,* 2005). Climate models suggest that storm intensity will increase, although other characteristics are more uncertain to ascertain (Meehl *et al.,* 2007). Other authors, however, include alterations in temporal patterns (timing, frequency and variance of meteorological events) in their predictions (e.g. Muller and Stone, 2001; Gutschick and BassiriRad, 2003). Besides some degree of uncertainty, there is large consensus on expecting that extreme storm activity is likely to accompany global warming (Aargaard *et al.,* 2004; Webster *et al.,* 2005; IPCC 2007; IPCC 2014 report (Wong *et al.*, 2014); Reguero *et al.,* 2013).

Ocean Acidification

Since the start of the industrial revolution that the atmospheric concentration of CO_2 has been rising, from a level of approximately 280 ppm to around 380 ppm today (Feely *et al.,* 2004). Almost half of all the anthropogenic CO_2 produced has been taken up by the world's ocean (Sabine *et al.,* 2004), reason why it's been heralded as a potential sink for atmospheric carbon dioxide. As this gas passively diffuses into the oceans it forms carbonic acid that causes the lowering of the oceans water's pH. This mechanism is, however, regulated by the carbonate buffering system where carbonate ions are used to maintain the global pH of the oceans. This buffering capability has always been considered as capable of sustaining the increase of CO_2, until recent (Caldeira and Wickett, 2003, for instance) models have demonstrated that the pH of the ocean (when compared with pre-industrial time) had already lowered by 0.1. The same studies project a fall of about 0.4 units before the year of 2100 and 0.7 by 2250. This ocean acidification process, as it is now understood, is not a consequence of the total amount of atmospheric CO_2 (although its increase is evident) but of the unprecedentedly high rate of increase instead (Widdicomb and Spicer, 2008).

The direct impacts of ocean acidification are numerous but focus primarily on calcifying and photosynthesizing organisms, with indirect impacts on associated species. Reef-building corals are among the ones more impacted as calcium carbonate availability is essential to the reef ecosystem. Other shelled organisms (as marine bivalves) have also been negatively impacted by the ocean's acidification. On the opposite direction, marine macrophytes appear to benefit from the larger CO_2 availability, which possibly interacts with their photosynthetic nature (in phytoplankton negligible effects were detected). Under the above illustrated circumstances, therefore, evaluating the effects of the human intervention on coastal ecosystems is a major focus of current ecological research and is essential to drive effective environmental managing decisions.

Objectives

The present research was aimed at experimentally assessing the ecological effects of different traits and combinations of human disturbances on sessile assemblages from rocky intertidal habitats of north Portugal, considered as a convenient and tractable study system. Analyses of previously collected data and three main new experimental studies, including a number of sub-objectives, were carried out. Overall, these studies were needed and essential to provide a deeper understanding of concomitant anthropogenic stressors that, individually or through complex interactions, affect and can greatly impact the examined systems.

The main components and objectives of the research were:

- *Interplay of experimental harvesting and climate-related disturbance on benthic assemblages of rocky seashores* (18 months study).

    - identifying the variability of extreme climatic (i.e. storm) events that naturally affected the NW coast of Portugal in the last decade;

    - using the collected information on natural storms to establish realistic levels of experimental treatments of mechanical disturbance varying in mean intensity and temporal variability, according to predictions from existing climate models;

    - combining, in a multi-factorial manipulative experiment, mechanical disturbance with levels of removal of a commercially harvested habitat-forming species (the blue mussel *Mytilus galloprovinciallis*) to examine how such species can buffer the effects of concomitant climate-related disturbance.

- *Urban vs. extra-urban environments: Scales of variation of intertidal benthic assemblages in north Portugal* (13 months study).

    - identifying and quantifying multi-scale patterns of distribution, abundance and diversity of populations and assemblages within or in the close proximity to urban centers, in comparison to areas far from sources of urbanization-related stress.

- *Patterns of recovery of intertidal organisms after compounded anthropogenic disturbances* (15 months study).

    - following temporal patterns in the structure of whole assemblages, in the richness of taxa and in the abundance of individual taxa after the end of experimental disturbances established in the first study, in order to assess whether traits of past disturbance could affect the end-points and the temporal trajectories of recovery of those response variables.

In addition, a preliminary task of the present PhD project, involved the performing of new statistical analyses of data collected by the Author during his MSc thesis, with the aim of preparing an international peer-reviewed publication. This work led to the publication of the paper entitled "*Type and timing of disturbance modify trajectories of recovery of rockpool assemblages at Aguda (NW Portugal)*" in the Journal of Experimental Marine Biology and Ecology, which provided the first and foremost achievement during the first year of the present PhD thesis.

Specific details on most of the above listed tasks and goals were included in three peer-reviewed papers published between 2011 and 2014 in indexed (ISI) journals, as reported in the next chapter.

Original Research

Although the experimental field work contributing to the following first paper was carried out previously, and in consideration of the close links between the corresponding topics, the re-analysis of past data and their publication-aimed elaboration were considered one of the main objectives of the first year of the PhD course.

The sequence of papers here presented is based exclusively on their chronological order and is suitable to show the temporal progress and evolution of the work done during the whole PhD course.

The last study was not yet published and is presented in its final manuscript form as its formal submission (likely to Marine Ecology Progress Series) is expected only after this thesis has been presented.

I - Type and timing of disturbance modify trajectories of recovery of rockpool assemblages at Aguda (NW Portugal)

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Type and timing of disturbance modify trajectories of recovery of rockpool assemblages at Aguda (NW Portugal)

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ABSTRACT

Patterns of recovery of rock pool assemblages were experimentally investigated along the rocky shore of Praia da Aguda (NW Portugal). The temporal variance in the structure of whole assemblages and abundance of individual algal and invertebrate organisms was examined after two experimental disturbances differing in intensity, i.e. one able to eliminate all the biota from the substratum, the other able to create a mosaic of bare patches interspersed within the remaining organisms. Two experimental trials were carried out (starting in March 2007 and July 2007, respectively), with a duration of the study of 13 months each. Multivariate results indicated a temporally consistent positive relationship between the intensity of disturbance applied and the temporal variance of the structure of assemblages. A similar response was documented at the univariate level by the encrusting coralline alga *Lithophyllum incrustans* and the foliose green algae of the genus *Ulva*, while both experimental disturbances decreased temporal fluctuations of the abundance of mussels, *Mytilus galloprovincialis*, compared to unmanipulated control. The present study has implications for understanding and predicting responses of intertidal assemblages to natural disturbances whose intensity is predicted to increase in the future, including climate events.

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1. Introduction

The marine environment, particularly in coastal areas, is increasingly subject to natural and anthropogenic pressures and threats (Bryant et al., 1996; Hixon et al., 2001; Burbridge and Humphrey, 2003). Therefore, assessing the recovery abilities of marine populations and assemblages after disturbances is key to understand and predict biodiversity dynamics, with implications for ecosystem functioning (Hooper et al., 2005) and sustainable exploitation of organisms (Botsford et al., 1997; Castilla, 2000).

Disturbance is a primary source of free space on rocky shores, shaping the structure of natural communities through complex mechanisms (Dayton, 1971; Paine and Levin, 1981; Sousa, 1984; Pickett & White, 1985; Petraitis et al., 1989; Shea et al., 2004). It may directly remove organisms from the substratum, thus allowing the colonization and growth of potential competitors that may take advantage of resources previously unavailable (Dayton and Hessler, 1972; Grime, 1973; Connell, 1978; Huston 1979, 1994; Connell et al., 1997). The intensity of events of disturbance, the timing of creation of free space and species' life traits can drastically affect patterns of recovery (Underwood et al., 1983; Benedetti-Cecchi & Cinelli, 1993; Blanchette, 1996) and the structure of intertidal assemblages (Sousa, 1979; Keough, 1984; Lively et al., 1993; Dayton et al., 1984; Reed, 1990; Breitburg, 1985; Airoldi, 2000; Benedetti-Cecchi, 2000; Bertocci et al., 2005). In fact, spatially and temporally variable processes, including propagule availability (Sousa, 1984; Hoffmann and Ugarte, 1985), physical stress (Moore, 1972; Bell, 1993) and predation (Garrity and Levings, 1981; Underwood and Jernakoff, 1984), may allow different species to use the resources (primarily space) made available at different times. Although a number of previous studies have investigated the relationships between changes in intensity and timing of different disturbances, including scouring (Shanks and Wright, 1986; McQuaid and Dower, 1990; McCook and Chapman, 1993; van Tamelen, 1996; Vaselli et al., 2008), heat extremes (Menge and Sutherland, 1987) and storms (Dayton, 1971; Alvarado et al., 2001), with patterns of convergence (e.g. increase of similarity over time) of the structure of assemblages in disturbed areas towards that of assemblages in adjacent undisturbed areas (Underwood, 1989), the unpredictable nature of events of disturbance still poses difficulties for the understanding of the generality of patterns of recovery of intertidal assemblages in space and time. Manipulative experiments

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are key to increase our ability to understand the responses of natural assemblages to disturbance and to predict their magnitude and direction under different circumstances. In addition, the majority of previous studies have investigated the effects of intensity and timing of disturbance on average values of response variables (Sousa, 1980; Hawkins, 1981; Keough, 1984; Kim and De Wreede, 1996; McCabe and Gotelli, 2000; Mackey and Currie, 2001; Shea et al., 2004; Vaselli et al., 2008), while how changes in such traits of disturbance affect the temporal trajectories of recovery of assemblages and individual taxa has been rarely examined experimentally (Collins, 1992, 2000; Bertocci et al., 2005; Araújo et al., 2009). Here, we intended temporal trajectories as the shape of the curves describing variations in the abundance of individual taxa or in the structure of assemblages over the course of the study, independently of the direction of such changes (see, for instance, Bevilacqua et al., 2006). Nevertheless, understanding the causes of temporal fluctuations in patterns of abundance and recovery of organisms is of overwhelming importance to predict trends in functional traits of natural systems, including productivity (Johnson et al., 1996; Grime et al., 2000) and stability (Micheli et al., 1999; Lundberg et al., 2000; Inchausti and Halley, 2003).

In the present study, intertidal assemblages inhabiting small rock pools of the NW Portuguese coast were used as a model system to investigate experimentally the simultaneous and interactive effects of two physical disturbances changing in intensity and timing. Previous research on intertidal habitats in this region has been limited to descriptive studies aimed at characterizing existing biota (Araújo et al., 2005, 2006) or to manipulative studies on responses of relevant species to specific anthropogenic disturbances (Araújo et al., 2009), while recovery of assemblages has never been examined explicitly. Specifically, we examined, by mean of a manipulative field experiment, the hypotheses that (1) two physical disturbances with different intensities (able to remove all organisms from the substratum or to create a mosaic of bare patches interspersed within remaining organisms, respectively) determined different temporal fluctuations in patterns of recovery of rockpool assemblages, and (2) these patterns were consistent with the timing of application of disturbance. These hypotheses were tested in terms of the structure of whole assemblages, the total number of taxa and the abundance of individual taxa as response variables.

2. Materials and methods

2.1. Study site

This study was performed between March 2007 and August 2008 at Praia da Aguda (41° 2.710′N, 8° 39.203′W), north-western Portugal, in small rock pools located at mid-tide level (between 1 m and 2 m above Chart Datum). The tidal regime along the Portuguese coast is semi-diurnal, with a maximum range of spring tides of 3.5–4 m, and the rocky shore is typically granitic (Araújo et al., 2005). Diversified rock pool assemblages occur at the examined level on the shore, with the domination by red algae, such as *Chondrus crispus* Stackhouse, the encrusting coralline *Lithophyllum incrustans* Philippi and articulated corallines of the genus *Corallina*, brown algae, such as *Bifurcaria bifurcata* R. Ross, and green algae, such as *Ulva* spp., particularly abundant during summer. Other common species are the filamentous red algae *Leptosiphonia schousboei* (Thuret) Kylin and *Ceramium* spp. and the invasive brown alga *Sargassum muticum* (Yendo) Fensholt (Araújo et al., 2006). The most common invertebrates are mussels (*Mytilus galloprovincialis* Lamarck) and grazing gastropods, such as limpets (*Patella* spp.) and trochids (*Gibbula* spp.). The examined pools are periodically affected by severe disturbances, including the mechanical impact of waves during storms and intense desiccation due to concomitant conditions of low tide, irradiation and high aerial temperatures, that can create patches of open space ranging in size from a few cm^2 to almost the whole pool area (personal observation).

2.2. Experimental design and collection of data

A total of 30 rock pools interspersed along about 1 km of coast were selected at the beginning of the study (March 2007). Rock pools were similar in size (mean and SE = $0.17 \pm 0.02\ m^2$), maximum depth (mean and SE = 0.63 ± 0.03 m) and distance to the sea at low tide (mean and SE = 2.15 ± 0.25 m). Fifteen out of the 30 pools were assigned at random to a first experimental trial, starting in March 2007. Five pools were assigned at random to each of the following treatments: Control (C), Scraping (S) and Total Removal (TR). Control pools were marked and left unmanipulated. In S pools, the whole surface was disturbed once with a paint scraper, taking care of maintaining the intensity of disturbance comparable across all pools assigned to this treatment. This manipulation was able to remove some organisms, leaving unaffected the most resistant ones or those that were more strongly attached to the substratum. Such an effects was likely modulated by physical features of pools, such as the presence of crevices of small-scale variations in the nature of the rock, but our experimental interspersion and level of replication of different rockpools reasonably allowed to distribute such sources of intrinsic natural variability across all treatments, thus providing an unconfounded test for our original hypotheses. In TR treatment, water was drained out and all organisms were removed by hand (mobile animals) or hammer and chisel (sessile species), then the whole surface of pools was scorched with a gas torch in order to reduce the substratum to bare rock. The effects of our experimental disturbances were analogous to those of natural processes previously documented on rocky shores, particularly of strong waves whose mechanical impact may directly or indirectly remove from the substratum only the less resistant organisms (Paine and Levin, 1981; Foster and Sousa, 1985) or all species (Sousa, 1985; Huston, 1994; Connell et al., 1997), for example through the breaking of portions of rock and the exposure of bare patches varying in size (Benedetti-Cecchi, 2000).

The second trial of the study started in July 2007, with the same experimental procedures applied on a new set of 15 randomly chosen pools interspersed along the study site.

The abundance of organisms over the whole surface of each pool was measured before experimental manipulations and monthly over a period of 13 months from the start of each experiment trial (from March 2007 to April 2008 and from July 2007 to August 2008 for Trial 1 and Trial 2, respectively) using photographic sampling. Logistical constraints prevented to prolong the study, therefore it could just capture the initial stages of recovery of assemblages after experimental disturbances. Digital imaging was found to be a repeatable, robust and sensitive method to quantify cover values of sessile organisms in intertidal habitats (Meese and Tomich, 1992) and it was considered particularly effective in the present study due to time constraints of sampling depending on tides and the general lack of canopy-forming organisms with understory associated assemblages in our experimental plots. Digital photographs of the whole area of each pool were taken using a Sony S40 camera with a x5.1–x15.3 objective. Photographs were then projected on a screen onto a grid large enough to cover the whole area of the pool. The cover of sessile organisms was estimated visually by assigning to each taxon in each sub-quadrat a score ranging between 0 (absence) and 4 (entire sub-quadrat covered), then summing over all sub-quadrats superimposed to each pool and expressing final values as percentages (Dethier et al., 1993; Benedetti-Cecchi et al., 1996). Mobile animals were quantified as density in each pool (number of individuals/m^2).

Organisms were identified to the finest level of taxonomic resolution achievable visually, in some cases lumping different species

into broader taxonomic or morphological categories (Littler and Littler, 1980; Steneck and Dethier, 1994).

2.3. Analysis of data

Univariate and multivariate analyses were performed to examine the effects of the type and timing of treatments on the temporal heterogeneity in the abundance and total number of taxa and in the structure of whole assemblages over the period of the study. For univariate analyses, the temporal heterogeneity was quantified as measures of temporal variance in the abundance of the most common organisms and of the total number of taxa (a proxy for species richness) among all sampling dates, calculated separately for each pool (Searle et al., 1992). This produced five replicate values of temporal variance for each treatment and trial, which were analysed with a two-way ANOVA model including the fixed factor Treatment (three levels: C, S and TR), crossed with the random factor Trial (two levels: Trial 1 vs Trial 2). Univariate measures of temporal variance were log-transformed to make variances homogenous (After Cochran's test). ANOVA was also used to examine the effects of experimental factors on multivariate analogues of the univariate measures (Anderson and Millar, 2004). In this case, we calculated multivariate pseudo-variances among all sampling dates on the basis of Bray–Curtis dissimilarities (Bray and Curtis, 1957) with PERMANOVA (Permutational Multivariate Analysis of Variance, Anderson 2001). Five replicate pseudo-variances were calculated for each treatment and trial and analyses with the same ANOVA model described for the univariate case. The multivariate measures of temporal heterogeneity were not transformed as variances were homogenous. In order to assess whether experimental treatments affected the studied organisms in terms of identity of species rather than relative abundances, the same analysis was performed on pseudo-variances calculated on the basis of presence–absence data.

Student-Newman-Keuls (SNK) tests were used for *a posteriori* comparisons of means that differed significantly in ANOVAs.

In order to examine pre-existing biological differences among pools assigned to each experimental condition, assemblages sampled before manipulations at each trial were compared using PERMANOVA, with the same model described above.

3. Results

3.1. Comparisons among assemblages before experimental manipulations

PERMANOVA did not detect any significant differences among assemblages allocated to each treatment within each trial at the beginning of the study ($MS_{TRIAL\times TREATM}=262.32$, pseudo-$F=1.15$, $p=0.349$; $MS_{TREATM}=370.59$, pseudo-$F=1.41$, $p=0.289$; $MS_{TRIAL}=512.2$, pseudo-$F=2.25$, $p=0.053$).

3.2. Patterns of recovery of assemblages

The temporal variance of assemblages based on abundance data varied consistently as a function of the disturbance treatment applied and the experimental trial (Table 1). Specifically, such response variable decreased progressively from the experimental removal of all organisms to the scraping treatment and to the unmanipulated control for both Trial 1 and Trial 2 (Fig. 1). Moreover, the temporal heterogeneity of the structure of assemblages from Trial 1 was larger than that from Trial 2, independently of the treatment applied (Fig. 1). These findings were analogous to those obtained from the analysis based on presence/absence data (not shown).

Table 1
ANOVA on multivariate temporal variance in structure of assemblages over the course of the experiment. Significant effects are indicated in bold.

Source of variation	df	MS	*F*	*P*
Treatment = Tr	2	304,860,245.8	21.16	**<0.05**
Trial = Tl	1	86,255,172.0	20.18	**<0.001**
Tr × Tl	2	14,406,945.5	3.37	>0.05
Residual	24	4,273,645.1		
Total	29			
Cochran's *C* test	$C=0.416$, $P>0.05$			
Transformation	None			

3.3. Patterns of recovery of individual taxa and total number of taxa

The temporal heterogeneity of the abundance of individual dominant taxa and of the total number of taxa was not affected by the interaction between the treatment applied and timing of disturbance (Table 2). A main effect of Treatment was detected on *L. incrustans* and *M. galloprovincialis* (Table 2), but with different patterns. Scraping caused a larger temporal variability in the abundance of *L. incrustans* compared to the other two experimental treatments at both Trial 1 and Trial 2 (SNK test: S>TR=C, SE=0.141; Fig. 2A), while the temporal variance of *M. galloprovincialis* was larger in the unmanipulated control than in both manipulated conditions (SNK test: C>TR=S, SE=0.294; Fig. 2D). All other individual taxa examined were not affected by experimental factors (Table 2, Fig. 2B, E, F), with the exception of green algae of the genus *Ulva*, whose abundance over the period of the study was more variable for Trial 1 than for Trial 2 (Table 2, Fig. 2C). For Trial 2, however, the total removal treatment tended to cause larger temporal fluctuations of this variable compared to scraping and control (Fig. 2C). A similar trend was shown by the total number of taxa (Fig. 2G). Patterns of abundance of individual taxa and the total number of taxa throughout the study are illustrated in Appendix A.

4. Discussion

This study indicated a temporally consistent positive relationship between the intensity of the disturbance applied and patterns of recovery, described in terms of the temporal variance of the structure of assemblages, supporting both our initial hypotheses. Moreover, such a response variable was larger for Trial 1 than for Trial 2, independently of the experimental treatment. Analogous response patterns were shown at the univariate level by the encrusting coralline alga *L. incrustans* and by the foliose green algae of the genus *Ulva*. On the contrary, both disturbance treatments decreased the temporal fluctuations of the abundance of mussels, *M. galloprovincialis*, compared to the unmanipulated control, while temporal variance was not significantly affected by any experimental factor for any of the other individual taxa examined or the total number of taxa.

A relationship between relatively higher intensities of disturbance and larger fluctuations in patterns of recovering assemblages has been

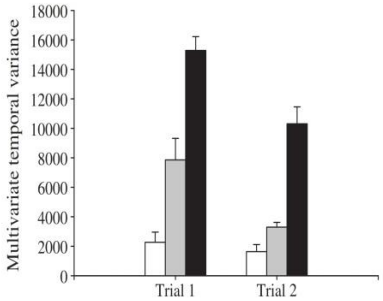


Fig. 1. Multivariate temporal variance of assemblages (mean + S.E.) for control (white), scraping (grey) and total removal (black) pools, separately for each trial of the experiment. Data averaged across five replicate pools in each treatment.

Table 2
ANOVA on measures of temporal variance in abundance of dominant organisms and in the number of taxa in rock pools. Significant effects are indicated in bold.

		Lithophyllum incrustans			*Corallina* spp.			*Ulva* spp.			*Mytilus galloprovincialis*		
Source of variation	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Treatment = Tr	2	8.33	42.14	<**0.05**	7.95	0.93	>0.5	29.98	4.79	>0.1	72.50	84.17	<**0.05**
Trial = Tl	1	0.13	0.08	>0.7	10.05	3.46	>0.05	25.49	11.19	<**0.01**	1.47	1.57	>0.2
Tr × Tl	2	0.20	0.12	>0.8	8.56	2.95	>0.05	6.26	2.75	>0.05	0.86	0.91	>0.4
Residual	24	1.69			2.90			2.28			0.94		
Total	29												
Cochran's *C* test		$C = 0.384$, $P > 0.05$			$C = 0.216$, $P > 0.05$			$C = 0.402$, $P > 0.05$			$C = 0.495$, $P > 0.05$		
Transformation		Ln(x + 1)			Ln(x + 1)			Ln(x + 1)			Ln(x + 1)		

		Gibbula spp.			*Patella* spp.			Number of taxa		
Source of variation	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Treatment = Tr	2	0.59	1.01	>0.4	3.97	4.33	>0.1	0.76	11.80	>0.05
Trial = Tl	1	0.59	0.87	>0.3	0.47	0.70	>0.4	0.02	0.26	>0.6
Tr × Tl	2	0.58	0.86	>0.4	0.92	1.37	>0.2	0.06	0.87	>0.4
Residual	24	0.67			0.67			0.07		
Total	29									
Cochran's *C* test		$C = 0.306$, $P > 0.05$			$C = 0.429$, $P > 0.05$			$C = 0.273$, $P > 0.05$		
Transformation		Ln(x + 1)			Ln(x + 1)			Ln(x + 1)		

established by a number of previous studies (Dethier, 1984; Bertocci et al., 2005; Benedetti-Cecchi et al., 2006). In principle, high intensities of disturbance could be expected to prevent large temporal fluctuations by determining local extinctions of most organisms or, anyway, drastic reductions of their abundances to such low levels that would make intrinsically impossible large variances due to the well known mean/variance relationship (Taylor, 1961), as several authors have exhaustively reviewed (Hurlbert, 1990; McArdle et al., 1990; McArdle and Gaston, 1992; Gaston and McArdle, 1993). Such a model was likely supported by patterns documented for mussels only. Although the recolonization of disturbed patches within mussel beds can start immediately after disturbance due to combinations of different processes, including a leaning of edge individuals, their inward migration and recruitment of dislodged adults or planktonic larvae, complete recovery may occur on temporal scales larger than those spanned by the present study (Paine and Levin, 1981; Menge and Sutherland, 1987), thus maintaining the abundance of mussels relatively low in our experimentally disturbed patches and driving the low temporal variability of such a response variable. Analogous patterns were not shown for most of the examined organisms, suggesting some possible mechanisms occurring in this system. First, it is evident that most of the organisms, although obviously not directly resistant to the most extreme intensity of disturbance applied, were able to recolonize quickly in the disturbed pools, in agreement with previous studies conducted in other intertidal systems (Hutchinson and Williams, 2001, 2003; Bertocci et al., 2005). This was further supported by the consistency of results between the multivariate analyses based on abundance or on presence/absence data, indicating that the observed patterns were likely driven by changes in the relative abundance of taxa, rather than changes in the identity of the taxa involved. Second, the fact that the abundance of species such as *L. incrustans* and *M. galloprovincialis* generally decreased in total removal compared to unmanipulated pools, while the percentage cover of other taxa, such as *Ulva* spp., drastically increased (see Appendix A), suggests that for some taxa the availability of resources, primarily space, produced by disturbance was more relevant than direct mortality in driving temporal patterns of distribution and abundance. In particular, as indicated in Appendix A, *Ulva* spp. might have benefited from the space made available at the beginning of the study by the more intense experimental disturbance that reduced the abundance of potential competitors, including mussels and encrusting corallines, in concomitance with the period of larger recruitment of these green algae (Araújo et al., 2006). Previous studies have indicated that the distribution of green algae of the genus *Ulva* and *Enteromorpha* is often associated with open patches of substratum, particularly in the lack of intense grazing (Williams et al., 2000). Such a response is likely made possible due to the fast growth of these ephemeral algae, which, once settled, can rapidly occupy relatively large patches of the substratum in a short time (Wai and Williams, 2006). Therefore, a combination of direct and indirect processes was likely responsible for the response of the whole assemblage to disturbance, with the reduction in the abundance of potentially stronger competitors and the subsequent facilitation, through the utilization of free space, of other taxa.

Differences in the effects of timing of disturbance independently of the experimental treatment could be explained in terms of abiotic and/or biological processes, whose direct examination was beyond the goals of the present study, that are known to contribute to small-scale variations in patterns of distribution of intertidal organisms. Factors such as the availability of larvae and propagules (Caffey, 1985; Connell, 1985; Gaines and Roughgarden, 1985; Roughgarden et al., 1988; Raimondi, 1990), post-settlement mortality (Denley and Underwood, 1979) and the patchy occupation of the substratum by adults (Sousa, 1984) could have differed between Trial 1 and Trial 2 as a consequence of their temporal variability (Foster, 1990; Kennelly and Underwood, 1992).

Our findings have potential implications when considering the possible ecological consequences of the larger temporal fluctuations in the abundance of some taxa determined by intense disturbance, in agreement with previous studies indicating that environmental fluctuations may promote the coexistence of species by reducing the intensity of biotic interactions (Hutchinson, 1961; Pickett and White, 1985; Chesson, 1986; Chesson and Huntly, 1997). Specifically, in any system subject to disturbance, no single taxa will possess all the life traits that would make it proliferate under every condition, with the consequence of creating a mosaic of resources that may prevent the dominance by one or a few species (Connell, 1978; Huston, 1979; 1994).

On the other hand, however, an increased temporal variance in the abundance of species may be related to increased risk of local extinctions of populations (Pimm, 1991; Lande, 1993; Vucetich et al., 2000). In the present study, such an outcome was documented only for mussels, while all other organisms were able to colonize quickly even after the most intense disturbance and could persist over the time scale of the experiment. This is in agreement with previous studies that have questioned the generality of such a relationship (Inchausti and Halley, 2003; Bertocci et al., 2005) and may suggest that in systems where most organisms have large recovering abilities

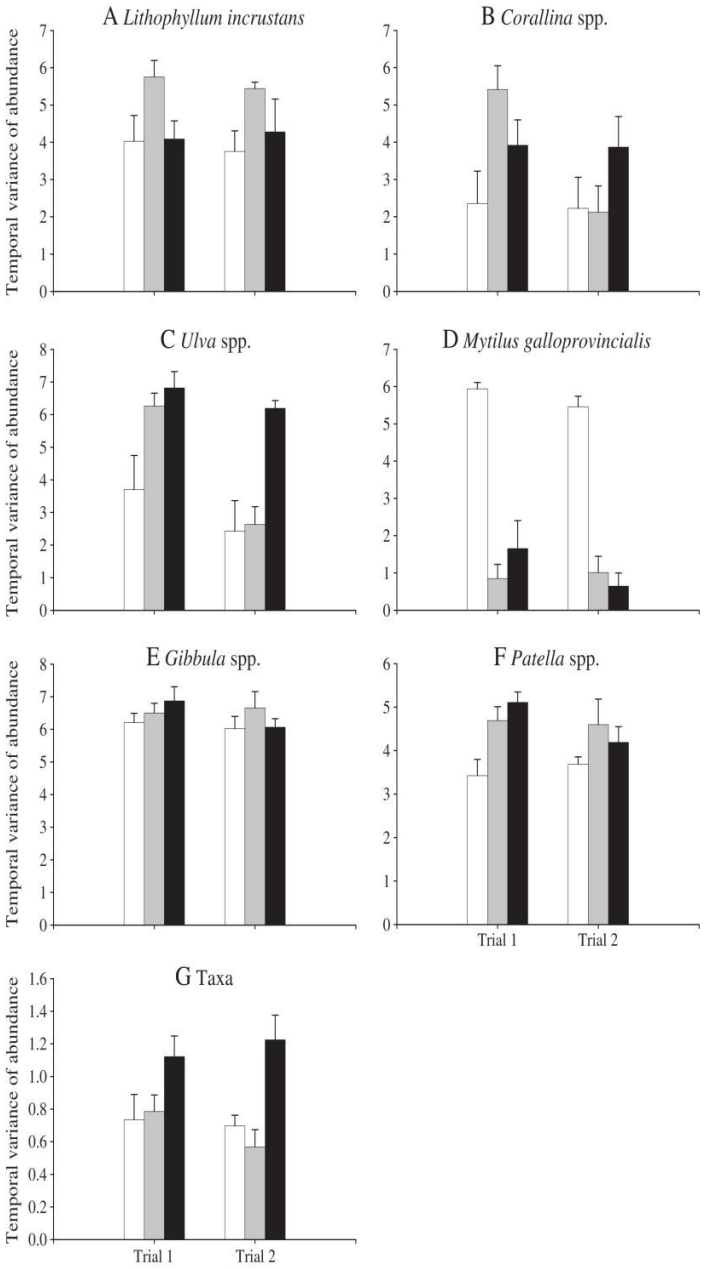


Fig. 2. Univariate measures of temporal variance of abundance (log transformed, mean + S.E.) for dominant taxa and total number of taxa, separately for each trial of the experiment. Data averaged across five replicate pools in each treatment (symbols as in Fig. 1).

after disturbance, intensity of disturbance may be positively related to temporal variance in abundances due to changes in the relative abundance of taxa rather than to changes in the species composition of assemblages. This would be further supported, for instance, by the lack of significant effects of experimental treatments on the total number of taxa.

Although a direct assessment of climate issues would require experimental studies performed over spatial and temporal scales larger than those included in the present study, it is worth noting that extreme meteorological events, such as storms, may exert complex direct and indirect effects analogous to our experimental disturbances (Underwood, 1998, 1999; Bertness et al., 1999; Jenkins et al., 2004; Lilley and Schiel, 2006). An increase of intensity and temporal variability of extreme storms is predicted by models of climate change (Emanuel, 1987; O'Brien et al., 1992; Raper, 1993; Michener et al., 1997; Easterling et al., 2000). The detailed occurrence and effects of such disturbances are largely unpredictable, but our findings suggest that these events are potentially able to introduce higher

variance in temporal patterns of recovery of intertidal assemblages through higher extinction risk of some species and increased abundance of other taxa, at least at the spatial scales of the present study. This appears as being a general feature of rocky intertidal habitats (Sousa, 2001) and terrestrial systems, such as grasslands (McNaughton, 1985; Cooper et al., 1999), that are characterized by a number of physical and biological disturbances, but highly resilient assemblages (Collins, 2000; Bertocci et al., 2005).

Supplementary materials related to this article can be found online at doi:10.1016/j.jembe.2011.01.023.

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II - Interplay of experimental harvesting and climate-related disturbance on benthic assemblages of rocky seashores

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Interplay of experimental harvesting and climate-related disturbance on benthic assemblages of rocky seashores

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ABSTRACT: Assessing responses of assemblages to compounded perturbations is a crucial goal of research on ecological impacts of current and predicted environmental changes. We examined the interactive effects of changes in temporal variance of 'storm-like' mechanical disturbance and in harvesting intensity of mussels (*Mytilus galloprovincialis*) on sessile assemblages associated with mussel beds on rocky shores in north Portugal. Response variables were measures of temporal variance in the abundance of individual taxa and in the structure of assemblages. Events of disturbance regularly distributed over the period of the experiment determined a lower temporal heterogeneity in assemblage structure than events characterized by larger temporal variance, but only in the presence of an intact mussel bed. The opposite pattern was documented in plots where mussels were completely removed. Univariate results indicated taxon-specific responses, with macroalgae, such as *Corallina* spp. and *Porphyra linearis*, showing patterns analogous to the multivariate one and grazing invertebrates displaying an opposite trend. This is one of the first manipulative examples aimed at examining effects of temporal variability of climate-related disturbance on systems increasingly depleted by concomitant stressors. The main finding was that effects of temporal variability of climate-related events of mechanical disturbance are mediated by the presence of intact stands of a foundation species. This may contribute to understanding and predicting responses of populations and assemblages under scenarios of concomitant climatic and non-climatic changes.

KEY WORDS: Climate change · Foundation species · Multiple disturbances · *Mytilus galloprovincialis* · Human impact · Intertidal · Temporal variance

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INTRODUCTION

Disturbance is a major driver of patterns of distribution, abundance and diversity of natural assemblages (Pickett & White 1985, Fraterrigo & Rusak 2008). The rate and scale of anthropogenic disturbances, however, are often much larger than those of natural ones, providing a serious threat to the maintenance of biodiversity and of its basic processes (see Gosling et al. 2011 for a review). Under observed and predicted climate change (Helmuth et al. 2006, Rosenzweig et al. 2008), there is special interest in responses of populations and assemblages to extreme events (Smith 2011).

In spite of the number of available studies on ecological effects of climate-related disturbance, several

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relevant issues still remain to be addressed. Most previous studies examined the effects of variations in mean intensity of environmental variables (Zavaleta et al. 2003, Stuart-Smith et al. 2010) or extreme events (Zimmerman et al. 2010). However, climate change also implies alterations in temporal patterns of meteorological events, including their timing, frequency and variance (Muller & Stone 2001, Gutschick & BassiriRad 2003). While the traditional approach for experimentally examining the effects of temporal variability of disturbance focused on manipulating the frequency of events (Collins 2000, McCabe & Gotelli 2000), the consideration that the temporal variation and the total intensity of disturbance applied over the period of the experiment can be confounded (Benedetti-Cecchi 2003) led to temporal variance of disturbance being taken into account (Bertocci et al. 2005, Benedetti-Cecchi et al. 2006, Vaselli et al. 2008, Molinos & Donohue 2011, Buckley & Kingsolver 2012). Few studies, however, specifically examined how variable regimes of disturbance can affect temporal fluctuations of assemblages (e.g. Collins 1992, 2000, Bertocci et al. 2005). In addition, in some cases, different traits of a single type of disturbance were manipulated simultaneously (McCabe & Gotelli 2000, Bertocci et al. 2005, Vaselli et al. 2008, Pincebourde et al. 2012), but little effort was put on experimentally examining the effects of variable regimes of disturbance combined with other concomitant perturbations that themselves alter local populations and assemblages. This is of overwhelming importance under real global change scenarios and current ecological theories on disturbance (Wernberg et al. 2012). Natural assemblages are normally exposed to multiple disturbances, potentially determining biological responses that are difficult to predict as being linked in complex ways to life traits of species (Bertness et al. 1999). Global decline of top predators (Heithaus et al. 2008) and foundation species (Bruno & Bertness 2001, Ellison et al. 2005), for instance, is likely to exacerbate the sensitivity of natural populations and assemblages to further disturbances whose patterns are modified by other factors. This would occur in all cases where natural populations depressed by anthropogenic factors (e.g. Connell et al. 2008, Lotze et al. 2011) are simultaneously exposed to climate change, including increases in frequency, variance and intensity of storms and other environmental extremes (Paine et al. 1998).

This study investigated the compounded effects of changes in temporal variability of climate-related disturbance (i.e. simulating the mechanical impact of major storms) and of varying intensity of loss of a foundation species (i.e. subject to human harvesting), using the mussel beds dominating rocky intertidal habitats in north Portugal as a study system. Mussel beds host diverse algal and invertebrate assemblages (e.g. Chapman 2005) and may protect understorey organisms from physical disturbance, analogously to other foundation species such as canopy-forming macroalgae (Lilley & Schiel 2006, Bertocci et al. 2010). In fact, they can be removed or damaged by waves (McQuaid & Lindsay 2000) whose impact is likely positively related to the integrity of the mussel bed as isolated mussels are more vulnerable to lifting and dislodging by waves (Denny 1987, Romme et al. 1998). In addition, mussels are intensively harvested for different purposes at several locations (Lasiak 1991, Airoldi et al. 2005, Atkinson et al. 2010). As classic studies documented, however, the loss of mussel beds may also make space available for organisms, such as green and red macroalgae, that are less efficient in the competition for space (e.g. Paine 1974). No previous studies have specifically examined hypotheses on the compounded effects of temporal variance of disturbance events (we refer here to the variation of a predictor variable) and of different levels of experimental harvesting of a foundation species on the temporal variation of associated organisms (here, we refer to the variation of the dependent variable). Filling this gap is needed as temporal changes in patterns of distribution, abundance and diversity of species are related to changes in important properties of natural systems, including productivity, stability and risk of local extinctions (Lundberg et al. 2000, Inchausti & Halley 2003).

We hypothesized that an intact mussel bed could buffer the effects of physical disturbance, leading us to anticipate an interaction between experimental harvesting of mussels and the climate-related mechanical treatment. Specifically, we expected that temporally variable (i.e. regularly vs. irregularly distributed over time) events of mechanical disturbance would exert limited effects on associated assemblages in the presence of an intact mussel bed, with the exception of the particularly stressful case where consecutive events, aggregated in short periods of time, were themselves able to remove the mussel bed. At the other extreme, the complete removal of the mussel cover would allow the dominance of fast-growing organisms, such as turf-forming algae, in the absence of disturbance, while increasing the aggregation of disturbance events would reduce such effects due to the sensitivity of turfs to mechanical disturbance (Bulleri 2006), indirectly facilitating

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more resistant species, such as encrusting algae and invertebrates (Lubchenco & Menge 1978). At intermediate levels of mussel removal, the responses of associated organisms would depend on the interactions among the available space, the ability of organisms to colonize and the temporal variability of concomitant mechanical disturbance. In fact, levels of removal of the mussel bed could be proportionally related to losses of associated organisms from experimental plots, but fast-growing species would rapidly colonize free patches. This would increase temporal fluctuations in patterns of abundance of these species, but such effects could be predicted to decrease with increasing variance of disturbance. Repeated events of disturbance clustered in time may drastically reduce the mean abundance of organisms for a relatively longer period than regularly distributed events, at the same time reducing their temporal fluctuations due to the mean-variance scaling relationship (Taylor 1961). These hypotheses were tested with an experimental design where the irregular treatment of disturbance was replicated through 2 random sequences (Vaselli et al. 2008) and where the same experiment was repeated at 2 different sites.

MATERIALS AND METHODS

Study site

The study was performed between February 2011 and July 2012 at 2 rocky sites (about 20 km of sandy coast apart), in north Portugal: Praia da Aguda (41° 2.71′ N, 8° 39.20′ W) and Praia do Marreco (41° 14.00′ N, 8° 43.27′ W). Each site is hundreds of meters long and occurs within an almost continuous sandy beach extending for nearly 200 km south of Aguda and 70 km north of Marreco. Both sites are characterized by the same south to north orientation and exposure of the coastline, gentle slope of typically granitic substratum and a semi-diurnal tidal regime with maximum range of spring tides of 3.5 to 4 m. Experimental plots were established at mid intertidal level, between 1 m and 2 m above chart datum. At this height, assemblages are dominated by mussels *Mytilus galloprovincialis* Lamarck, associated with diverse algal organisms. These include red algae such as *Chondrus crispus* Stackhouse and encrusting and articulated corallines (e.g. *Lithophyllum incrustans* Philippi and *Corallina* spp., respectively). Other algae are noticeably more abundant during summer, such as the brown *Bifurcaria bifurcata* R. Ross and the green *Ulva* spp. The most common grazers are topshells and limpets in the genera *Gibbula* and *Patella*, respectively. Details on distributional and demographic patterns of mussels over a range of scales at the study region can be found in Bertocci et al. (2012). Both sites are affected by severe natural and anthropogenic disturbances, including the mechanical impact of waves during storms, aerial exposure at low tide and harvesting of organisms for commercial and recreational purposes.

Experimental design

The interactive effects of changing the harvesting intensity of mussels and the temporal variation of storm-related mechanical disturbance on animal and algal assemblages associated with the *Mytilus galloprovincialis* bed were examined through a manipulative experiment. A total of 39 plots (35 × 35 cm, 10s of cm to meters apart) were marked at the beginning of the experiment at each site, within the belt dominated (i.e. cover in each plot ≥90%) by *M. galloprovincialis*. Three plots were left unmanipulated as controls. Nine plots were randomly assigned to each of the 4 treatments of experimental removal of *M. galloprovincialis*, simulating increasing intensities of human harvesting: (1) mussels intact (0% removal: '0'), (2) 70% cover of mussels remaining (about 30% removal: '30'), (3) 40% cover of mussels remaining (about 60% removal: '60') and (4) mussels completely eliminated (100% removal: '100'). Removal was performed using a knife, with particular care taken to leave the surrounding organisms unaffected. Mussels were removed at random until the desired level of abundance, but with focus on the largest individuals (i.e. those most likely targeted by human harvesting) occurring in each plot. Each harvesting treatment was crossed, in 3 out of the 9 plots, with 3 treatments manipulating the temporal variation of mechanical disturbance, one (regular treatment) characterized by an homogenous temporal distribution of events over the experiment, the other 2 (irregular treatments) characterized by a heterogeneous temporal distribution of the same total number of events. In the first treatment, a total of 6 events of disturbance were performed every 3 mo over the period of the experiment, generating a variance of 0 for the time intervals between consecutive events. In the other 2 treatments, the same 6 events in total were arranged in 2 randomly chosen sequences where some events were aggregated over short periods of time, separated by prolonged periods without disturbance, both producing the same value of variance for

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the time intervals between consecutive events. Replicating sequences within the same level of variance allowed us to separate the effects of temporal variation of disturbance from those of the particular sequence of events used to produce that desired level of variation (Vaselli et al. 2008). Climate-related mechanical disturbance was simulated by hand using a small weeding hoe and battering the plot in 2 orthogonal directions. The intensity of this disturbance was standardized to half of the number of hits able to remove, on average, all biomass of intertidal organisms from the substratum, as identified after a preliminary study (see also Bertocci et al. 2010). The chosen level of intensity, however, was considered as an 'extreme' event as it was potentially able to create patches of free substratum interspersed within intact assemblages, analogously to the effects of the mechanical impact of waves during severe storms and with patterns comparable to those observed in other intertidal systems (e.g. Benedetti-Cecchi & Cinelli 1994). Therefore, our study was designed to examine the effects of changes in the temporal regime of extreme disturbance events (combined with different intensities of harvesting) and not those of variations in the intensity of the mechanical disturbance itself.

Obviously, our mechanical disturbance was able to remove some mussels from experimental plots, including those assigned to the intact bed treatment, but such an effect was analogous to that of any natural disturbance occurring on assemblages subject to previous events. Thus, we assumed that this could not hinder our ability to test the model of interactions between mussel harvesting and mechanical disturbance in an unconfounded way.

Details on the design and on the choice of the total number of experimental disturbance events disturbance on the basis of the natural occurrence of storms at the study area are illustrated in Fig. 1 and Supplement 1 at www.int-res.com/articles/suppl/m495p131_supp.pdf, respectively.

Sampling and analysis of data

Organisms in each plot were sampled on each of the 9 dates selected at each site. Owing to the structure of the experiment, on each sampling date, the time elapsed since the previous disturbance event differed between levels of temporal variation or sequences of disturbance. In order to eliminate this potentially confounding factor, the 9 dates were selected in a way that the average time between a disturbance event and the next sampling was the same for all treatments (Fig. 1). As a consequence, the treatment effects could only be examined on response variables integrated over the whole course of the study, but not on single sampling dates.

Organisms were sampled visually utilizing a 30 × 30 cm frame, divided into twenty-five 6 × 6 cm subquadrats. The abundance of sessile organisms was estimated by assigning to each taxon a score from 0 to 4 in each sub-quadrat, adding up the 25 estimates and expressing final values as percentage covers (Dethier et al. 1993). Mobile animals were quantified as number of individuals. Each taxon was identified to the finest level of taxonomic resolution achievable in the field, in some cases lumping different species into broader taxonomic or morphological categories.

Univariate and multivariate procedures were carried out to test for the effects of experimental treatments on the temporal variance of the abundance and number of individual taxa and of the structure of whole assemblages. Analyses were based on an asymmetrical design including a first partitioning of the total variability into a Control vs. Treatments contrast and the Among treatments variation. The latter was then partitioned into the main effects of harvesting intensity (4 levels), disturbance variability (3 levels, provided by the regular, i.e. low variability, treatment and the 2 irregular, i.e. high variability, sequences) and their interaction. The main effect of temporal variability and the interaction term were further partitioned into a Low vs. High variability contrast and the Among sequences variation and into an Intensity × Low vs. High variability term and an Intensity × Sequences term, respectively. Dependent variables were measures of temporal variance over the period of the experiment in the abundance of common organisms and of the total number of taxa (a proxy for species richness) and their analogues for multivariate data. Each response variable was ana-

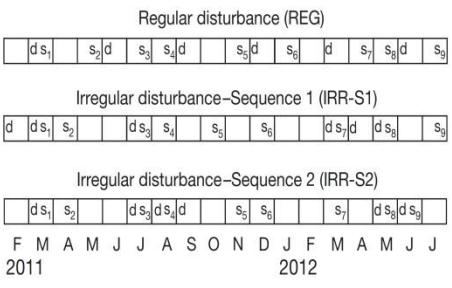


Fig. 1. Experimental and sampling design (d = disturbance event; s_1 to s_9 = sampling dates)

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lyzed with analysis of variance (ANOVA). When relevant, Student-Newman-Keuls (SNK) tests were performed for post-hoc comparisons of means. Details of statistical methods are described in Supplement 2.

RESULTS

Structure of assemblages

A total of 37 (16 animal and 21 macroalgal) taxa associated with the *Mytilus galloprovincialis* bed were identified and used for multivariate analyses. At both sites, assemblages regularly disturbed over the period of the experiment showed lower temporal heterogeneity than those subjected to disturbance events irregularly distributed in the presence of an intact mussel bed, while an opposite pattern was documented in plots where the mussel bed was completely removed (Fig. 2). At intermediate levels of mussel harvesting, no differences occurred in the effects of disturbance variability on the temporal heterogeneity in the structure of assemblages (Fig. 2). In addition, only Marreco control plots hosted assemblages less temporally variable compared to those sampled in plots assigned to all other treatments (Fig. 2B). These patterns were supported by a significant interaction of Intensity × Low vs. High variance at Aguda and by the significant Control vs. Treatments contrast at Marreco. At this second site, in spite of graphically evident differences and of the relatively large value of the *F*-test, the same interaction was not significant, likely due to the low power of the test. Temporal patterns of the structure of assemblages and results of statistical analyses are illustrated in detail in Figs. S1 & S2 in Supplement 3 and Table S2 in Supplement 5, respectively.

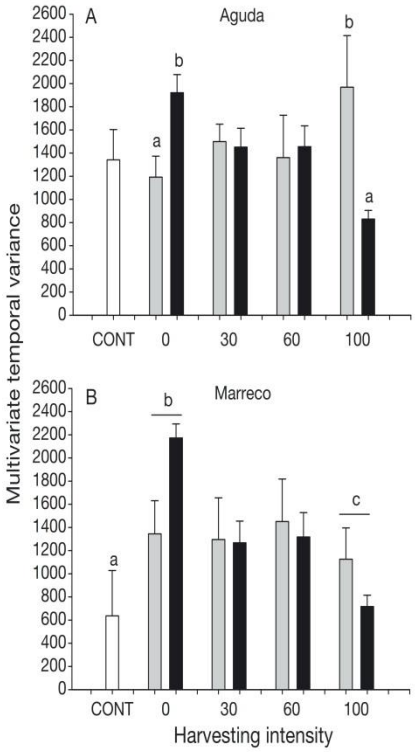


Fig. 2. Mean values (+1 SE) of multivariate temporal variance in unmanipulated controls (white, data averaged over 3 replicate plots) and in each combination of experimental harvesting intensity (0: intact mussel bed; 30 and 60: mussel bed reduced to 70% and 40% cover after removing about 30% and 60%, respectively; 100: mussel bed completely removed) and temporal variability of mechanical disturbance (grey: regular treatment, data averaged over 3 replicate plots; black: irregular treatment, data averaged over 3 replicate plots and 2 sequences) at (A) Aguda and (B) Marreco. Different lowercase letters above bars represent means that differed significantly (at $p < 0.05$, SNK test)

Total number of taxa and individual taxa

Although their total number was not affected by any experimental factor, individual taxa showed different responses to experimental treatments (Figs. 3 & 4). Interactive effects of harvesting intensity of mussels and disturbance variability were detected for topshells *Gibbula* spp., limpets *Patella* spp. and articulated coralline algae *Corallina* spp., at both sites and for the red alga *Porphyra linearis* at Marreco. The temporal variance in the abundance of *Gibbula* spp. was larger under regularly, compared to irregularly, distributed disturbance in the presence of a slightly reduced cover of mussels (treatment '30') at both sites (Figs. 3B & 4B; SNK tests) or of an intact mussel bed (treatment '0') at Marreco (Fig. 4B; SNK test). Such a response was reversed in Aguda for plots where the mussel bed was completely removed (Fig. 3B; SNK test). At Aguda, besides a significant effect of the experimental sequence of irregularly distributed disturbance events (see Table S3 in Supplement 5), the temporal variance of *Patella* spp. was reduced under irregular, compared to regular, disturbance combined with the lowest experimental level of mussel harvesting ('30' treatment) (Fig. 3C; SNK test). At Marreco, a similar pattern was documented in plots where the mussel bed was left intact, while it was clearly reversed in plots where it was fully

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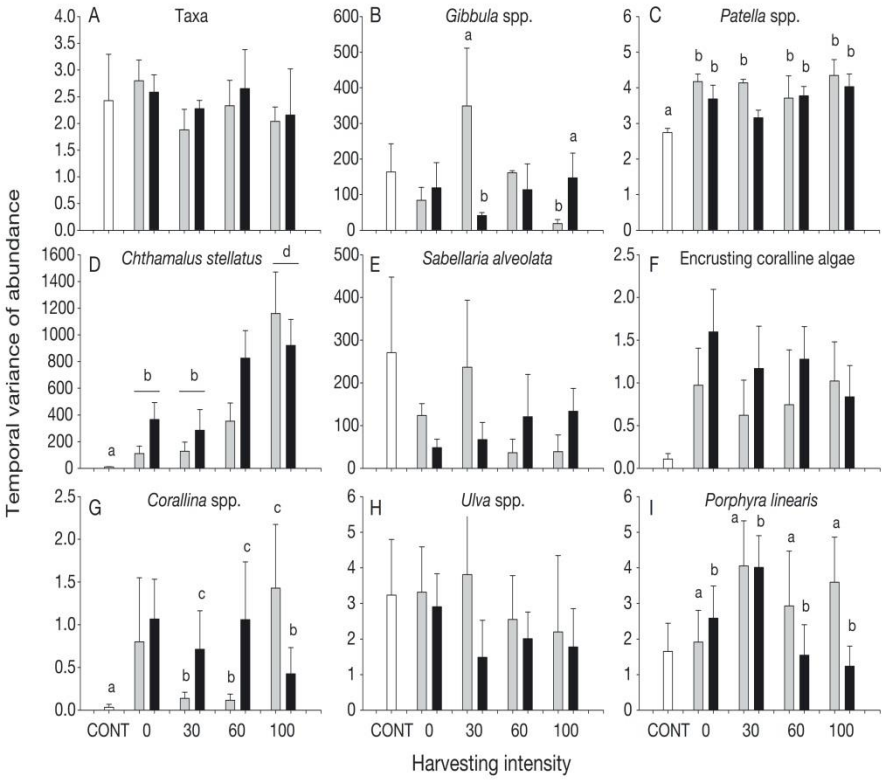


Fig. 3. Mean values (+1 SE) of univariate measures of temporal variance of abundance (A, B, D and E untransformed; C, F, G, H and I in logarithmic form) for the total number of taxa and the most abundant taxa sampled at Aguda. Abbreviations, symbols and sample sizes as in Fig. 2 (C and D: data averaged over sequences in spite of their significant effect for ease of reading and consistency)

removed (Fig. 4C; SNK tests). Temporal variance in the percentage cover of *Corallina* spp. was clearly reduced, at Aguda, under irregularly, compared to regularly, distributed disturbance events combined with both intermediate levels of mussel removal, while the opposite response occurred in plots where mussels were completely removed (Fig. 3G; SNK test). Analogous significant responses were not documented at Marreco. There, however, unharvested plots were still characterized by lower temporal variance of *Corallina* spp. when the regular treatment was compared with the irregular ones, while plots entirely free of mussels showed an opposite pattern (Fig. 4G; SNK test). The temporal variance of *P. linearis* at Marreco was affected by the interaction of harvesting intensity with the particular experimental sequence of events, rather than with the temporal variability of the disturbance itself (Fig. 4I and Table S4 in Supplement 5). Smaller fluctuations of this variable over the period of the experiment, in particular, occurred under disturbance events applied according to Sequence 1 in plots where mussels were completely eliminated compared to all plots where mussels were only partially removed (treatments '30' and '60', with the treatment having an intact mussel bed inconsistently ranked: SNK test). Disturbance events applied according to Sequence 2, instead, determined lower variance of *P. linearis* in extremely (treatment '100') and highly (treatment '60') harvested compared to not harvested plots (with treatment '30' inconsistently ranked: SNK test). An effect of disturbance variability on *P. linearis*, independent of harvesting intensity of mussels, was detected at Aguda, with the regular treatment determining lower temporal variance of this variable compared to the irregular one (Fig. 3I; SNK test). The barnacle *Chthamalus stellatus* Poli showed, consistently between sites, a pattern of increasing temporal variance with increasing harvesting intensity (Figs. 3D & 4D), although SNK tests could only detect signifi-

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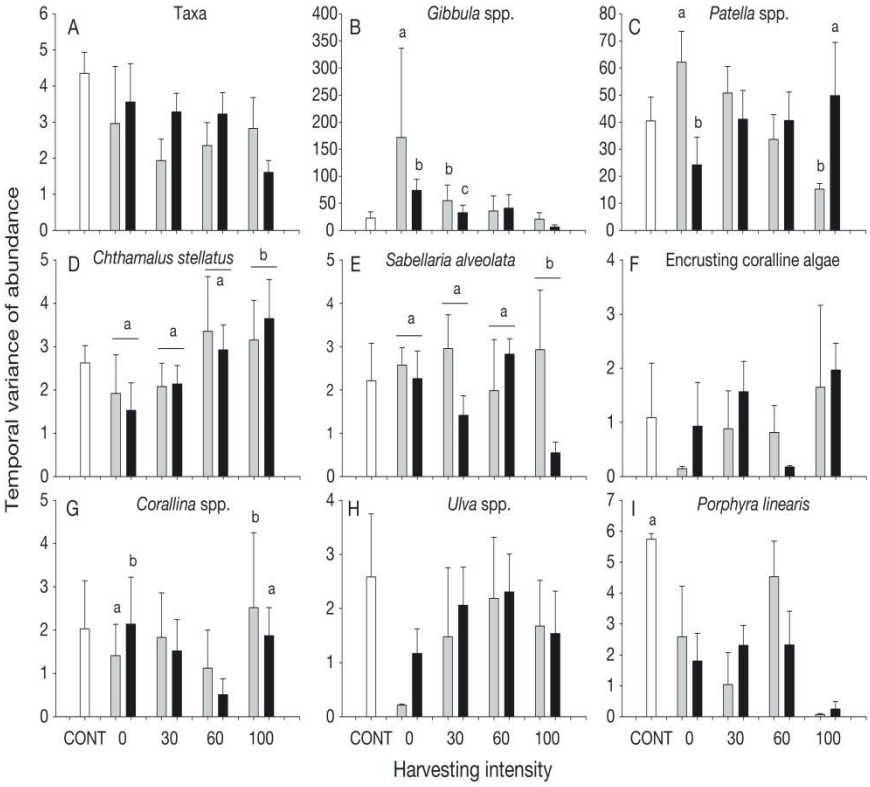


Fig. 4. Mean values (+1 SE) of univariate measures of temporal variance of abundance (A, B, and C untransformed; D, E, F, G, H and I in logarithmic form) for the total number of taxa and the most abundant taxa sampled at Marreco. Abbreviations, symbols and sample sizes as in Fig. 2 (H: data averaged over sequences in spite of their significant effect for ease of reading and consistency)

cant differences between the complete removal treatment vs. not or mildly harvested treatments ('0' and '30', respectively) and between the '60' vs. the '0' treatment at Aguda, and between the complete removal treatment vs. other treatments at Marreco. Only at Aguda was the temporal variance of *C. stellatus* reduced in unmanipulated plots compared to all other treatments and was affected by the sequence of disturbance events (Fig. 3D and Table S3 in Supplement 5). No significant responses to experimental treatments were provided by the honeycomb worm *Sabellaria alveolata* L., encrusting coralline algae and the green alga *Ulva* spp. at Aguda (Fig. 3E,F,H and Table S3 in Supplement 5). Nevertheless, a trend towards larger temporal variance of *S. alveolata* subject to regular, compared to irregular, disturbance combined with no or mild mussel harvesting was evident, while an opposite trend was observed in plots more intensively harvested (Fig. 3E). The temporal variance of encrusting corallines, instead, tended to be reduced in unmanipulated plots compared to all other treatments (Fig. 3F). A significant main effect of harvesting intensity on the temporal variance of *S. alveolata* was detected at Marreco, where this variable was reduced in completely compared to less or no harvested plots (Fig. 4E, Table S4 in Supplement 5 and SNK test). Finally, a main effect of disturbance variability was detected for *Ulva* spp. at Marreco, but this was driven by a response of this taxon to the particular irregular sequence of events rather than to variation of the level of temporal variability itself (Fig. 4H and Table S4 in Supplement 5). Univariate temporal patterns are illustrated in Fig. S3 in Supplement 4.

DISCUSSION

Our proposed model involving a buffering effect of an intact mussel bed on physical disturbance led us

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to predict an interaction between experimental mussel harvesting and climate-related mechanical disturbance. This was generally supported by multivariate findings, indicating that disturbance events regularly distributed over the period of the experiment determined lower temporal heterogeneity in the structure of assemblages than events characterized by larger temporal variance, but only in the presence of an intact mussel bed. The opposite pattern was documented in plots where the mussel bed was completely removed. These results were in most cases consistent across sites. As expected, an intact mussel bed, analogously to other habitat-forming organisms (e.g. Bertocci et al. 2010, Maggi et al. 2012), could mitigate the effects of regularly distributed disturbance events, thus maintaining the structure of associated assemblages relatively stable over the experiment. The application of several disturbance events clustered in shorter periods of time could overwhelm such buffering ability, creating patches of available resources, mainly space, that allowed the colonization by organisms otherwise excluded as inferior competitors per se. This could logically explain the larger temporal heterogeneity in the structure of assemblages when subjected to the irregular disturbance treatment, in general in agreement with previous findings on freshwater benthic assemblages under constant vs. temporally variable sediment disturbance (Molinos & Donohue 2011). Such a mechanism has also been documented in terrestrial systems through a progressive reduction of the ability of organisms to resist or recover from increasingly stressful disturbance, leading to a destabilization of assemblages and to an increased variability of the system (Collins 2000, Fraterrigo & Rusak 2008). In contrast, in plots where mussels were completely removed, irregularly distributed disturbance events reduced the temporal heterogeneity of assemblages compared to regularly distributed ones, analogously to findings of Burgmer & Hillebrand (2011) on phytoplankton assemblages exposed to increasing temperature variance in a microcosm experiment. This result is consistent with the expected effects of increasing disturbance intensity, defined as the physical force of the event per unit of area and time (Pickett & White 1985). Intense disturbances can reduce the variability of assemblages by eliminating most organisms and thus homogenizing patches, while weaker disturbances can maintain a more heterogeneous mosaic of available resources and local organisms, thus enhancing the spatial and temporal patchiness of the system (e.g. Collins et al. 2001, Bertocci et al. 2005). Although the total intensity of applied disturbance was kept constant among all our experimental treatments, a similar mechanism was hypothesized also in the present study. In fact, disturbance events clustered in time could drastically reduce the abundance of organisms for a longer period than regularly distributed events, thus reducing their temporal fluctuations due to the scaling relationship between the mean and the variance (Taylor 1961). The homogenization of damaged patches and their prolonged maintenance under the relatively more severe treatment might explain the reduced heterogeneity of assemblages subjected to irregular disturbance in the absence of mussels (see also Fraschetti et al. 2001). In fact, the mean cover of bare rock (b.r.) over the whole period of the experiment was much lower for the regular treatment than for the irregular ones at both sites (Aguda: $b.r._{Reg}$ = 33.7%, $b.r._{Irr}$ = 60.1%; Marreco: $b.r._{Reg}$ = 44.1%, $b.r._{Irr}$ = 71.2%).

Univariate responses analogous to the multivariate one were displayed by *Corallina* spp., at both sites, and by *Porphyra linearis*, particularly at Aguda. Opposite effects of temporal variability of disturbance were exerted on *Corallina* spp. depending on the presence of an intact or fully removed mussel bed, conditions where this taxon tended to maintain relatively lower and larger abundance, respectively. Such patterns are consistent with previous findings indicating that algal turfs often tend to become dominant after the loss of a foundation species, such as canopy-forming macroalgae (e.g. Benedetti-Cecchi et al. 2001, Lilley & Schiel 2006, Eriksson et al. 2007), likely due to the greater competitive abilities of turfs under the new harsher conditions (reviewed by Airoldi et al. 2008). A combination of reduced competition and large mechanical resistance can also facilitate articulated coralline algae to become dominant at sites characterized by intense disturbance, including wave exposure (Bulleri & Airoldi 2005). In the present case, the abundance of *Corallina* spp. within an intact mussel bed was particularly low and tended to remain relatively steady under regularly distributed disturbance events which could likely be buffered by the mussel bed. As expected, the irregular treatment, where the occurrence of disturbance events were clustered in relatively shorter periods of time, could lead to more resource availability, i.e. mostly space, for coralline algae within the mussels. As a consequence, *Corallina* spp. could increase slightly in abundance and their temporal heterogeneity over the period of the experiment could increase relative to regular treatment plots. Irregular disturbance events applied to plots where mussels

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were completely removed were consistently associated with a larger observed cover of *Corallina* spp. The maintenance of steady values over the course of the experiment resulted in lower temporal heterogeneity of this taxon under irregular, compared to regular, treatments after the removal of the mussel bed. An opposite response to the removal of mussels could explain *P. linearis* results. Although not exclusively associated with mussels as has been observed elsewhere (e.g. Crowe et al. 2011), we found this alga clearly more abundant within the mussel bed than in plots where mussels had been removed. It was not surprising that the complete removal of a relevant component of the habitat, besides a drastic reduction of *P. linearis*, also determined, through the mean/variance relationship (Taylor 1961), a reduction of its temporal heterogeneity in all treatments, compared to the control, at Marreco and under the irregular vs. regular treatment at Aguda. Consistently, at both sites, also where mussels were heavily, although not completely, removed (i.e. 60% treatment), the more stressful irregular treatment could reduce the temporal heterogeneity of *P. linearis* relative to the regular one.

Grazers of the genus *Gibbula* and *Patella* showed responses to experimental treatments substantially reversing those of turf-forming algae. The temporal heterogeneity of both taxa was reduced by irregularly, compared to regularly, distributed disturbance events applied to an intact mussel bed, while the opposite pattern was observed in plots where mussels were completely removed. However, mussel-intact plots tended to be associated with a larger abundance of *Gibbula* spp. and lower abundance of *Patella* spp. compared to mussel-depleted plots, suggesting possible different mechanisms for comparable effects. For topshells, we could hypothesize that a fully or almost intact mussel bed could provide a favorable habitat in terms of protection from desiccation, waves and predators, analogously to previous findings documenting strong reductions of whelks after the removal of a canopy-forming macroalga (Underwood 1998). Under these conditions, the abundance of such animals could be maintained relatively high, while their temporal heterogeneity could be mostly driven by temporal patterns of relevant abiotic and biological factors (e.g. Broitman et al. 2008). Intense disturbance events could then reduce, directly or indirectly, the abundance of topshells, logically determining lower fluctuations of this response variable over the period of the experiment observed under the irregular vs. regular disturbance treatment. Limpets, instead, could be more resistant than topshells to disturbance, including desiccation and mechanical impacts, while they could be excluded from substrates monopolized by sessile organisms (Underwood & Jernakoff 1981). Thus, they tend to increase in abundance once free patches were made available by relatively intense disturbances (e.g. Bertocci et al. 2005). In the present system, although more evidently at Marreco, the larger and more persistent availability of free space determined by the irregular treatment could have also made limpets more homogenously distributed over the period of the experiment within the mussel bed. Once mussels were completely removed, temporal patterns of limpets could have remained relatively stable due to the increased abundance and homogenous distribution of these animals driven by their resistance/recovery abilities, and enhanced in variability by the irregular treatment through its harsher effect on such processes. Increases in grazing intensity after the removal of habitat-forming species have been previously documented (Jenkins et al. 2004).

Independent effects of harvesting intensity and of the sequence of disturbance were documented for barnacles *Chthamalus stellatus*. Increased temporal variance of *C. stellatus* with increasing intensity of mussel harvesting could be a function of increased amounts of available space after mussels and possibly associated algal turfs were eliminated (e.g. Bertness et al. 2001). Not mutually exclusive direct and indirect mechanisms could explain the effect of the particular sequence of disturbance events at Aguda. First, *C. stellatus* showed its largest peak in abundance in July 2011, likely falling within the period of greatest recruitment of this species, as documented for nearby locations (O'Riordan et al. 2004). Second, potential better competitors for space were reduced during the same period, likely leaving room for colonizing barnacles. It could be hypothesized that a disturbance occurring in the same period could affect recruits and abundance patterns of barnacles independent of its irregular vs. regular trait, potentially determining significant effects of the sequence of disturbance alone. In the present experiment, the IRR-S2, but not the IRR-S1, treatment involved a disturbance event in August 2011, i.e. still within peak recruitment of *C. stellatus*, and this might have driven the significant effect of experimental treatments characterized by the same overall variance, but different temporal arrangements of events. The same results were not obtained at Marreco, leading us to reject our hypothesis of consistent responses of some individual taxa over 2 sites kilometers apart and characterized by a number of comparable abiotic

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factors that were controlled in the experimental design. Variability in the patterns of colonization of sessile organisms was also documented in other systems over similar scales and was attributed, for instance, to variation in availability of larvae (Raimondi 1990).

In spite of even large fluctuations in the abundance of individual taxa, in a system such as the present one, where most organisms are able to effectively resist or recover from disturbance, the overall number of taxa over the examined temporal scale remained relatively stable, as the variability of assemblages was due to large changes in relative abundances rather than in the identity of taxa. However, this contrasts with empirical and theoretical evidence that environmental variability might increase richness by reducing competition and promoting the coexistence of species with different life-traits (Chesson & Huntly 1997), or decrease it through increased risk of extinction for most species (Vucetich et al. 2000). Here, most organisms could persist over the period of the experiment (or they could rapidly re-colonize after temporary extinctions), suggesting caution in assuming general relationships between disturbance traits and richness of assemblages (see also Inchausti & Halley 2003).

The present findings could be applied to other systems characterized by organisms able to resist or recover quickly after disturbance events, particularly when these occur to populations and assemblages already maintained in altered states. This could be the case for populations collapsed due to overfishing, chronic pollution or climate-related factors, including changes in the regime of storms (e.g. Paine et al. 1998). In addition, our results are relevant to understanding responses of organisms to environmental variations. Climatic models forecast an increase in the occurrence of extreme events (e.g. Smith 2011), with changes in temporal patterning so that they would cluster in short periods of time, interspersed within calm periods (D'Odorico & Bhattachan 2012 and references therein). Different effects of multiple traits of single disturbances have been previously documented, but it is obvious that any disturbance occurs simultaneously and interacts with other natural or human perturbations drastically threatening biodiversity. This complicates predictions arising from the evaluation of each disturbance separately (Paine et al. 1998). We provided one of the first manipulative examples of opposite responses of assemblages associated with intact vs. depleted foundation species to changes in environmental variance. Although climate events occur on scales larger than the present ones, this study can contribute to predicting responses of assemblages under scenarios of combined climate and non-climate processes (Rosenzweig et al. 2008).

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III - Urban vs. extra-urban environments: Scales of variation of intertidal benthic assemblages in north Portugal

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Urban vs. extra-urban environments: Scales of variation of intertidal benthic assemblages in north Portugal

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ABSTRACT

Littoral areas are subject to severe and increasing pressures resulting from human activities occurring along or next to the coast. In this study, patterns of variability in the structure of rocky intertidal benthic assemblages and in the abundance of individual taxa were compared between locations close to the coastal cities of Porto and Vila Nova de Gaia (north Portugal) and reference locations far from it in much less urbanized conditions over a temporal scale of fourteen months and multiple spatial scales. Present findings indicated that assemblages were more heterogeneously distributed in the urban than in the extra-urban condition. The total number of taxa and several individual taxa displayed, in general, this same pattern of variability. This could be interpreted as the beginning of a habitat deterioration process with largely unpredictable consequences. The adopted sampling design supports the need for simultaneously including a range of temporal and spatial scales when evaluating responses of coastal marine biodiversity to anthropogenic disturbances.

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1. Introduction

Littoral areas are subject to severe pressures resulting from activities of the large proportion (i.e. more than 60%) of the human population living in coastal areas. This proportion is predicted to increase up to 75% in the next decades due to further demographic growth and to the tendency to move to coastal zones (Vitousek et al. 1997; Airoldi and Beck, 2007). This urban development typically modifies original habitats, causing impacts on local animals and plants, which are often replaced by non-native species (Lindegarth and Hoskins, 2001; Magura et al. 2009). In general, anthropogenic pressures are not easy to unequivocally identify and quantify as they interact in complex ways with natural processes and can be produced by a wide range of activities, including chemical pollution, sewage discharges, fishing and aquaculture (e.g. Fraschetti et al. 2011). Nevertheless, there is evidence that they can affect local, regional and even global patterns of distribution, abundance and diversity of benthic organisms, from individuals and populations to the ecosystem level (Lotze et al. 2006; Halpern et al. 2008; Fraschetti et al. 2011; Martins et al. 2012).

It has been frequently addressed (Olden and Rooney, 2006; Bevilacqua et al. 2012; Guarnieri et al. 2012; Tamburello et al. 2011) that a major result of anthropogenic pressures is a reduction of species diversity and evenness, as rare and more sensitive species are replaced by more resistant ones, leading to assemblages including less taxa, each represented by a relatively larger number of individuals. This can also lead to a homogenization of the spatial distribution, in terms of composition and abundance of species, among sites, i.e. the beta diversity (e.g. Balata et al. 2007a). Empirical tests of such expectation, however, have documented inconsistent findings. In particular, homogenization could occur in heavily disturbed systems (Archambault et al. 2001; McKinney, 2008), while in moderately disturbed ones the results varied considerably. In fact, several authors reported that human disturbances could modify local assemblages towards increases of their heterogeneity (e.g. Caswell and Cohen, 1991; Warwick and Clarke, 1993; Fraschetti et al. 2001), for example through the addition of non-native species that replace native species faster than these are lost (McKinney, 2002, 2006). Therefore, formally testing whether local anthropogenic disturbances drive a homogenization or a larger heterogeneity of populations and assemblages is extremely important to detect actual impacts of human activities in previously

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unstudied systems and to provide a basis for their possible mitigation (e.g. Elmqvist et al. 2003). An overwhelmingly relevant issue in such assessments concerns the scale at which a certain human impact is examined (Fraschetti et al. 2001, 2005; Menconi et al. 1999; Tamburello et al. 2011), as different processes can generate variability at different scales (Underwood and Chapman, 1996). In such a context, the use of hierarchical sampling designs able to capture variability over a range of scales has been widely proposed as a key tool to identify the extent of an impact (e.g. Vanderklift and Lavery, 2000; Piazzi et al. 2004; Pardi et al. 2006; Balata et al. 2007b, 2008) and to ensure that comparisons between disturbed and reference locations are done over scales appropriate to detect the actual impacts in an unconfounded way (Underwood, 1993, 1994; Glasby and Underwood, 1996; Bishop et al. 2002).

In this study, patterns of variability in the structure of rocky intertidal benthic assemblages and in the abundance of individual taxa were compared between urban (i.e. close to large coastal cities on the NW Portuguese coast) and reference (i.e. far from large coastal cities, in less urbanized areas) locations over a temporal scale of fourteen months and multiple spatial scales, ranging from 10s cm to 10s km. The hypothesis was that temporal and spatial patterns of distribution and abundance of whole assemblages and individual taxa were significantly affected by urbanization. The direction of such change towards a reduced or increased homogenization, however, could not be anticipated in detail as such issues had not been previously addressed in the present system despite the obvious local human pressure due to its proximity to the largest cities, commercial and industrial activities occurring along the Portuguese shore.

2. Materials and methods

2.1. Study site

The study took place between February 2012 and April 2013 at four rocky locations (100s m long) interspersed along about 30 km of coast in NW Portugal: Praia da Aguda (41° 2.71′N, 8° 39.20′W), Lavadores (41° 7′52.31″N, 8°40′12.31″W), Castelo do Queijo (41°10′4.82″N, 8°41′25.96″W) and Mindelo (41°18′36.19″N, 8°44′32.77″W). Lavadores and Castelo do Queijo are adjacent to highly urbanized areas (Vila Nova de Gaia and Porto, located next to the south and north side of Douro River's estuary, respectively). A map of the study system is reported in Fig. 1. Resource limitation prevented to include a larger number of replicate locations in each condition, which would have allowed a more accurate estimation of patterns of variability at each scale. However, our design was aimed at assuring that possible sources of uncontrolled variability, particularly those related to the geographical positioning of the sampled units relatively to the urban areas were equally distributed between the urban and the reference condition. Praia da Aguda and Mindelo occur in areas with small resident population lacking large commercial and industrial plants and are located south and north of Lavadores and Castelo do Queijo, from which they are separated by about 10–15 km of sandy beach. The shore at all locations is gently sloping, with a typical granitic substratum, analogous exposure to prevalent wind and waves, similar accessibility and a semi-diurnal tidal regime with maximum range of spring tides of 3.5–4 m. At each location, sampling was carried out in the mid intertidal habitat, i.e. about 1–2 m above Chart Datum. Assemblages at this shore level are dominated by mussels (*Mytilus galloprovincialis* Lamarck) in association with several algal species, commonly including encrusting (e.g. *Lithophyllum* spp.) and articulated corallines (e.g. *Corallina* spp.), other erect red algae such as *Gelidium* spp., *Chondracanthus teedei* (Mertens ex Roth) Kützing and *Chondracanthus acicularis* (Roth) Fredericq, green foliose species of the genus *Ulva* and the brown tuning fork weed *Bifurcaria bifurcata* R. Ross (Araújo et al. 2005). Grazing gastropods such as *Patella* spp. and *Gibbula* spp. are also very common.

2.2. Experimental design and data collection

At each of the two urbanized and reference locations, we randomly selected two sites (about 100 m long, about 50 m apart), with two 10 m areas (about 10 m long, 3–5 m apart) in each site and 15 quadrates (20 × 20 cm, 10s cm apart) in each area. The same sampling was repeated at each of four dates, established at random over a scale of 14 months: February 2012, May 2012, November 2012 and April 2013. New sets of independent sites, areas and quadrates were established at random at each sampling date.

At each date, organisms in each replicate quadrate were sampled visually utilizing a 20 × 20 cm frame, divided into twenty-five 4 × 4 cm sub-quadrates. The abundance of sessile organisms in

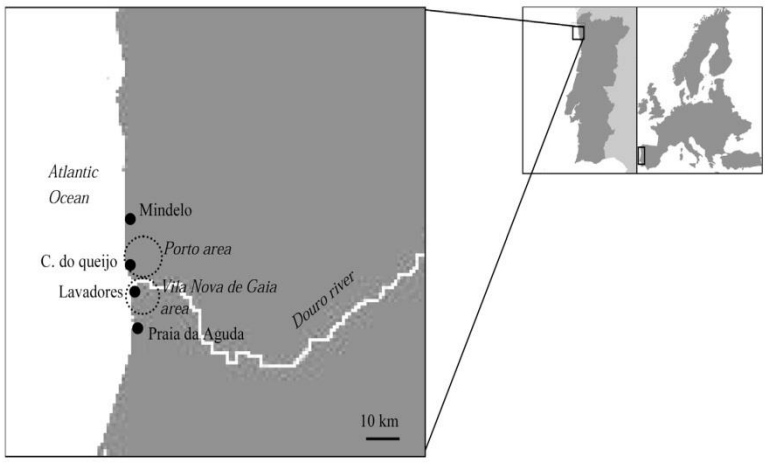


Fig. 1. Map of the study system.

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each quadrate was estimated by assigning to each taxon a score ranging from 0 to 4 in each sub-quadrate, adding up the 25 estimates and expressing the final values as percentage covers (Dethier et al. 1993). Densities of mobile animals were quantified as number of individuals per quadrate. Each taxon was identified to the lowest level of taxonomic resolution achievable in the field, in some cases lumping different species into broader taxonomic or morphological categories (Littler and Littler, 1980; Steneck and Dethier, 1994).

2.3. Analysis of data

In order to quantify and compare between the urbanized and the reference condition the patterns of variability of the structure of whole assemblages, of the total number of taxa (a surrogate measure of species richness) and of the abundance of individual taxa at each examined spatial and temporal scale, independent estimates of pseudo-variance and variance components, respectively, were calculated. First, five replicate quadrates out of the fifteen sampled at each date in each area, site and location were assigned to each of three randomly established groups. For each condition (urban *vs.* extra-urban), date of sampling and group, this produced a 3-way model including the factors Location (2 levels, random), Site (2 levels, random, nested within Location) and Area (2 levels, random and nested within Location and Site), with 3 replicates. Univariate variance components were estimated from analysis of variance (ANOVA) by equating observed and expected mean squares (Winer et al. 1991; Underwood, 1997). This method for partitioning variation was extended to multivariate data following the procedure described in Terlizzi et al. (2007). Specifically, permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001) based on Bray–Curtis (Bray and Curtis, 1957) untransformed dissimilarities was performed, separately for each condition, date and group, including the same factors and levels of ANOVA. Pseudo-variance components for each term in the model were then calculated using the multivariate analogues to the univariate estimators (Searle et al., 1992; Terlizzi et al. 2007). The above described procedures produced three independent replicates of variance or pseudo-variance components at each of four scales (i.e. between quadrates, areas, sites and locations) for each condition and date of sampling. In a few cases, negative estimates of variance were obtained. These were interpreted as sample underestimates of very small or null variances and set to zero (Searle et al. 1992; Underwood, 1996). Variance and pseudo-variance components were finally analyzed with a 3-way ANOVA including the crossed factors Date (4 levels, random), Condition (2 levels, fixed) and Scale (4 levels, fixed). Before each ANOVA, Cochran's C-test was used to test for homogeneity of variances and data were Ln $(x + 1)$ transformed when necessary. When this was not possible, untransformed data were analyzed and results were considered robust if not significant (at $P < 0.05$) or, to compensate for the increased probability of type I error, significant at $P < 0.01$ (Underwood, 1997). When relevant, Student–Newman–Keuls (SNK) tests were used for *a posteriori* multiple comparisons of means.

In order to assess whether patterns of variability at multiple scales were due to differences in the identity or in the relative abundance of taxa between the urban and the extra-urban condition, we had repeated our multivariate analysis (and the following ANOVAs) using a matrix of presence/absence data as the input.

Individual taxa for the univariate analyses were selected through the SIMPER procedure (Clarke, 1993), determining the percentage contribution (δ_i %) of each taxon to the Bray–Curtis dissimilarity between assemblages from the urban and the reference condition. Taxa considered important were those whose cumulative contribution to the total dissimilarity was ≥70%.

3. Results

3.1. Structure of assemblages

A total of 45, including 17 animal and 28 macroalgal taxa, were identified over all samplings and used for the multivariate analysis. The significant interaction 'Time × Condition × Scale' indicated different patterns of temporal and spatial variability in the structure of whole assemblages between the urbanized and the reference condition (Table 1). Specifically, urban assemblages were more heterogeneous than extra-urban assemblages at the scale of areas and sites at Date 1 of sampling (February 2012) and at the scale of locations at Date 3 (November 2012) and Date 4 (March 2013), while non-significant differences were observed at all other combinations of scales and dates (Table 1, Fig. 2 and SNK tests). Moreover, in both conditions and at each date of sampling, the variability of assemblages tended to be larger at the smallest (i.e. among quadrates) and the largest (i.e. between locations) spatial scale examined than at the intermediate scales (i.e. between areas and between sites; Fig. 2).

Analyses conducted on presence/absence data indicated that urban *vs.* reference differences tended to disappear, suggesting that the documented differences were actually driven mostly by changes in the relative abundance of taxa between conditions. This conclusion was also supported by SIMPER results (see the following sub-chapter, 'Total number of taxa and individual taxa').

3.2. Total number of taxa and individual taxa

Patterns of variance of the total number of taxa were similar to those described for the structure of assemblages and represented by a significant interaction 'Time × Condition × Scale' (Table 3). At all dates of sampling, but November 2012, this response variable was larger at the urban compared to the reference locations, while no significant differences were detected at the other scales (Fig. 3 A and SNK tests).

The SIMPER analysis identified 11 (including 5 animal and 6 macroalgal) taxa as those most contributing to the 'urban *vs.* reference' dissimilarity of assemblages. Among animals, mussels (*M. galloprovincialis*) and limpets of the genus *Patella* were relatively more abundant in the urban condition, while the honeycomb worm polychaete *Sabellaria alveolata* Linnaeus, snails of the genus *Gibbula* and barnacles (*Chthamalus stellatus* (Poli)) were more abundant in the reference condition. Among macroalgae, articulated corallines of the genus *Corallina* and the encrusting '*Petrocelis*' (the crustose sporophyte, previously known as *Petrocelis cruenta* J. Agardh, alternating with the foliose upright gametophyte of the red alga *Mastocarpus stellatus* (Stackhouse) Guiry; see Guiry and West 1983) were found with larger cover values under the urban

Table 1
ANOVA examining temporal and spatial differences in the variance of the structure of assemblages under the urban and the reference condition (ns = not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

Source of variation	df	MS	*F*	Denominator
Time = Ti	3	152653186.37	43.40***	Residual
Condition = Co	1	1234181.28	6.02	Ti × Co
Scale = Sc	3	6195934.70	13.74***	Ti × Sc
Ti × Co	3	205098.03	5.83**	Residual
Ti × Sc	9	450891.33	12.82***	Residual
Co × Sc	3	661731.76	1.95	Ti × Co × Sc
Ti × Co × Sc	9	339640.25	9.66***	Residual
Residual	64	35170.50		
Cochran's *C* test	$C = 0.125$, ns			
Transformation	None			

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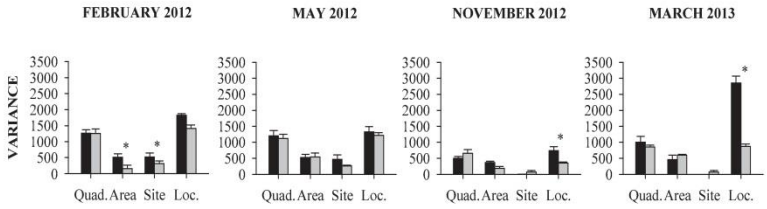


Fig. 2. Multivariate untransformed variance (mean + SE, $n = 3$) in the structure of assemblages from the urban (black) and the reference (extra-urban, grey) condition at four spatial scales and four dates of sampling. Stars above bars represent means significantly different (SNK tests; note: only comparisons within each spatial scale are appropriate; * = $p < 0.05$, ** = $p < 0.01$).

condition, while foliose green algae of the genus *Ulva* and the red seaweeds *M. stellatus*, *Osmundea pinnatifida* (Hudson) Stackhouse and *C. acicularis* were more abundant under the reference condition (Table 2). Each of these taxa, in addition to the total number of taxa, was individually analyzed.

Patterns of spatial variability dependent on the level of urbanization were inconsistent through time for most taxa found to be relatively more abundant in the urban (Table 3: '*Petrocelis*' and *M. galloprovincialis*; Table 4: *Patella* spp.) or in the reference (Table 4: *Ulva* spp. and *O. pinnatifida*; Table 5: *S. alveolata* and *Gibbula* spp.).

Within the first group, such differences were generally in the direction of a relatively larger variance under the urban condition. This was the case, in particular, for: '*Petrocelis*' at all scales sampled in February 2012 and at the smallest and largest scale sampled in March 2013, as well as at almost all other scales and dates of sampling, although not detected as significant by the analysis (Fig. 3C and SNK tests); for *M. galloprovincialis* at the scales of quadrates and areas in February 2012 and at the scale of locations in November 2012 (Fig. 4A and SNK tests; and for *Patella* spp. at the scales of quadrates and locations in February 2012, of areas in November 2012 and of quadrates and areas in March 2013 (Fig. 4B and SNK tests). The only exception was provided by *M. galloprovincialis* sampled in May 2012, when the variability of this species between areas and locations was larger under the extra-urban compared to the urban condition (Fig. 4A and SNK

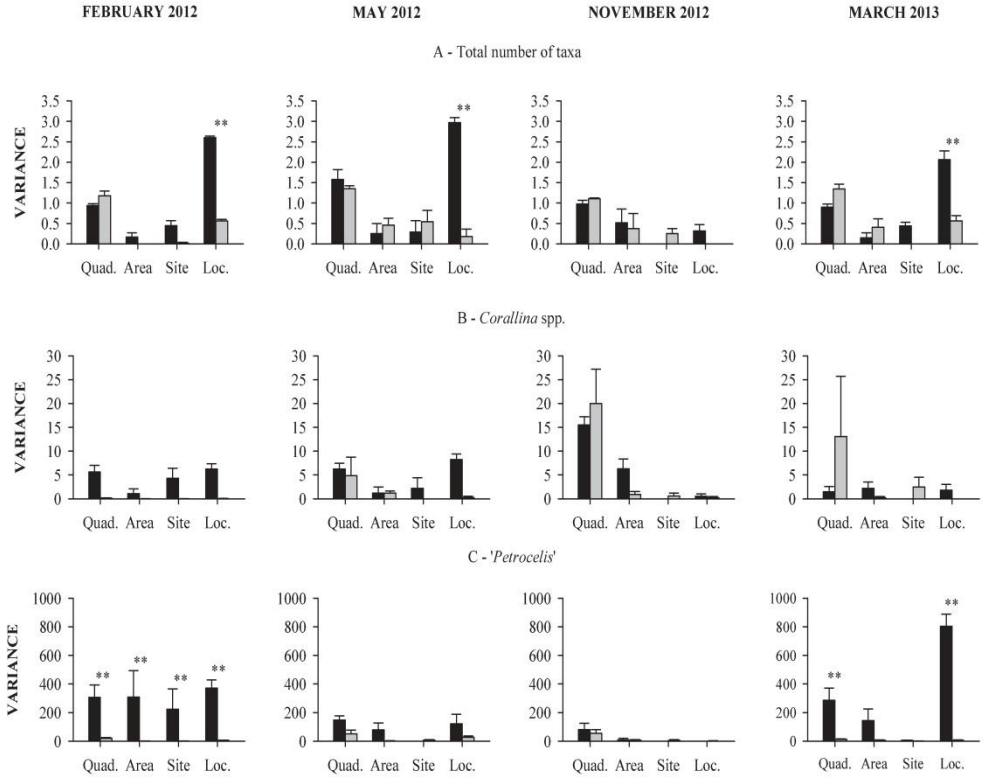


Fig. 3. Univariate measures (mean + SE, $n = 3$) of variance of the total number of taxa and of the abundance of individual taxa from the urban and the reference condition at four spatial scales and four dates of sampling. Symbols as in Fig. 1. SNK results illustrated only for panel C. Panel A is on a logarithmic scale, panels B and C are on an untransformed scale.

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Table 2
Contribution (δ_i) of individual taxa to the total Bray–Curtis dissimilarity of assemblages between the urban (U) and the extra-urban (reference: R) condition. Only taxa cumulatively contributing ≥70% are included.

Species	Average abundance			
	U	R	δ_i	δ_i %
S. alveolata	27.11	35.12	21.15	28.71
'*Petrocelis*'	12.93	2.68	8.71	11.82
Mytilus galloprovincialis	11.08	8.28	8.33	11.31
Ulva spp.	5.25	11.87	7.64	10.37
Mastocarpus stellatus	1.97	5.4	4.18	5.68
Gibbula spp.	4.29	6.24	4.15	5.63
Osmundea pinnatifida	1.35	6.09	3.85	5.23
Chondracanthus acicularis	2.14	5.42	3.74	5.07
Chthamalus stellatus	0.74	2.68	2.06	2.8
Patella spp.	2.63	1.26	1.93	2.63
Corallina spp.	2.00	1.11	1.58	2.14

tests). A significant spatial and temporal variation, independently of urbanization, was detected for the cover of *Corallina* spp. (Table 3). At both November 2012 and March 2013, this taxon was more variable among quadrates than at all the larger examined scales, while comparable patterns of variability at each scale were found at the other dates of sampling (Fig. 3B; SNK_{TixSc}: Loc = Quad = Site = Area in Feb 2012, Quad = Loc = Area = Site in May 2012; Quad > Area = Loc = Site in Nov 2012, Quad > Site = Area = Loc in Mar 2013).

Within the second group, the variability of *Ulva* spp. was larger in the extra-urban compared to the urban condition at the scales of quadrates and locations in February 2012. A similar, but non-significant, trend was displayed at the other scales at the same date of sampling, at the scale of locations in May 2012 and at all scales in March 2013. On the contrary, the variability of these algae

Table 3
ANOVA examining temporal and spatial differences in the variance of the richness of taxa and of the abundance of individual taxa under the urban and the reference condition (ns = not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

Source of variation	df	Taxa		*Corallina* spp.		'*Petrocelis*'		*M. galloprovincialis*	
		MS	*F*	MS	*F*	MS	*F*	MS	*F*
Time = Ti	3	1.05	13.03***	52.95	2.16	117198.84	12.88***	18524.63	12.61***
Condition = Co	1	3.66	7.41	33.29	0.60	676254.22	4.69	122.13	0.01
Scale = Sc	3	6.41	8.88**	272.21	3.46	85357.07	2.11	27373.04	3.74
Ti × Co	3	0.49	6.12***	55.71	2.28	144098.51	15.83***	10556.23	7.19***
Ti × Sc	9	0.72	8.96***	78.58	3.21**	40402.92	4.44***	7324.45	4.99***
Co × Sc	3	4.36	8.37***	42.62	1.89	71571.91	1.8	2177.39	0.49
Ti × Co × Sc	9	0.52	6.46**	22.59	0.92	39749.52	4.37***	4455.35	3.03***
Residual	64	0.09		24.49		9101.40		1468.56	
Cochran's *C* test		$C = 0.160$, ns		$C = 0.608$ ($p < 0.01$)		$C = 0.348$ ($p < 0.01$)		$C = 0.256$ ($p < 0.01$)	
Transformation		Ln ($x + 1$)		None		None		None	

Table 4
ANOVA examining temporal and spatial differences in the variance of the richness of taxa and of the abundance of individual taxa under the urban and the reference condition (ns = not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

Source of variation	df	*Patella* spp.		*Ulva* spp.		*M. stellatus*		*O. pinnatifida*	
		MS	*F*	MS	*F*	MS	*F*	MS	*F*
Time = Ti	3	0.95	4.12**	41198.22	29.15***	195.76	27.68***	8948.50	15.44***
Condition = Co	1	36.20	4.26	13306.90	1.14	271.49	2.91	33775.03	3.57
Scale = Sc	3	80.25	10.11**	17070.22	1.85	251.15	8.51**	2475.96	1.18
Ti × Co	3	0.85	3.69*	11720.09	8.29***	93.43	13.21***	9461.62	16.33**
Ti × Sc	9	0.79	3.45**	9251.23	6.55***	29.51	4.17***	2095.77	3.62***
Co × Sc	3	0.83	1.44	9514.31	1.99	0.70	0.69	1703.87	0.75
Ti × Co × Sc	9	0.57	2.49*	4783.06	3.38**	10.11	1.43	2271.80	3.92***
Residual	64	0.23		1413.28		0.71		579.45	
Cochran's *C* test		$C = 0.111$, ns		$C = 0.223$ ($p < 0.05$)		$C = 0.133$, ns		$C = 0.351$ ($p < 0.01$)	
Transformation		Ln ($x + 1$)		None		Ln ($x + 1$)		None	

Table 5
ANOVA examining temporal and spatial differences in the variance of the richness of taxa and of the abundance of individual taxa under the urban and the reference condition (ns = not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

Source of variation	df	*C. acicularis*		*S. alveolata*		*C. stellatus*		*Gibbula* spp.	
		MS	*F*	MS	*F*	MS	*F*	MS	*F*
Time = Ti	3	58.27	58.65***	9.06	4.16**	12.08	19.34***	2.53	4.07*
Condition = Co	1	55.56	4.64	0.56	0.05	21.64	2.23	1.16	0.45
Scale = Sc	3	11.53	13.43**	39.86	12.4**	6.51	12.09**	18.67	8.39**
Ti × Co	3	11.98	12.06***	11.28	5.17**	9.70	15.53***	2.57	4.13**
Ti × Sc	9	0.86	0.86	3.22	1.47	0.54	0.86	2.22	3.58**
Co × Sc	3	5.85	4.25*	13.21	2.54	0.72	5.38*	2.67	1.03
Ti × Co × Sc	9	1.38	1.39	5.19	2.38*	0.13	0.22	2.59	4.17***
Residual	64	0.99		2.18		0.62		0.62	
Cochran's *C* test		$C = 0.194$, ns		$C = 0.165$, ns		$C = 0.151$, ns		$C = 0.137$, ns	
Transformation		Ln ($x + 1$)		Ln ($x + 1$)		Ln ($x + 1$)		Ln ($x + 1$)	

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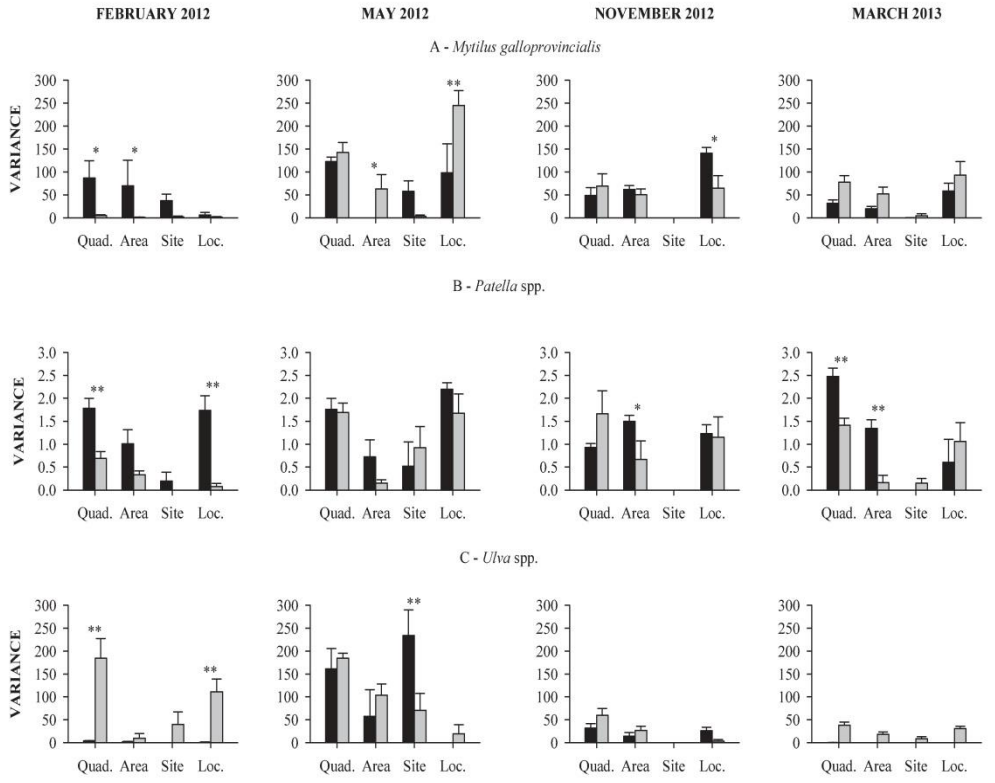


Fig. 4. Univariate measures (mean + SE, $n = 3$) of variance of the abundance of individual taxa from the urban and the reference condition at four spatial scales and four dates of sampling. Symbols as in Fig. 1. Panels A and C are on an untransformed scale, panel B is on a logarithmic scale.

was larger at the urban than at the extra-urban sites sampled in May 2012 (Fig. 4C and SNK tests).

Significant patterns of variability of *O. pinnatifida* were always in the direction of a more heterogeneous distribution under the extra-urban than under the urban condition, such as, in particular, at the scale of quadrates in February 2012, May 2012 and March 2013, at the scale of sites in May 2012 and at all scales, but sites, in March 2013 (Fig. 5B and SNK tests).

The direction of urban vs. extra-urban differences in the variability of both *S. alveolata* and *Gibbula* spp. changed with the date of sampling. The sedentary polychaete was more heterogeneously distributed under the urban compared to the reference condition at both the scales of areas and locations in February 2012, while the opposite pattern was observed at the scale of sites in May and November 2012 and at the scale of areas in March 2013 (Fig. 6A and SNK tests). The variability of snails was relatively larger under the urban condition at the scale of locations in February 2012 and at the scale of areas in May 2012 and March 2013, under the extra-urban condition at the scale of locations in November 2012 (Fig. 6 C and SNK tests).

Effects of urbanization were found for all other analyzed taxa, i.e. *M. stellatus*, *C. acicularis* and *C. stellatus*, but never simultaneously variable over time and spatial scales. The 'Time × Condition' interaction was significant in each case, while the 'Condition × Scale' interaction for *C. acicularis* and *C. stellatus* (Tables 4 and 5). Specifically, independently of the spatial scale, at each date of sampling when significant differences were found, the variance of all these three species was relatively larger under the extra-urban condition (Fig. 5A and C and Fig. 6B; SNK_{TixCo}: reference > urban in February 2012 (*M. stellatus* and *C. acicularis*), May 2012 (all species) and March 2013 (*C. acicularis* and *C. stellatus*)). Similarly, the extra-urban condition was associated, consistently over time, to a larger variability of both *C. acicularis* (Fig. 5C; SNK_{CoxSc}: reference > urban for quadrates, sites and locations) and *C. stellatus* (Fig. 6B; SNK_{CoxSc}: reference > urban for quadrates, areas, sites and locations) at all spatial scales, with the only exception of the 'between areas' one for *C. acicularis*.

Finally, temporally variable, but consistent between conditions, patterns of distribution of *M. stellatus* at different scales were detected (Table 4 and Fig. 5A; SNK_{TixSc}: Quad = Loc > Site = Area in Feb 2012, Loc = Quad = Area > Site in May 2012, Quad > Area = Site = Loc in Nov 2012, Loc > Area = Site and Quad > Site, with the other means inconsistently ranked, in Mar 2013).

4. Discussion

It was expected that urbanization could generally modify temporal and spatial patterns of distribution and abundance of whole assemblages and individual taxa. Unfortunately, the most probable direction for this effect could not be hypothesized in detail *a priori* as no reference studies were available for the study areas and the

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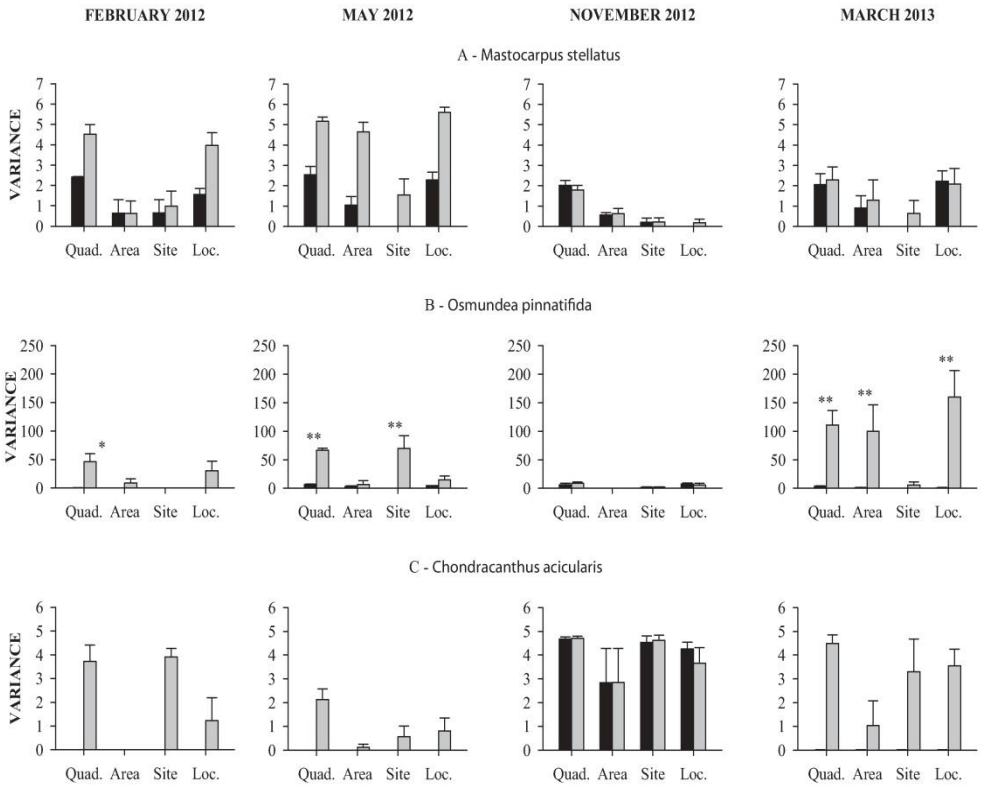


Fig. 5. Univariate measures (mean + SE, $n = 3$) of variance of the total number of taxa and of the abundance of individual taxa from the urban and the reference condition at four spatial scales and four dates of sampling. Symbols as in Fig. 1. SNK results illustrated only for panel B. Panels A and C are on a logarithmic scale, panel B is on an untransformed scale.

existing literature includes evidence, depending on the case, either for urbanization homogenizing biodiversity (Olden and Rooney, 2006; Bahartan et al. 2010; Martins et al. 2012) or in the opposite direction (Sousa, 2001; Hewitt and Thrush, 2009; Hewitt et al. 2010).

Present findings indicated that, in the examined systems, assemblages, as a whole, were more heterogeneously distributed in the urban than in the extra-urban condition. The total number of taxa and some individual taxa displayed, in general, this same pattern of variability, although, in some cases, inconsistent through time or dependent on the spatial scale. These results are in agreement with several recent papers showing a drastic influence of urbanization on coastal biodiversity (Bevilacqua et al. 2012; Guarnieri et al. 2012; Martins et al. 2012). Although the distinction between urbanized and "pristine" environments can sometimes represent an oversimplification of the complex patterns produced by urbanization (McKinney, 2008), the patterns of variability here documented were clearly associated to the proximity to the second largest Portuguese city. As it could be somehow expected, most taxa showed a significant larger variability under the condition where they were more abundant, namely '*Petrocelis*', *M. galloprovincialis* and *Patella* spp. in the urban and *Ulva* spp., *O. pinnatifida*, *S. alveolata* and *Gibbula* spp. in the extra-urban condition. A similar, though non-significant, trend was displayed by several other taxa. These included *Corallina* spp., with larger abundance and variance under the urban condition, and *M. stellatus*, *C. acicularis* and *C. stellatus*, with larger heterogeneity and percentage cover values under the extra-urban condition. Such general response could be related to the known scaling relationship between the mean and the variance, as lower average abundances can hinder large fluctuations (Taylor, 1961). Nevertheless, several processes acting at different spatial and temporal scales can be invoked when debating urbanization's influence over marine assemblages. Local factors, such as water turbidity, hydrodynamics (Balata et al. 2008), chemical pollution from sewage or industrial effluents (McIntyre, 1995; Koop and Hutchins, 1996), inputs of suspended particulate material (Clarke and Kirkman, 1989; Giensen et al. 1990; Balestri et al. 2004) and dissolved nutrients (Carpenter et al. 1998), acting independently or in complex interactions with each other and/or with other natural and anthropogenic disturbances (Short and Wyllie-Echeverria, 1996; Short and Neckles, 1999), can drastically differ and drive large modifications of patterns of abundance and distribution of marine benthic organisms between urban and "pristine" areas. For example, storm disturbance, although potentially occurring in analogous ways both in urbanized and non-urbanized environments, can cause varying effects in coastal areas when coupled with anthropogenic pressures (e.g. Oliveira et al. 2014).

Identifying and testing the actual sources of pressure over local assemblages was beyond the goals of this study. However, the adopted sampling design was able to detect differences between

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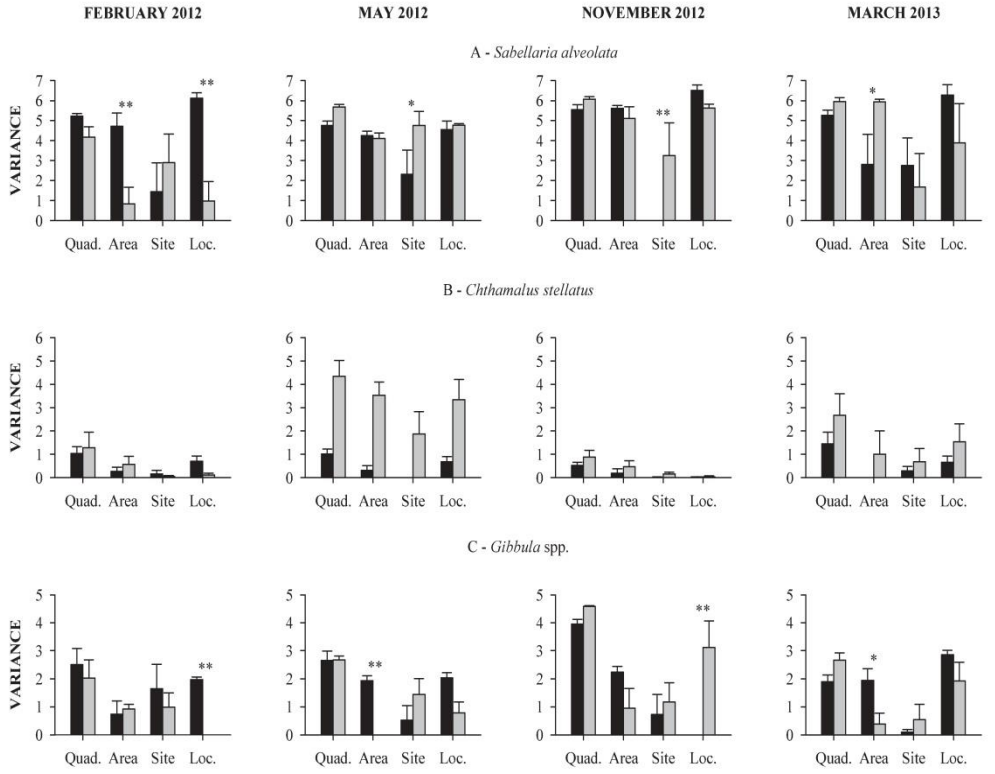


Fig. 6. Univariate measures (mean + SE, $n = 3$) of variance of the total number of taxa and of the abundance of individual taxa from the urban and the reference condition at four spatial scales and four dates of sampling. Symbols as in Fig. 1. SNK results illustrated only for panels A and C. All panels are on a logarithmic scale.

locations close to large urban areas and those located in much less to non-urbanized areas. Large reductions of both the overall diversity and the variance of local assemblages were typically documented in response to very high levels of urbanization-related stress (McKinney, 2008 and references therein; Bevilacqua et al. 2012). On the contrary, under relatively low levels of anthropogenic disturbance, variance and, in some cases, diversity, can be larger under 'pristine' conditions (Warwick and Clarke, 1993; Balestri et al. 2004; Fraschetti et al. 2005; Guarnieri et al. 2012). Several, not mutually exclusive, hypotheses can account for an increased variability under low to medium intensity disturbances, including the presence of mosaics of patches in different successional stages (Hewitt et al. 2010), increased local productivity (Shochat et al. 2006) or the addition of non-native species replacing local organisms faster than they are lost (McKinney, 2002, 2006). Although, such as in the present situation, these mechanisms could be responsible for effects of urbanization in terms of increases of biodiversity and assemblages might resist to or recover from disturbances at rates large enough to prevent persistent impacts (Lindegarth and Hoskins, 2001), these responses could represent the first stages of the degradation of local systems, eventually followed by the onset of processes of homogenization through a reduction of beta diversity (Underwood and Chapman, 1996; Glasby and Underwood, 1996; Lardicci et al. 1999; Balata et al. 2007a).

Presently documented patterns of variability were not consistent across all the analyzed spatial scales, with the smallest and largest ones (i.e. Quadrate and Location, respectively) generally showing larger variability than the intermediate ones (i.e. Areas and Sites). The repeated evidence of relatively larger heterogeneity at small spatial scales led to an active debate on the underlying factors (Fraschetti et al. 2005). Most views agree that small-scale processes such as biotic interactions, physical habitat heterogeneity and local variations in patterns of settlement and recruitment can produce a patchy distribution of benthic marine organisms (e.g. Underwood, 1985; Bourget et al. 1994; Menconi et al. 1999). In fact, this was also eventually considered as a general property of benthic assemblages in marine coastal habitats (Fraschetti et al. 2005). Of course, several other processes could explain the larger scale (10s of Km, such as between the sampled locations) variability, including patterns of circulation of water, the availability of food, the type of substratum (Raimondi, 1988, 1990; Bertness et al. 1991; Sanford et al. 1994), patterns of dispersal of larvae and propagules (Dayton, 1973; Paine, 1979; Chapman, 1986; Reed et al. 1988; Santelices, 1990; Arrontes, 1993) and differences in physical factors that may affect the rates of recruitment, growth and mortality of macroalgae at that scale (Santelices, 1990).

Although the responsible mechanisms, including their possible complex interactions, are yet to be elucidated, this study represents a relevant contribution to document urbanization effects on both whole assemblages and individual taxa in a system and a geographical area where such issues were not addressed before. According to present findings, it could be hypothesized that, in spite of the large extent of urban areas and the local presence of

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important commercial and industrial plants in North Portugal, their actual effects on rocky intertidal assemblages are still low to moderate and, thus, likely to produce levels of environmental pressure sustainable by local organisms. At the same time, however, the demonstrated increase in variability could also be interpreted as the beginning of a habitat deterioration process with largely unpredictable consequences. Through a hierarchical sampling design, this study supports the notion that several spatial scales must be simultaneously taken into account when evaluating responses of coastal marine biodiversity to anthropogenic disturbances.

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IV – Patterns of recovery of intertidal organisms after compounded anthropogenic disturbances

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Patterns of recovery of intertidal organisms after compounded anthropogenic disturbances

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ABSTRACT: Coastal habitats are exposed to increasing human and natural disturbances, including extraction of organisms and extreme climatic events. Patterns of recovery (i.e. convergence towards an unmanipulated control) of the structure of benthic assemblages, the total number, and the abundance of individual taxa were examined over a period of 15 mo after the end of a previous experiment. In that experiment, crossed manipulations of levels of mussel harvesting and of the temporal patterns of storm-related mechanical disturbance (in terms of changes in variance but not in the overall intensity) were performed on north Portugal rocky shores. Effects of past disturbances were mostly detected at 3 and 9 mo after the end of disturbances, while no significant differences between unmanipulated and treated assemblages were present after 15 mo. These findings confirm that intertidal assemblages subjected to even extreme combinations of past disturbances can recover in a relatively short time. Important effects present after 3 to 9 mo were associated with the timing of disturbance, likely depending on interactions with life-history traits such as peaks in reproduction and recruitment. Contrary to theories considering disturbances as opportunities for some organisms to replace competitors that dominate space in undisturbed conditions, we observed changes in the abundances of the same taxa rather than their replacement. The present evidence contributes to the understanding of how multiple anthropogenic pressures affect the ability of intertidal assemblages to recover after disturbance.

KEY WORDS: Climate change · Human impact · Multiple disturbances · Foundation species · Intertidal · Recovery patterns · Abundance · Temporal variance

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INTRODUCTION

Marine systems are globally exposed to considerable and increasing anthropogenic and natural perturbations operating over a range of scales (Sala et al. 2000, Halpern et al. 2008). Coastal areas, besides being exposed to anthropogenic activities that increase inputs of contaminants and nutrients (Vitousek et al. 1997), extract natural habitats (Gray 1997, Airoldi & Beck 2007) and promote biological invasions (e.g. Williams & Smith 2007), have been used throughout human history as sources of food (Siegfried et al. 1994, Griffiths & Branch 1997, Castilla 1999). Populations of different groups of animals are intensely collected all over the world for human consumption, animal feeding or baiting (Keough et al. 1993, Underwood 1993, Addessi 1994, Crowe et al. 2000). These activities, which normally exert their direct effects on a local scale, occur simultaneously and potentially interact with events occurring on larger scales, such as those related to climate change. In fact, empirical observations and models have

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shown and predicted drastic alterations of the regime of meteorological variables (including intensity, timing, frequency and variance) for the near future (Easterling et al. 2000, Muller & Stone 2001, Meehl et al. 2007, Wong et al. 2014).

Both natural and anthropogenic disturbances, in general, are key forces structuring natural assemblages, as they can modify patterns of species distribution, abundance and diversity through complex interactions with life traits of organisms (Sousa 1984, Connell et al. 1997). On rocky shores, in particular, disturbances represent important mechanisms for releasing resources (i.e. freeing space first, but also light and nutrients) and maintaining heterogeneous assemblages by eliminating the most sensitive and competitively dominant species (Dayton 1971, Connell 1978, Lubchenco & Menge 1978, Paine & Levin 1981, Sousa 1984). Separate and interactive effects of the intensity and timing of disturbance and of the life traits of organisms can drastically affect not just the structure of intertidal assemblages (Keough 1984, Breitburg 1985, Reed 1990, Airoldi 2000, Benedetti-Cecchi 2000, Bertocci et al. 2005), but also their functional characteristics, including their ability to recover once the disturbance is over (Underwood et al. 1983, Blanchette 1996, Bevilacqua et al. 2006, Oliveira et al. 2011). This last property is strictly related, although not necessarily scaled linearly (e.g. Cervin et al. 2005), with the intensity and timing of disturbance (Speidel et al. 2001, Carr et al. 2003) and interacts with other abiotic and biological processes that are variable in space and time. For instance, the availability of propagules (e.g. Hoffmann & Ugarte 1985) and the presence of physical stress (e.g. Bell 1993) can drastically affect the way different species use resources made available by disturbance (Connell & Keough 1985, Watling & Norse 1998) and, therefore, how they replace each other during the subsequent recovery (O'Connor & Anderson 2010, Maggi et al. 2011). Most previous studies have examined the effects of changes in multiple attributes of single disturbances, such as scouring (e.g. van Tamelen 1996, Vaselli et al. 2008), temperature extremes (e.g. Menge & Sutherland 1987) and storms (e.g. Alvarado et al. 2001), on patterns of convergence of disturbed assemblages towards those from reference areas (Underwood 1989). There is a general lack of empirical studies specifically designed to examine the effects of attributes of compounded perturbations (e.g. Paine et al. 1998) on the recovery process (but see Tamburello et al. 2014), particularly in terms of temporal trajectories (the shapes of the curves describing fluctuations in the abundance of individual taxa or in the structure of whole assemblages over the examined period (e.g. Bevilacqua et al. 2006) of recovery. Such manipulative experiments are needed since the wide range of multiple disturbances natural assemblages are currently exposed to, and their often unpredictable nature, make it almost impossible to extrapolate general patterns of recovery of populations and assemblages. Realistic manipulations of multiple anthropogenic stressors are essential in understanding and, possibly predicting, the causes of temporal variations in assemblages, which can affect ecologically relevant properties such as productivity (Johnson et al. 1996, Grime et al. 2000), stability and extinction risk (Micheli et al. 1999, Lundberg et al. 2000, Inchausti & Halley 2003).

A previous manipulative experiment performed at multiple rocky shores in north Portugal was aimed at examining the effects of local harvesting of the habitat-forming mussel *Mytilus galloprovincialis* Lamarck, and of storm-related mechanical disturbance varying in temporal variance, on benthic assemblages associated with mussel beds (Oliveira et al. 2014). That experiment involved crossed manipulations of 4 levels of mussel harvesting with 2 levels of temporal patterning (evenly vs. irregularly distributed events, once the total intensity of disturbance was maintained over the period of the experiment) of storm-like mechanical disturbance. These crossed manipulations aimed to assess how an intact or increasingly damaged mussel bed could protect associated organisms from the impact of extreme storms, whose temporal patterns of occurrence would vary due to climate change. In the present study, the same experimental sites and units of the previous manipulative experiment were tracked over 15 mo after the end of all manipulations, in order to follow patterns of recovery (i.e. the degree of convergence towards the unmanipulated control) of assemblages and individual taxa, and to assess how these patterns were affected by the different combinations of past disturbances. Specifically, the main finding from previous manipulations was that mussels could effectively buffer the effects of mechanical disturbance, particularly when the mussel bed was left intact and disturbance was relatively less stressful (e.g. under the regular treatment, compared to that involving multiple events aggregated over short periods). From this finding, it could be hypothesized that assemblages from patches that were previously allocated to the experimental combination of an intact mussel bed and regularly distributed events of mechanical disturbance, would be characterized by the greatest similarity and fastest convergence towards unmanip-

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ulated assemblages. At the other extreme, manipulated patches, where mussels were completely removed in combination with irregular disturbance events, would host the most different assemblages compared to the unmanipulated patches and would take the longest time to convergence to the unmanipulated patches. Intermediate outcomes would be expected for the other combinations of past experimental disturbances. Such hypotheses were tested in the present study; response variables included both average responses measured at the beginning (3 mo of recovery), in the middle (9 mo) and at the end (15 mo) of the examined period, and temporal trajectories (quantified as variances calculated over 5 sampling periods) of the structure of whole assemblages, the total richness of taxa and the abundance of individual taxa.

MATERIALS AND METHODS

Collection of data

Data were collected between October 2012 and October 2013 at the same study sites (Aguda and Marreco: 41° 2.71′ N, 8° 39.20′ W and 41° 14.12′ N, 8° 43.45′ W, respectively) and on the same units of a previous manipulative experiment which ended in July 2012. The study system and past experimental procedures are illustrated in detail in Oliveira et al. (2014). In summary, the intensity of mussel harvesting and the temporal variance of storm-related mechanical disturbances were manipulated according to a partially asymmetrical design. This design crossed 4 levels of harvesting ('0', '30', '60' and '100', corresponding to no harvest, mussels reduced to 70% cover, mussels reduced to 40% cover, and mussels completely removed, respectively) with 1 regular and 2 irregular temporal patterns of mechanical disturbance produced with a hand rake. The regular treatment involved a total of 6 events evenly distributed over a period of 18 mo, while the irregular patterns involved the same 6 events clustered in shorter periods, separated by prolonged periods without disturbance. Two different sequences of events were replicated in the irregular treatment, but not in the regular one (Fig. 1). Three independent plots (35 × 35 cm) were allocated to each combination of these factors and 3 additional plots were left unmanipulated as control.

In the present study, the same 39 plots at each site were sampled during 5 periods (October 2012, January, April, June and October 2013) corresponding to 3, 6, 9, 12 and 15 mo after the end of experimental manipulations. At each sampling period, the percentage cover of sessile taxa and the number of individuals of mobile animals were visually estimated using a 30 × 30 cm frame placed in the centre of each plot following the same procedures described in Oliveira et al. (2014).

Data analyses

A total of 27 taxa (including 15 macroalgae and 12 animals) were identified throughout the study. Multivariate and univariate statistical techniques were used to compare patterns of recovery of the structure of whole assemblages, the richness of taxa and the abundance of the most common individual taxa between plots allocated to different combinations of past disturbances. Response variables were exam-

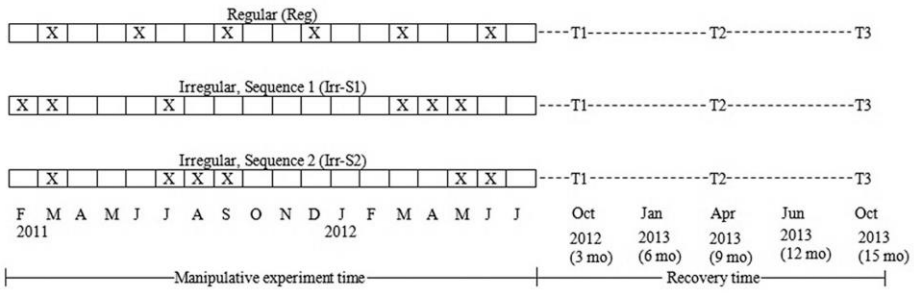


Fig. 1. Design of the temporal arrangements of events (X) of mechanical disturbance (crossed with 4 levels of intensity of mussel harvesting in the full experiment, as described in detail in Oliveira et al. 2014) of the previous manipulative experiment and sampling design of the present recovery study. The recovery study included 5 sampling times, out of which 3 (T1, T2 and T3) were statistically analysed

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ined both in terms of average responses at the beginning (October 2012), in the middle (April 2013) and at the end (October 2013) of the examined period, and of temporal variances calculated over the 5 sampling periods (see Fig. 2). All analyses were based on the same model that included a first partitioning of the total variability into the Control vs. Treatments contrast and the Among treatments variation. The second term was then partitioned into the main effects of harvesting intensity (I), mechanical disturbance variability (V), and their interaction, with each term involving temporal variability further partitioned into a Regular vs. Irregular contrast and Between sequences (S) variation (see Oliveira et al. 2014 for details and Table 1 for an illustration of all tested sources of variability).

Permutational multivariate analysis of variance (PERMANOVA, Anderson 2001) based on Bray-Curtis untransformed dissimilarities was used to examine differences in the structure of whole assemblages at each of the 3 recovery periods reported above. When the low number of permutations prevented enough powerful tests, a p-value was calculated using 999 Monte Carlo draws from the appropriate asymptotic permutation distribution (Anderson & Robinson 2003). When relevant, Student's t tests were used for post-hoc comparisons.

Differences in temporal trajectories of whole assemblages were tested by calculating measures of multivariate variance over the 5 sampling periods following an approach for partitioning variation among factors that was successfully extended from univariate to multivariate data (Terlizzi et al. 2007). Estimates of temporal variance of the structure of assemblages were obtained by performing a 1-way PERMANOVA separately for each plot, with time as a factor. Multivariate pseudo-variance components were then calculated analogously to univariate ANOVA estimators, i.e. by equating observed mean squares and mean squares expected from the linear model of the analysis (Searle et al. 1992, Underwood, 1997, Terlizzi et al. 2007). Negative estimates of pseudo-variance were set to zero, being interpreted as sample underestimates of very small or null variances (Searle et al. 1992, Underwood 1996). This procedure generated 3 replicate measures (one for each plot) of pseudo-variance components for each combination of past experimental treatments, including the unmanipulated control, that were finally analyzed with ANOVA according to the model illustrated in Table 1.

Analogous procedures based on ANOVA were used to test for differences in mean values and temporal variances of the richness of taxa and of the abundance of individual taxa. Before each ANOVA, the assumption of homogeneity of variances was checked with Cochran's C test and data were $\log(x+1)$ transformed when necessary. When the transformation could not make variances homogeneous, untransformed data were analyzed and results considered robust if not significant (at $p > 0.05$) or, considered significant at $p < 0.01$ to compensate for the increased probability of Type I error (Underwood 1997). When relevant, Student-Newman-Keuls (SNK) tests were used for post-hoc comparisons of means.

Differences in the structure of whole assemblages were visualized by calculating centroids of assemblages according to results of the analyses for October 2012, April 2013 and October 2013 and those for multivariate temporal variance. For this goal, principal coordinates were calculated from the Bray-Curtis dissimilarity matrix of original data. Centroids were obtained by averaging principal coordinates according to each relevant experimental condition and sampling period (McArdle & Anderson 2001). Finally, non-metric multidimensional scaling (nMDS) ordination plots based on Euclidean distances were displayed.

Multivariate analyses were carried out using the PRIMER 6.0 and PERMANOVA package (Anderson et al. 2008). Univariate analyses were carried out using GMAV-5 for Windows (Underwood et al. 2002).

RESULTS

Whole assemblage

Three months after the end of the disturbance experiment, assemblages differed between the unmanipulated plots and, on average, those allocated to manipulations in both Aguda (Table 1A, Fig. 2) and Marreco (Table 1B, Fig. 2). In addition, the significant I × S interaction observed only in Aguda indicated an existing effect of the intensity of harvesting that was not consistent between the 2 sequences of irregular disturbance (Table 1A, Fig. 2). Assemblages from unmanipulated plots, in particular, differed from those in plots subjected to the lowest level of mussel removal (treatment '30') combined with sequence 1 (Student's $t = 2.241$, $p < 0.05$) of irregular disturbance events, and those in plots completely deprived of mussels (treatment '100') combined with sequence 2 (Student's $t = 3.590$, $p < 0.01$) (Fig. 2). Nine months after the end of the experiment, control and manipulated plots still differed only in Marreco, while the

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Table 1. PERMANOVA on the structure of assemblages sampled at 3, 9 and 15 mo after the end of the disturbance experiment in (A) Aguda and (B) Marreco. Significant effects in **bold**

Source of variation	df	3 mo (Oct 2012) MS	pseudo-*F*	p	9 mo (Apr 2013) MS	pseudo-*F*	p	15 mo (Oct 2013) MS	pseudo-*F*	p	Permutable units and denominator
(A) Aguda											
Among experimental levels	12	0.12	2.24	**0.007**	0.09	1.75	0.068	0.05	0.75	0.810	39 replicate plots
Control vs. treatments	1	0.14	2.67	**0.048**	0.12	2.34	0.091	0.05	0.70	0.526	39 replicate plots
Among treatments	11	0.12	2.20	**0.007**	0.09	1.69	0.082	0.05	0.75	0.794	39 replicate plots
Temporal variability = V	2	0.12	2.27	**0.047**	0.11	2.17	0.087	0.01	0.16	0.981	39 replicate plots
Regular vs. Irregular	1	0.19	4.35	0.128 MC	0.21	13.02	**0.044** MC	0.01	1.46	0.423 MC	3 among seq. cells[a]
Between sequences = S	1	0.04	0.85	0.464	0.02	0.31	0.751	0.01	0.02	0.975	39 replicate plots
Harvesting intensity = I	4	0.12	2.35	**0.043**	0.13	2.49	**0.048**	0.09	1.31	0.222	39 replicate plots
I × V	6	0.11	2.09	**0.028**	0.06	1.13	0.355	0.05	0.67	0.802	39 replicate plots
I × Regular vs. Irregular	3	0.08	0.60	0.750 MC	0.06	0.98	0.398 MC	0.05	0.94	0.507 MC	9 I × S cells[b]
I × S	3	0.14	2.62	**0.021**	0.06	1.14	0.379	0.05	0.69	0.722	39 replicate plots
Residual	26	0.05			0.05			0.07			
(B) Marreco											
Among experimental levels	12	0.13	1.51	**0.033**	0.12	1.18	0.216	0.10	1.27	0.291	39 replicate plots
Control vs. treatments	1	0.47	5.27	**0.002**	0.50	4.71	**0.002**	0.07	0.91	0.349	39 replicate plots
Among treatments	11	0.10	1.17	0.231	0.09	0.86	0.712	0.10	1.31	0.276	39 replicate plots
Temporal variability = V	2	0.07	0.80	0.629	0.04	0.39	0.955	0.11	1.50	0.154	39 replicate plots
Regular vs. Irregular	1	0.10	2.42	0.183 MC	0.08	14.76	**0.011** MC	0.10	0.79	0.609 MC	3 among seq. cells[a]
Between sequences = S	1	0.04	0.47	0.816	0.01	0.05	0.992	0.13	1.68	0.162	39 replicate plots
Harvesting intensity = I	4	0.15	1.70	0.061	0.13	1.19	0.270	0.11	1.52	0.114	39 replicate plots
I × V	6	0.09	1.03	0.431	0.09	0.85	0.661	0.09	1.14	0.370	39 replicate plots
I × Regular vs. Irregular	3	0.07	0.67	0.762 MC	0.09	0.98	0.503 MC	0.04	0.29	0.778 MC	9 I × S cells[b]
I × S	3	0.11	1.23	0.245	0.09	0.86	0.608	0.13	1.77	0.052	39 replicate plots
Residual	26	0.09			0.11			0.08			

[a]One regular and 2 irregular sequences were used as permutable units, but only irregular sequences were used for calculating denominator MS

[b]One regular and 8 intensity × sequence cells were used as permutable units, but only intensity × sequence cells were used for calculating denominator MS

[MC]p-values calculated using the Monte Carlo method

temporal variability of past disturbance was associated with different assemblages at both study sites, independent of harvesting intensity (Table 1). A main effect of harvesting intensity was instead detected in Aguda; this was driven by the difference between unmanipulated assemblages and those from plots where the mussel bed had been fully removed (Table 1A, Fig. 2; Student's $t = 2.948$, $p < 0.05$). No main or interactive effects of past experimental disturbances were detected at 15 mo of recovery time at both sites (Table 1, Fig. 2).

In both Aguda and Marreco, no significant effects of past disturbances were documented on the multivariate temporal variance of the structure of assemblages over the course of the recovery study; however, the relatively high *F*-value obtained for the contrast Regular vs. Irregular could suggest a relevant effect of the temporal patterning of past disturbance, which was probably not detected as significant due to the low power of the test that involved only one degree of freedom at both the numerator and the denominator (Table 2). Nevertheless, MDS plots showed a large degree of overlap between the temporal trajectories of assemblages that were previously subjected to regular and irregular events of disturbance; these temporal trajectories generally tended to also run parallel to that of control assemblages (Fig. 3).

Individual response variables

Three months after the end of the manipulative experiment, the total number of taxa in Aguda differed between plots previously subjected to regular events of disturbance and those where disturbance was applied irregularly over time (Table 3A). The irregular treatment, in particular, was associated with a higher richness of taxa compared to the regular treatment. However, the absolute difference (although proportionally important) was just by a single taxon (i.e. on average, ~5 taxa in the Regular and

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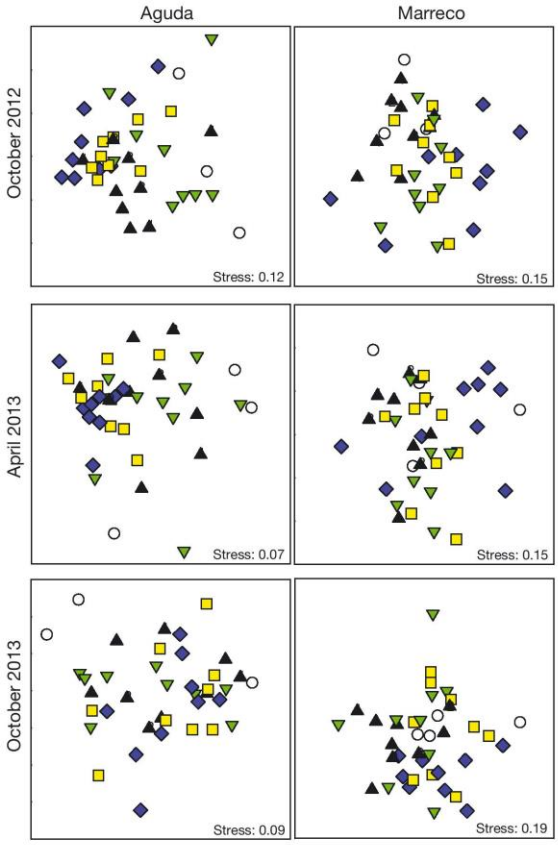


Fig. 2. nMDS plots comparing assemblages from the 2 study sites (Aguda and Marreco) at 3, 9 and 15 mo (October 2012, April 2013 and October 2013, respectively) after the end of experimental manipulations. Each symbol represents the centroid of assemblages calculated over replicate unmanipulated plots (○) and plots assigned to each past combination of intensity of mussel harvesting (black: '0' % removal; green: '30' % removal; yellow: '60' % removal; blue: '100' % removal; Oliveira et al. 2014) at different temporal variabilities of mechanical disturbance (□: Regular treatment; △: Irregular treatment, sequence 1; ▽: Irregular treatment, sequence 2)

6 taxa in the Irregular treatment; Fig. 4A). At the same site, effects of the intensity of mussel harvesting were found in April 2013 (9 mo of recovery), although effects were variable depending on the irregular sequence of mechanical disturbance. The '30' level of mussel removal combined with sequence 1 resulted in a higher number of taxa than any other intensity, while no significant differences were caused by the intensity of harvesting when combined with sequence 2 of events of disturbance (Table 3A, Fig. 4A; SNK test). Differences in the average total number of taxa were not yet evident in Aguda in October 2013 (Table 3A, Fig. 4A), and were never detected in Marreco (Table 3B, Fig. 5A). However, variations in the total number of taxa over the whole examined period of recovery drove significant differences in the temporal variance of this variable, with patterns being inconsistent between sites. In Aguda, temporal fluctuations in the richness of taxa were larger in unmanipulated than in treated plots on average (Table 4, Fig. 4A). In Marreco, regularly distributed past events of disturbance were associated with a larger temporal variance of the total number of taxa of recovering assemblages compared to irregular events (Table 4, Fig. 5A).

Significant differences in the mean abundance of the target species (*Mytilus galloprovincialis*) of the past experimental harvesting were detected at each of the 3 analyzed sampling periods. In October 2012, the cover of mussels was higher in control than in treated plots and was affected by the intensity of harvesting in both Aguda (Table 3A) and Marreco (Table 3B). In Aguda, mussels were more abundant in plots that were initially subjected to the lower level of harvesting ('30' treatment) than in plots allocated to the complete removal, with the unmanipulated control and the '60' treatment being inconsistently ranked between the '30' and '100' levels (Fig. 4B; SNK test). In Marreco, the cover of mussels in control plots was almost double that in plots assigned to both intermediate levels of harvesting, and was further reduced to approx. one-fourth in complete removal plots (Fig. 5B; SNK test). In April 2013, the cover of *M. galloprovincialis* was affected by the intensity of harvesting, with patterns being similar in both Aguda and Marreco; values progressively decreased from unmanipulated plots to those allocated to both intermediate harvesting levels to those where mussels had been completely removed (Table 3,

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Table 2. ANOVA on multivariate temporal variance of the structure of assemblages over the recovery period at each study site.

Source of variation	df	Aguda			Marreco			Denominator for *F*
		MS	*F*	p	MS	*F*	p	
Among experimental levels	12	0.075	0.98	>0.4	0.255	1.38	>0.2	Residual
Control vs. treatments	1	0.009	0.12	>0.7	0.002	0.01	>0.9	Residual
Among treatments	11	0.080	1.06	>0.4	0.278	1.51	>0.2	Residual
Temporal variability = V	2	0.040	0.53	>0.5	0.186	1.01	>0.3	Residual
Regular vs. Irregular	1	0.078	41.21	>0.05	0.352	16.83	>0.1	Among sequences
Between sequences = S	1	0.002	0.03	>0.8	0.021	0.11	>0.7	Residual
Harvesting intensity = I	4	0.065	0.86	>0.4	0.220	1.19	>0.3	Residual
I × V	6	0.101	1.34	>0.2	0.338	1.83	>0.1	Residual
I × Regular vs. Irregular	3	0.081	0.67	>0.6	0.599	3.25	>0.1	I × S
I × S	3	0.121	1.60	>0.2	0.077	0.42	>0.7	Residual
Residual	26	0.076			0.184			
Cochran's test		*C* = 0.251, p > 0.05			*C* = 0.313, p > 0.05			
Transformation		ln(*x*+1)			ln(*x*+1)			

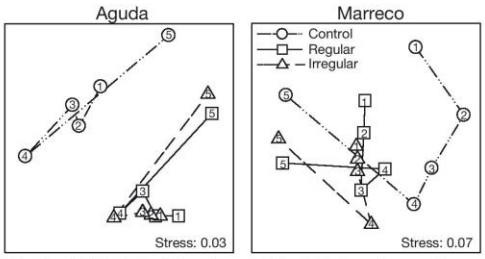


Fig. 3. nMDS plots of the temporal trajectories of assemblages from unmanipulated (control) plots (centroids calculated over replicate plots) and plots assigned to past Regular (centroids calculated over replicate plots and 4 levels of mussel harvesting) and Irregular (centroids calculated over replicate plots, 2 temporal sequences and 4 levels of mussel harvesting) treatments of mechanical disturbance, sampled 5 times (from 1 to 5: October 2012, January, April, June and October 2013, respectively corresponding to 3, 6, 9, 12 and 15 mo of recovery)

Figs. 4B & 5B; SNK test). Together with a higher cover of mussels under the irregular treatment applied according to sequence 1 compared to sequence 2, exactly the same pattern as in April 2013 was still present in October 2013 in Marreco only (Table 3B, Fig. 5B; SNK test), while a difference between control and treated plots was again evident in Aguda, in the same direction as that displayed 1 yr earlier (Table 3A, Fig. 4B). Overall, temporal fluctuations in the abundance of the recovering mussels were larger in control than in treated plots at both study sites (Table 4, Figs. 4B & 5B).

Articulated coralline algae of the genus *Corallina* responded to past treatments in a very similar way as the total number of taxa. In Aguda, this taxon was present in October 2012 in plots allocated to regularly distributed events of disturbance and absent in those allocated to the irregular pattern of disturbance (Table 3A, Fig. 4C). The Harvesting intensity × Sequence interaction was significant in April 2013, with *Corallina* spp. being present only in '30' treatment plots subjected to sequence 1, and with the same abundance in control and '60' treatment plots subjected to sequence 2 of disturbance events (Table 3A, Fig. 4C). No significant differences were documented for the mean abundance of this algal group in Aguda at 15 mo and in Marreco at 3, 9 and 15 mo after the end of the manipulative experiment (Table 3, Figs. 4C & 5C). Analogously, no effects of any past manipulations were shown in terms of temporal variance of the abundance of *Corallina* spp. (Table 4, Figs. 4C & 5C).

Although absolute values were very low, the cover of encrusting coralline algae in Aguda was, on average, 15× higher in unmanipulated than in treated plots sampled 3 mo after the end of experimental disturbances, but this difference disappeared 6 and 12 mo later (Table 3A, Fig. 4D). In Marreco, this taxon contrastingly did not show significant responses to any past disturbance in October 2012, while interactive effects of the intensity of harvesting and the temporal variability of disturbance became evident later during the recovery period (Table 3B). In April 2012, encrusting corallines were absent in plots where the mussel bed had been left intact or slightly reduced (control and '30' treatment, respectively) if regularly distributed events of disturbance had been applied, while they increased in abundance (with mean cover values ~50% higher in the '30' treatment compared to the control) where disturbance had been irregularly applied. The opposite pattern was displayed in plots subjected to higher intensities of

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Table 3. Summary of significant results from the ANOVA on the total number of taxa and the abundance of individual taxa in (A) Aguda and (B) Marreco. T1 = 3 mo (October 2012), T2 = 9 mo (April 2013), T3 = 15 mo (October 2013). $^{\bullet}p < 0.05$, $^{\bullet\bullet}p < 0.01$, $^{\bullet\bullet\bullet}p < 0.001$. Abbreviations as in Table 1

Variable	Time	Source of variation	MS	*F*	Transf.
(A) Aguda					
Total number of taxa	T1	Regular vs. Irregular	0.06	555.0$^{\bullet}$	None
	T2	I × S	3.82	3.0$^{\bullet}$	None
Mytilus galloprovincialis	T1	Control vs. treatments	1720.43	6.1$^{\bullet}$	None
		Harvesting intensity	792.31	2.8$^{\bullet}$	
	T2	Harvesting intensity	0.77	3.5$^{\bullet}$	ln(*x*+1)
	T3	Control vs. treatments	205.34	4.5$^{\bullet}$	None
Encrusting corallines	T1	Control vs. treatments	4.77	13.8$^{\bullet\bullet}$	None[a]
Corallina spp.	T1	Regular vs. Irregular	4.53	56.3$^{\bullet}$	None[a]
	T2	I × S	2.57	3.3$^{\bullet}$	None
Chthamalus spp.	T1	Control vs. treatments	3573.21	16.8$^{\bullet\bullet\bullet}$	None
		Between sequences	2295.97	10.8$^{\bullet\bullet}$	
		Harvesting intensity	2360.69	11.1$^{\bullet\bullet\bullet}$	
	T2	Control vs. treatments	3250.76	9.1$^{\bullet\bullet}$	None
		Harvesting intensity	2654.17	7.4$^{\bullet\bullet\bullet}$	
Patella spp.	T1	Control vs. treatments	716.33	6.7$^{\bullet}$	None
		I × Regular vs. Irregular	302.24	17.4$^{\bullet}$	
	T2	Control vs. treatments	302.09	6.1$^{\bullet}$	None[a]
Bare rock	T1	Between sequences	622.53	6.1$^{\bullet}$	None
		Harvesting intensity	510.77	5.0$^{\bullet\bullet}$	
	T2	Between sequences	350.17	5.1$^{\bullet}$	None
		Harvesting intensity	222.59	3.2$^{\bullet}$	
(B) Marreco					
Mytilus galloprovincialis	T1	Control vs. treatments	1624.24	8. 9$^{\bullet\bullet}$	None
		Harvesting intensity	1514.07	8.3$^{\bullet\bullet}$	
	T2	Control vs. treatments	1348.81	6.1$^{\bullet}$	None
		Harvesting intensity	762.87	3.4$^{\bullet}$	None
	T3	Between sequences	165.38	5.5$^{\bullet}$	None
		Harvesting intensity	107.56	3.6$^{\bullet}$	
Encrusting corallines	T2	I × Regular vs. Irregular	10.11	8.6$^{\bullet}$	None
	T3	I × V	0.16	2.7$^{\bullet}$	ln(*x*+1)
Chthamalus spp.	T3	Between sequences	181.50	7.4$^{\bullet}$	None
Gibbula spp.	T1	I × Regular vs. Irregular	1.44	15.9$^{\bullet}$	ln(*x*+1)
Patella spp.	T2	Regular vs. Irregular	0.27	180.3$^{\bullet}$	ln(*x*+1)
	T3	I × V	156.31	4.1$^{\bullet\bullet}$	None
Bare rock	T2	I × Regular vs. Irregular	596.80	12.0$^{\bullet}$	None

[a]Variances were heterogeneous (after Cochran's *C* test) and could not be stabilized by transformations

harvesting, with encrusting corallines being virtually absent where irregular events of disturbance had been performed, and present (with mean percentage covers reduced to approx. one-third in the '100' compared to the '60' treatment) where past disturbance had been established through regular events (Table 3B, Fig. 5C; SNK test). In October 2013, encrusting coralline algae were only present (with relatively doubled mean cover) in plots allocated to the complete removal of mussels and in those where the mussel bed had been left intact when combined with sequence 1 of irregular disturbance, with comparable values in the '60' and '100' treatments when combined with regular disturbance (Table 3B, Fig. 5C; SNK test). However, these patterns were not accompanied by significant differences in the temporal variance of the abundance of these algae at both sites (Table 4).

The mean abundance of the other abundant algal group analyzed (the green foliose *Ulva* spp.) did not show any effect of past disturbances in both Aguda (Table 3A, Fig. 4E) and Marreco (Table 3B, Fig. 5E). Nevertheless, sequence 1 of past events of disturbance was associated with larger temporal fluctuations in the abundance of *Ulva* in Marreco over the recovery period compared to sequence 2 (Table 4, Fig. 5E).

The mean abundances of barnacles, *Chthamalus* spp., were higher in treated than in control plots sampled in Aguda at 3 and 9 mo, but not at 15 mo after the end of the previous experiment (Table 3A, Fig. 4F). Moreover, in October 2012, Aguda showed a higher cover of barnacles in plots that were previously disturbed using sequence 2 of past disturbance events compared to sequence 1, and in plots where the mussel bed had been completely removed than in plots that were subjected to any other level of experimental harvesting, including the control (Table 3A, Fig. 4F). A similar main effect of harvesting intensity was also evident in April 2013, with barnacles being more abundant in both treatments where mussels had been more intensely removed ('60' and '100' treatments) than in the '30' level of harvesting and the control (Table 3A, Fig. 4F; SNK test). In Marreco, the only significant difference in the mean cover of *Chthamalus* spp. was observed in October 2013, when disturbance sequence 1 was associated with higher values than sequence 2 (Table 3B, Fig. 5F). Significant effects of past disturbances on the temporal variance of barnacles were detected only in Aguda (Table 4). Specifi-

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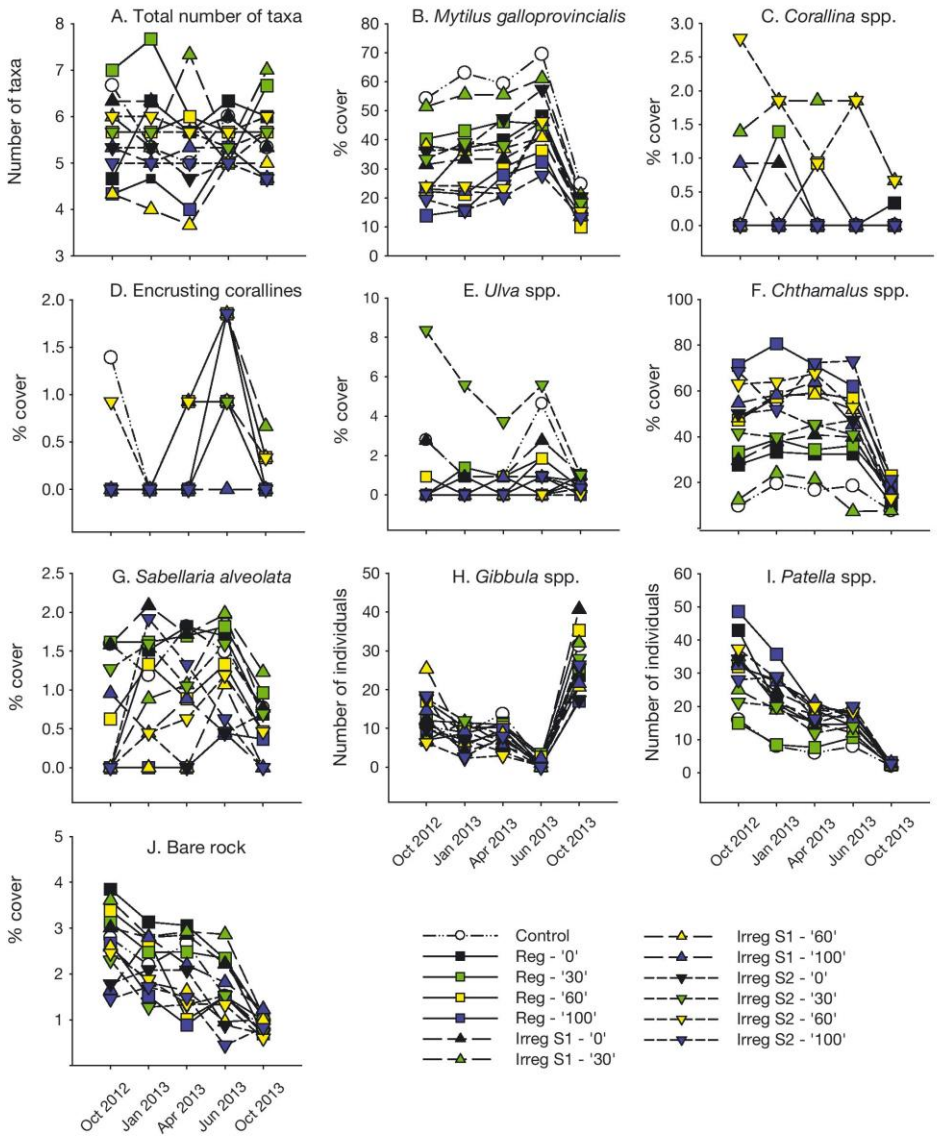


Fig. 4. (A) Mean total number of taxa and (B–J) mean abundance of individual taxa at each sampling period in Aguda. Data averaged over 3 replicate plots assigned to the unmanipulated control and to each combination of past experimental treatments (regular disturbance or irregular disturbance sequence [S] 1 or 2, with '0', '30', '60' or '100' % mussels removed; see Fig. 1). Standard error bars removed for clarity

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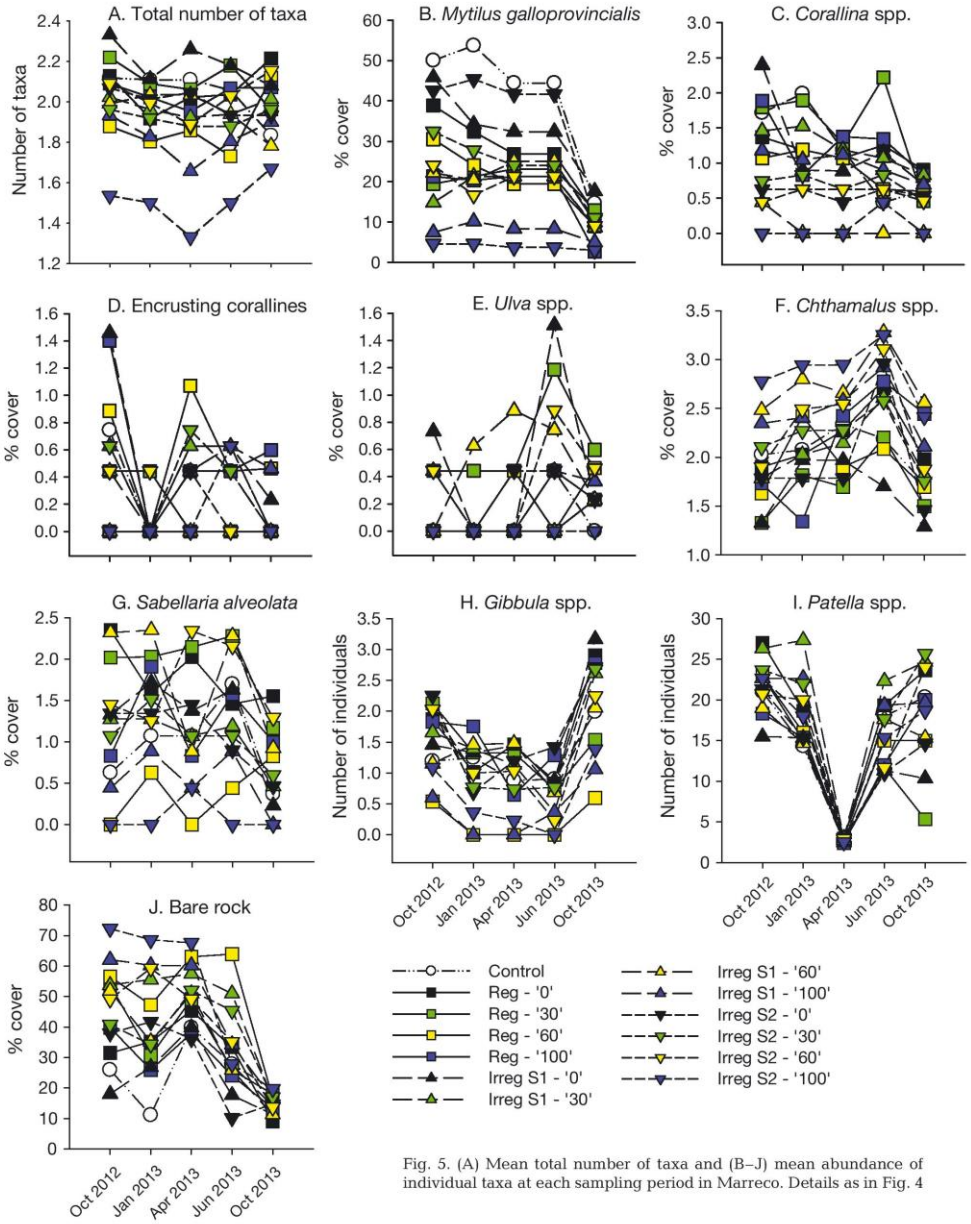


Fig. 5. (A) Mean total number of taxa and (B–J) mean abundance of individual taxa at each sampling period in Marreco. Details as in Fig. 4

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Table 4. Summary of significant results from the ANOVA on the temporal variance of the total number of taxa and the abundance of individual taxa in Aguda (A) and Marreco (M). $^{\bullet}p < 0.05$, $^{\bullet\bullet}p < 0.01$, $^{\bullet\bullet\bullet}p < 0.001$

Variable	Site	Source of variation	MS	*F*	Transf.
Total number of taxa	A	Control vs. treatments	3.80	$6.3^{\bullet\bullet}$	None[a]
	M	Regular vs. Irregular	0.37	$120.2^{\bullet}$	ln(*x*+1)
Ulva spp.	M	Between sequences	1.37	$4.4^{\bullet}$	ln(*x*+1)
Mytilus galloprovincialis	A	Control vs. treatments	133245.53	$6.6^{\bullet}$	None
	M	Control vs. treatments	79288.83	$7.3^{\bullet}$	None
Chthamalus spp.	A	Control vs. treatments	120966.50	$4.5^{\bullet}$	None
		Between sequences	150552.86	$5.5^{\bullet}$	
		Harvesting intensity (I)	294040.71	$10.8^{\bullet\bullet\bullet}$	
Sabellaria alveolata	A	Regular vs. Irregular	3.23	$38.8^{\bullet}$	ln(*x*+1)
	M	I × Regular vs. Irregular	5.72	$63.1^{\bullet\bullet}$	ln(*x*+1)
Gibbula spp.	M	I × Regular vs. Irregular	6.92	$17.3^{\bullet}$	ln(*x*+1)
Patella spp.	A	Regular vs. Irregular	12500.97	$1479.6^{\bullet}$	None

[a]Variances were heterogeneous (after Cochran's *C* test) and could not be stabilized by transformations

cally, fluctuations were larger in treated than in control plots, in plots subjected to sequence 2 than in those subjected to sequence 1 of disturbance, and in completely harvested compared to the less intensely harvested ('60' treatment), further less harvested ('30' treatment) or mussel-intact plots (Fig. 4F; SNK test).

The mean cover of *Sabellaria alveolata* did not show any significant differences in both Aguda and Marreco at each of the 3 analyzed periods of recovery (Table 3, Figs. 4G & 5G). Nevertheless, the temporal variance in the abundance of this species was affected by the temporal variability of past disturbance in Aguda, with larger fluctuations being observed in previously irregularly than in regularly disturbed plots (Table 4, Fig. 4G), and by the interactive effect of the temporal variability and the harvesting intensity in Marreco (Table 4). The pattern of recovery of *S. alveolata* in Marreco was more variable over time in plots that had been subjected to regular events of mechanical disturbance when disturbance had been combined with the total removal of mussels than when mussels had been left untouched, or slightly ('30' treatment) harvested, or when they had been more intensely ('60' treatment) harvested. In contrast, in plots subjected to irregular disturbance, the largest fluctuations in *S. alveolata* abundance were observed in combination with the '60' treatment, with progressively lower variance values in combination with the unmanipulated, the '30' and the '100' treatments (Fig. 5G; SNK test).

Patterns of recovery of gastropods of the genus *Gibbula* in Aguda did not show significant effects of past disturbances both in terms of mean abundances (in October 2012, April 2013 and October 2013) and of temporal variance (Tables 3A & 4, Fig. 4H). In contrast, a significant Intensity × Regular vs. Irregular interaction was detected in Marreco in October 2012 (Table 3B), with the mean abundance *of Gibbula* spp. decreasing progressively from control plots to treatments '30', '100' and '60' when combined with regular events of disturbance, and from treatment '60' to the control to treatments '30' and '100' when combined with irregular events of disturbance (Fig. 5H; SNK test). Such differences disappeared in April 2013. Temporal variations in abundance over the recovery period were affected by combinations of harvesting intensity and the temporal variability of disturbance (Table 4). In plots that had been regularly disturbed, temporal fluctuations in *Gibbula* spp. abundance progressively decreased from treatment '100', to the control, to treatments '30' and '60', while they progressively decreased from treatment '60', to the control, to treatments '30' and '100' in plots that had been irregularly disturbed (Fig. 5H; SNK test).

The mean abundance of limpets (*Patella* spp.) in Aguda was higher in treated than in control plots in October 2013 and April 2013 (Table 3A, Fig. 4I). Moreover, this variable also varied during the first sampling period in plots allocated to combinations of experimental levels of harvesting and temporal patterns of disturbance (Table 3A). Specifically, where the pattern of disturbance was regular, the most intensely harvested treatments ('100' and '60') had more limpets than the unmanipulated control, or the '30' treatment. Conversely, where events of disturbance were irregularly distributed, limpets were less abundant in the '30' treatment than in any other level of harvesting, including the unmanipulated control (Fig. 4I; SNK test). In Marreco, no significant

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differences were observed in the abundance of limpets in October 2012, while effects of past treatments were detected later (Table 3B). In April 2013, independent of the intensity of harvesting, plots subjected to the irregular level of disturbance hosted more limpets than those subjected to the regular level (Fig. 5I). In October 2013, the patterns of mean abundance of *Patella* spp. were very variable depending on the combination of harvesting intensity and temporal pattern of disturbance. Regularly distributed disturbance events were associated with a relatively higher abundance of limpets in both the unmanipulated plots and in those where mussels had been completely removed than in plots allocated to the '60' treatment, with a further reduction in '30' plots. When irregularly distributed events of disturbance had been applied, their sequence was also important 15 mo after the end of the disturbance experiment, as sequence 1 was associated with a higher number of limpet individuals in the control and in the '30' and '100' treatments compared to the '60' treatment, while sequence 2 was associated with a higher abundance in treatments '30' and '60' compared to the other 2 levels (Fig. 5I; SNK test). In terms of temporal variance, patterns of limpet abundance in Aguda were characterized by larger fluctuations in plots that were previously regularly disturbed than in those subjected to irregular disturbance (Table 4, Fig. 4I).

Finally, past disturbance affected the availability of bare rock during the recovery process (Table 3). In Aguda, similar results were obtained in October 2012 and April 2013, with more bare rock becoming available in plots that were previously irregularly disturbed using sequence 1 than in those disturbed using sequence 2, and independently of the temporal variability of disturbance in the control or the relatively less harvested ('30') plots than in intensely harvested ('60' and '100') plots (Table 3A, Fig. 4J; SNK test). No significant effects were evident in October 2013. In Marreco, past combinations of harvesting intensities and temporal variability of disturbance were associated with significant differences in the amount of free substratum only in April 2013 (Table 3B). In regularly disturbed plots, the availability of bare rock was highest under the '60' treatment, decreased in both the '30' treatment and the control, and was further reduced where mussels had been completely removed. In contrast, in irregularly disturbed plots, the greatest amount of bare rock was observed under the complete harvesting treatment, with the amount of bare rock decreasing in both intermediate levels of harvesting and further diminishing where the mussel bed had been left untouched (Fig. 5J; SNK test). However, no significant differences in the temporal variance of the availability of bare rock were found in both study sites (Table 4, Figs. 4J & 5J).

DISCUSSION

This study followed a previous experiment that demonstrated the buffering ability of an intact mussel bed against the effects of physical disturbance (see Oliveira et al. 2014). The present work was aimed at evaluating the influence of each combination of past disturbances on the recovery process of assemblages, under the main hypotheses that assemblages associated with an intact mussel bed subjected to regularly distributed events of mechanical disturbance would be more similar and would take less time to converge towards unmanipulated assemblages than those associated with a partly damaged or completely removed mussel bed subjected to irregular disturbance.

The present findings suggest relatively short-term 'legacy' effects of experienced disturbances, although we were unable to fully exclude further subtle consequences of past experimental treatments which might become visible over temporal scales larger than that examined here. In fact, 15 mo after the end of all manipulations, no differences due to any combination of treatments were yet found for multivariate and most univariate response variables, suggesting a strong overall ability of the examined organisms to converge towards the unmanipulated control over such temporal scale. Effects of both the intensity of mussel harvesting and the variability of mechanical disturbance were evident at the earlier sampling dates, although patterns were not consistent between the 2 study sites. While whole assemblages differed between control and, on average, treated plots at both sites 3 mo after the end of the manipulative experiment, the interaction of harvesting intensity and sequence of irregularly distributed events of mechanical disturbance was significant only in Aguda, particularly when a small removal of the mussel bed was combined with sequence 1 of disturbance and when full removal was combined with sequence 2. Six months later, simultaneous, but separate, effects of harvesting intensity and temporal variability of disturbance on the structure of assemblages were still evident in Aguda, while assemblages in Marreco were affected only by a main effect of the temporal variability of disturbance. The

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present findings do not clearly support the proposed hypothesis, although a more intense removal of mussels and a larger temporal variability of disturbance seemed to be independently important in preventing a quick recovery in Aguda and Marreco, respectively.

It could be hypothesized that multivariate patterns might be driven by the responses of manipulated habitat-forming mussels, but effects on recovering *Mytilus galloprovincialis* only partly matched those on whole assemblages. In general, the abundance of mussels in Aguda was inversely related to the intensity of past harvesting, with more prolonged significant effects due to more intense past removal. This is consistent with the documented ability of mussels to occupy space on rocky shores and to relatively quickly re-colonize disturbed patches, particularly in the absence of effective consumers (e.g. Bertness et al. 2004). The drastic reduction in the cover of mussels observed in control plots in October 2013, however, was responsible for a clear reduction in differences in the cover of mussels relative to all treated plots at both study sites. More complicated to interpret is the significant effect of the sequence of mechanical disturbance events documented in Marreco in October 2013. This effect is likely due to the different timing of events of disturbance established according to each sequence and differences in interacting with some relevant life cycle stages of *M. galloprovincialis*. The recruitment and settlement of this species at Portuguese locations are known to occur throughout the year, but with peaks from May to September (Fragoso & Icely 2009). Over the duration of the manipulative experiment, just 2 events (one in July 2011 and another in May 2012) of disturbance were established according to sequence 1, while 2 sets of consecutive events (3 between July and September 2011, and 2 between May and June 2012) were established according to sequence 2. Such difference might have differentially affected phases of the life cycle of mussels that could be relevant to their ability to recover after disturbance. For instance, it has been shown that extremely stressful conditions occurring at the time of recruitment of several sessile species, including *M. galloprovincialis*, can drastically affect their patterns of distribution and abundance (Wethey et al. 2011). Analogously, clustered events of disturbance associated with sequence 2 might have hampered the attachment of mussel larvae to rocks, resulting in lower abundances of mussels (compared to sequence 1) recorded much later. Of course, the establishment of exactly the same treatments at both sites should have implied analogous findings also in Aguda, but this was not the case. This could be explained by differences between the 2 study sites in other physical and biological processes that were impossible to control despite the 2 sites being comparable in a number of identified factors. For example, complex and almost unpredictable variations in the availability of larvae of sessile organisms can be responsible for the variability in their patterns of colonization over a spatial scale comparable to that between Aguda and Marreco (e.g. Raimondi 1990).

Site-specific processes might have also been responsible for the inconsistent responses documented for the total number of taxa (a proxy for species richness). When the temporal variability of mechanical disturbance was the key factor, such as in Aguda 3 mo after the end of the disturbance experiment, the richness of taxa was higher in previously irregularly disturbed plots compared to regularly disturbed plots. This would be consistent with the supposed relatively greater ability of past irregular disturbance events to generate a mosaic of resources usable by a larger set of taxa (Dayton 1971, Connell 1978, Lubchenco & Menge 1978, Paine & Levin 1981, Sousa 1984). Absolute differences in the number of taxa, however, were always very small, suggesting that caution should be exercised in attributing crucial ecological importance to this mechanism. Moreover, site-specific inconsistencies suggest that any possible relationship between past disturbances and subsequent patterns of richness of recovering assemblages might not be as relevant as the effects of other processes. For example, in Aguda, the articulated coralline algae of the genus *Corallina* showed initial patterns of recovery that were affected by the temporal variability of past disturbance, this taxon being present only in regularly disturbed and absent in irregularly disturbed plots. In principle, it could be expected that events of disturbance clustered over reduced periods of time could be particularly stressful for most organisms whose abundance might be reduced to lower values than by regular disturbance, these organisms eventually needing more time to recover (Benedetti-Cecchi 2003, Bertocci et al. 2005). However, previous findings suggested that *Corallina* spp. may benefit from relatively stressful conditions due to its mechanical resistance and its concomitant release from competition with more sensitive species (Bulleri & Airoldi 2005, Oliveira et al. 2014). The present findings clearly disagree with these previous findings, which might suggest that the direct effect of disturbance would not be as relevant for their patterns of recovery as the indirect influence of other

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processes. These other processes might also include competitive and/or grazing relationships with other sessile organisms that are also affected by disturbance in a contrasting way. Intense grazing by limpets, in particular, can almost completely eliminate erect seaweeds, including articulated corallines, from patches of rocky substratum (e.g. Benedetti-Cecchi et al. 1996). In October 2012, limpets of the genus *Patella* were relatively more abundant (in contrast to *Corallina* spp.) in Aguda in plots where mussels had been intensely removed, and where regularly distributed events of mechanical disturbance had been applied, suggesting a possible grazing effect on these algae.

The interaction with different types of disturbance and the ability to occupy space in competition with co-occurring organisms was likely responsible for the patterns of recovery of encrusting coralline algae, although patterns were again inconsistent between sites. While control plots in Aguda were characterized by higher cover values of this taxon compared to treated plots only 3 mo after the end of the experiment and differences were no longer evident at the next analyzed periods of recovery, later differences were detected in Marreco depending on the interaction between the intensity of mussel harvesting and the variability of storm-like disturbances. Specifically, an intact mussel bed could buffer the effects of physical disturbances occurring through single events that are regularly distributed in time, while more aggregated events could create free patches suitable for the colonization and increase in abundance of encrusting corallines (see also Oliveira et al. 2014). This is consistent with the known resistance of these algae to mechanical disturbance and with their subsequent colonization of disturbed patches (e.g. Bulleri 2006). When mussels were intensely removed, however, the combination with clustered disturbance events (in principle the condition potentially able to create larger free patches) was surprisingly associated with the virtual absence of encrusting corallines, which, instead, increased in abundance in plots that were previously disturbed according to the regular pattern. These results are unlikely due to a negative effect of the most stressful combination of past experimental manipulations that exceeded the resistance ability of this algal group (see also Oliveira et al. 2014); however, possible explanations for the present outcome should be found in indirect effects of past treatments. Such mechanisms could be speculated based on the present results, but are beyond the goals of this discussion.

It is interesting to notice, however, that another taxon that is dependent on the availability of free space, i.e. the barnacle *Chthamalus* spp. (e.g. Bertness et al. 2004), was generally positively affected by the intensity of mussel harvesting. Especially in Aguda, both the mean abundance and the temporal variance of barnacle abundance tended to be higher in intensely harvested ('60' and '100' treatments) plots. Nevertheless, the patterns of availability of bare rock during the examined recovery period of assemblages could not support a simple interpretation of the present results, as a larger amount of free space tended to be available in plots that had been subjected to the lower levels of harvesting. This can be explained by the ability of other taxa to colonize large patches left by removed mussels and to exclude barnacles from them. In contrast, smaller patches could have represented a refuge for recovering barnacles, once their larvae were present at the suitable time (Keough 1984). In fact, the sequence of disturbance was also relevant for *Chthamalus* spp. Populations of *C. stellatus* from nearby locations were shown to have their peaks of recruitment during summer months (i.e. from June to late August; O'Riordan et al. 2004), when different numbers of experimental events of disturbance had been performed for each sequence (a single event in 2011 for sequence 1, but 2 consecutive events in both 2011 and 2012 for sequence 2). This might have affected the subsequent patterns of recovery of barnacles in disturbed plots.

A potential competitor of barnacles for substratum could be represented by the honeycomb worm *Sabellaria alveolata* which, particularly in Marreco, appeared to benefit from the protection provided by an intact mussel bed (and from its ability to live within and below mussel individuals) when events of disturbance had been clustered; when disturbance events had been regularly established, the worm increased in abundance after the full removal of mussels. In the absence of major disturbances, this reef-building polychaete is described as a dominant habitat-former (e.g. Wethey et al. 2011).

As could be expected, the top shells *Gibbula* spp. showed little effects of past disturbances, likely due to their mobility allowing them to quickly re-invade disturbed patches. In the first stages of recovery in Marreco, however, the integrity of the mussel bed was an important factor for these gastropods as their abundance still appeared to decrease with increase in harvesting intensity, reaching the lowest values in the most extremely disturbed plots (i.e. those where mussels had been completely reduced and those

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where irregular storm-like disturbance events had been performed). This was also the combination of past treatments that was associated with the relatively lowest value of the temporal variance of the abundance of *Gibbula* spp. over the examined period, in agreement with predictions from the scaling relationship between the mean and the variance, by which large fluctuations around consistently low values would not be intrinsically possible (Taylor 1961). For these animals, it is logical to hypothesize that an intact mussel bed could provide protection from physical disturbance and/or predators, while increasing mussel removal would expose them to increasing stress, leading to a decrease in abundances (e.g. Underwood 1998).

Independent of the specific mechanisms underlying the present outcomes, this study demonstrated that assemblages that mostly include organisms known to withstand 'extreme' combinations of types, mean intensity and temporal variability of disturbance can quickly converge toward unmanipulated ones. Although high resilience is a key property of intertidal organisms (Steneck 1986, Benedetti-Cecchi 2000, Bertocci et al. 2005), the present findings raise some warning considerations.

First, within the same level of temporal variance, the sequence of disturbances was shown to be responsible for modifications both in the mean abundance and the temporal variance of recovering organisms, likely due to its interference with biological traits such as reproduction, recruitment and growth seasons. This suggests the need to design manipulative experiments that take this point into account when examining hypotheses in ecological contexts similar to those of the present study (Vaselli et al. 2008, Oliveira et al. 2014, Tamburello et al. 2014). This also raises several questions regarding the timing of the implementation of planned disturbances in coastal areas, such as beach nourishment interventions (e.g. Schlacher & Thompson 2012) and other construction works (e.g. Airoldi & Beck 2007). Wherever possible, these operations should be done with reference to the timing of life cycles of the main exposed organisms in order to guarantee their better and faster recovery.

Second, ecological theories consider disturbance, with the consequent elimination of dominant organisms, as an opportunity for other, less competitive, species to colonize and grow in otherwise unsuitable conditions, which could eventually lead to an increase in the diversity (particularly species richness) of the system (Connell 1978, Huston 1994, Connell et al. 1997). In this study, the set and total number of taxa found in experimental plots before and during manipulations (Oliveira et al. 2014) and during the recovery period remained almost unaltered. This suggests that the main effects of disturbance can be in terms of changes in the relative abundance of the same taxa, rather than in changes in the identity or the overall richness of taxa (O'Connor & Crowe 2005, Cardinale et al. 2006, Stachowicz et al. 2007, Maggi et al. 2009, Bertocci et al. 2010).

In conclusion, this study contributes answers to questions about the growing influence of compounded anthropogenic perturbations on coastal areas, particularly on how different life traits can allow exposed organisms to cope with multiple disturbances through modifications of their patterns of recovery over a definite time frame.

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Discussion

The main objectives of this thesis, as debated in a previous chapter, were related with direct or indirect anthropogenic disturbances over sessile assemblages of coastal ecosystems in north Portugal. Two perspectives were used: one aimed at evaluating the effects of experimental human disturbances in intertidal areas (paper II and III) and the other at understanding the process of recolonization starting once the disturbing factor ceased to exist (paper I and IV). In order to assist the most focused reading, the main methodological characteristics and results of each component of the thesis are reported below before the general discussion of the most ecologically relevant issues addressed throughout the whole project.

The option to include the paper "Type and timing of disturbance modify trajectories of recovery of rockpool assemblages at Aguda (NW Portugal)", published in Journal of Experimental Marine Biology and Ecology by Elsevier, which was not part of the initial thesis plan, was mainly due to the added knowledge that it could introduce regarding the statistical procedures needed for the rest of the studies, but also because it could provide a description of one of the main experimental sites (Praia da Aguda in Vila Nova de Gaia) that were going to be used in all other experimental works.
In this paper, two main hypotheses were tested; the first related with the temporal fluctuations in patterns of recovery of tide pool assemblages previously subject to two disturbances with different intensity; the other associated to the consistency of these effects, when the timing of the implementation of the disturbances was altered. In this project, 15 tidepools were disturbed in March 2007 (Trial 1) and a second set, of equal number, in July of the same year (Trial 2) and their recolonization assessed monthly during 13 months (each trial). Five out of each set of 15 tidepools were left unmanipulated as control), while other 5 were assigned to

experimental disturbance according to each of two methods: scraping with a paint scraper (lower intensity) and scorching with a gas torch (higher intensity). The temporal variance of the whole assemblages and that of individual taxa were examined as response variables.
Results indicated that the temporal heterogeneity of the whole assemblage increased with the increase of disturbance intensity in both experimental trials. However, relatively larger fluctuations were detected in the trial that started in March of 2007, independently of the experimental treatment. The obtained results were in disagreement with predictions from Taylor's mean/variance scaling relationship (Taylor, 1961), which postulates that variance would be necessarily reduced when the mean value of a variable is low. Theoretically, disturbances of high intensity (as the one that was used) should be deterrent of large temporal fluctuations of abundance due to the strong effect they produce over the total number of individuals with local extinctions of most organisms. Although some taxa displayed this pattern (namely *M. galloprovincialis*), the recolonization process of most others (including the whole assemblage) must be interpreted according to alternative biological mechanisms. Specifically, as debated in the literature (e.g. Hutchinson and Williams, 2003; Bertocci *et al*., 2005), the ability of intertidal organisms to rapidly reoccupy free space and make use of other resources (e.g. light and nutrients) made available can be more relevant than the direct mortality caused by a disturbance in the modulation of temporal patterns of distribution and abundance.

Although not directly oriented to identify and test the reasons behind the differences detected in each experimental trial, this project documented that the recovery was affected by the timing at which a given impact occurred. Several different processes (alone or through complex interactions) could account for this variation, as temporally variable characteristics of life-cycles of organisms (e.g. growth season, availability of larvae or propagules, recruitment) can determine drastically variable responses to other temporally variable processes, such as oceanic currents

and the occurrence of upwelling events (Caffey, 1985; Connell and Keough, 1985; Gaines and Roughgarden, 1985; Roughgarden *et al.*, 1988; Raimondi, 1990; Nykjaer and Van Camp, 1994; Fiúza *et al.*, 1998).
The conclusions from this paper were strictly related with the intended goals of the next planned experimental work, representing, during the PhD's probationary year, a particularly effective form of using the knowledge and data from previous activities to build a bridge to the following studies.

The second paper, entitled "Interplay of experimental harvesting and climate-related disturbance on benthic assemblages of rocky seashores" and published in Marine Ecology Progress Series by Inter-Research Science Center, developed some of the main concepts addressed in the previous paper, with special focus on assessing the effects of compounded disturbances varying in intensity and temporal variability. This study was inspired by climatic observations and models (IPCC 2007) documenting and predicting changes in both the intensity and the temporal patterning of extreme meteorological events, and by the fact that experimental tests of the effects of compounded disturbances were relatively rare in the scientific literature.
In this study, in particular, interactive effects of different levels of mussel harvesting and mechanical "storm-related" disturbance varying in temporal variance were simultaneously used to investigate how the presence of a foundation species (*M. galloprovincialis*) can modulate the system's response to anthropogenic compounded disturbances.
Thirty-nine 35 x 35 cm plots dominated by *M.* galloprovincialis were selected at two different rocky beaches (Praia da Aguda in Vila Nova de Gaia and Praia do Marreco in Matosinhos) that were comparable in both biotic (existing taxa) and abiotic (e.g. wave exposure, shore height and orientation) factors. Three plots were left unmanipulated as control and the other 36 were divided into groups of 3 plots, each assigned to each combination of 4 levels of harvesting (0 %, 30 %, 60 % and 100 %) and 3

patterns of disturbance. In all patterns, 6 total events of mechanical disturbance were performed, but these were regularly distributed over the period of the study, while the other two were characterized by events aggregated over short periods, separated by prolonged periods without disturbance (generating, however, the same overall value of the variance of the time intervals between consecutive events). 9 sampling dates were established in a way that, on average, the time between a disturbance event and the next sampling was the same in all treatments. When the *M. galloprovincialis* bed was left intact, the whole associated assemblage demonstrated lower temporal heterogeneity when exposed to regularly distributed disturbances compared to when the disturbances were clustered, while this response was inverted when the mussel bed was fully removed. The aggregation of disturbances appeared to be the threshold for the buffering capability of mussels as it was the main factor able to create patches of free space within the mussel bed. Nevertheless, the increase of intensity, as defined by Pickett & White (1985), caused by the repetition of disturbance events at close intervals of time was responsible for a large removal of individuals and, likely as a consequence of the previously mentioned mean/variance relationship, for a reduced heterogeneity of assemblages.

In general, environmental variability is considered a vehicle to increase species richness via a reduction of competition and the promotion of coexistence of taxa with different functional characteristics (Chesson and Huntly, 1997) or a factor negatively affecting species diversity due to enhanced extinction risk (Vucetich *et al.*, 2000). In this case, most taxa persisted throughout the study, suggesting caution when attributing changes of richness to the effects of disturbance (Inchausti and Halley, 2003). These results were further important as they could be, at least in part, extended to other systems where other habitat-forming taxa (e.g. kelp, corals, other species of mussels) play a relevant role, particularly

once maintained in altered states due anthropogenic and/or natural perturbations.
In terms of PhD development, this study further expanded the knowledge on how natural communities react to compounded disturbances and how their interactions and specific traits can allow them to cope with simultaneous impacts.

The third paper "Urban vs. extra-urban environments: Scales of variation of intertidal benthic assemblages in north Portugal", published in Marine Environmental Research by Elsevier, examined the influence of anthropogenic disturbance on rocky intertidal systems through a different approach. Instead of performing experimental manipulations, the study was designed to assess possible effects of present urbanization-related disturbances in terms of modifications of patterns of spatial and temporal variation over a range of scales of populations and assemblages of algae and invertebrates.
Most of the human population is living in coastal areas (Vitousek *et al.*, 1997, Airoldi and Beck, 2007) and this behavior is expected to increase in the next decades. This is likely to increasingly subtract and modify natural habitats, directly impact native organisms and possibly replace them with alien species. However, these impacts are difficult to characterize as they are produced by several different activities (e.g. pollution, sewage and aquaculture) and can interact between them and with natural processes, with largely unpredictable consequences. For these reasons, scientific results have been inconsistent as some indicated, as a major outcome of these pressures (or combination of pressures), the reduction of diversity due to the substitution of several sensitive taxa by a few resistant ones (e.g. Olden and Rooney, 2006; Balata *et al.*, 2007; Bahartan *et al.*, 2010; Martins *et al.*, 2012). This mechanism would thus promote homogenization of the habitat. On the contrary, other studies suggested that moderate disturbances would be able to increase richness by providing a mosaic of resources available to a large set of species or even

by facilitating the introduction of exotic species faster than the endemic are eliminated (Sousa, 2001; McKinney, 2002, 2006; Hewitt and Thrush, 2009; Hewitt *et al*., 2010).

In this project a total of 4 study shores were selected: 2 classified as Urban (praia de Lavadores in Vila Nova de Gaia and Castelo do Queijo in Porto) and 2 classified as Extra urban (Praia da Aguda in Vila Nova de Gaia and Mindelo in Vila do Conde) according to the amount of resident population and the proximity to big urban centers and industrial activities. In each shore, 2 sites (approximately 100 m in extension and 50 m apart) were selected at random, with 2 areas (10 m long and 3 to 5 m apart) in each site and 15 plots in each area. Assemblages in each plot were sampled 4 times throughout the study, choosing new plots, areas and sites at each occasion.

In the present system, no previous data existed regarding these issues, preventing to propose any *a priori* hypothesis on the most likely direction of possible urbanization effects.

Results from this study demonstrated that, at most sampling dates, urban systems were characterized by larger heterogeneity than extra-urban ones, even though the largest variability was always found at the smallest (10's cm) and largest (10's of km) examined scales, rather than at the intermediate ones. Interestingly, the documented effects of urbanization were mostly driven by modifications in the relative abundance of taxa rather than in their identity. Heterogeneity tended, however, to be directly related with abundances, as higher variability was often associated to larger abundances of taxa, still according to expectations from the mean/variance scaling relationship (Taylor, 1961).

Extreme effects of urbanization, on both ends of the scale (very intense/negligible) have been documented by several authors. Under very strong anthropogenic pressures upon costal assemblages the typical response appeared to be a critical reduction of diversity (and simultaneously variance) as only few individuals are able to cope with such

high stress (McKinney, 2008; Bevilacqua *et al*., 2012). Under very low levels of pressure both abundances and species richness could remain unmodified or even increase. With intermediate levels of intensity of disturbance the available evidence is more inconsistent, probably due to variable responses associated to the patchy patterns of resources maintained by disturbance (e.g. McKinney, 2008 and references therein). This is actually a main issue of the Intermediate Disturbance Hypothesis (IDH: Connell, 1978), according to which intermediate intensities (or frequencies) of disturbance could promote species diversity by inhibiting the domination of space by more adapted or resistant organisms.

The issue of spatial scale of possible human impacts has also been addressed in this paper and results reinforced the notion that it should be taken into account when planning management and conservation measures in coastal systems. Several biological and physical processes can be invoked as responsible for the documented small-scale variation of organisms. Biotic interactions (e.g. predation, competition and symbiosis), habitat heterogeneity and local variations in patterns of settlement and recruitment can contribute to create local environmental conditions promoting patchiness in the occupation of space by intertidal benthos. Larger scale processes, including water circulation and patterns of dispersion of larvae and propagules, could explain variation at the scale of 10's of km.

The documented increase in the variability of urban compared with extra-urban assemblages could be viewed as a "positive" effect of urbanization. Nevertheless, this interpretation should be taken with caution as such evidence could represent the beginning of a process of environmental degradation, which would be likely to continue and exacerbate unless proper mitigation measures were implemented.

Finally, "Patterns of recovery of intertidal organisms after compounded anthropogenic disturbances" is the fourth paper concluded during this thesis and was submitted to Marine Ecology Progress Series. This project followed, during 15 months, the same plots used in the second described paper, aiming at evaluating the possible influence exerted by past disturbances on recovering populations and assemblages.

While several studies have examined how natural assemblages recover after the occurrence of single disturbances and return to a condition similar to that existing before (or, more properly, to a state similar to the present one of assemblages not subject to that disturbance), only a few aimed at evaluating how compounded disturbances could modify the recovery process (e.g. Paine *et al.*, 1998; Tamburello *et al.*, 2014). Filling this gap is urgently needed as simultaneous human disturbances are increasingly pervasive in natural systems concomitantly with increasing activities in coastal areas.

Focusing on the structure of whole assemblages and the abundance of individual taxa and on their temporal variances as response variables, this part of the PhD project evaluated how and, possibly, on what temporal scales, compounded disturbances (mussels harvesting and temporally variable climate-related mechanical impacts, as previously described) can shape patterns of recovery of organisms.

Results indicated that, while differences were observed by 9 months after the end of experimental disturbances, 15 months after almost no statistically relevant effects were yet present, both at praia da Aguda and Marreco, for the structure of whole assemblages. This cannot *per se* exclude, however, that subtle "legacy" effects of past disturbances could further become visible over temporal scales larger than those examined here. Most univariate results, instead, were inconsistent between study sites. The probable cause for this variability was that, even though care was taken to select sites comparable for a number of controllable traits, other site-specific differences related to some physical or biological

processes (e.g. availability of larvae of sessile organisms (Raimondi, 1990)) could still be present.
In general, the use of temporal variability of the structure of assemblages and of the abundance of taxa as response variables in studies involving compounded disturbances is not common. Nevertheless, it can definitely contribute to understand the causes of and, possibly, predict temporal variations of exposed organisms, which are relevant for a range of valuable ecological properties, including their productivity (Johnson *et al.*, 1996; Grime *et al.*, 2000), stability and extinction risk (Micheli *et al.*, 1999; Lundberg *et al.*, 2000; Inchausti and Halley, 2003).

The objectives of this thesis were all related to the broader theme of examining the effects of anthropogenic disturbances in coastal areas. Issues involving the intensity and timing of disturbances *per se*, the combined effects of harvesting habitat-forming organisms in concomitance with extreme climatic events, the consequences of urbanization pressures on patterns of intertidal biodiversity and the post-disturbance recovery are aspects relating to the dichotomy Man/Coastal ecosystems that were taken into account in order to contribute to a clearer picture of the ecological consequences of the intense and increasing use of marine system that took place in the last decades and is expected for the near future.

The issue of intensity and timing of disturbances, and the subsequent recovery process, have been studied for the last decades (e.g. Foster, 1975), but are still a fertile ground for ecological research. Even though the intensity of disturbance, once not exceeding the tolerance ability of most organisms, could be considered a driver of ecosystem stability and richness by reducing the amount of biomass and providing resources usable by new individuals or colonies (Sousa, 1984a; Pickett and White, 1985), this factor should not be analyzed independently of the timing in which it occurs. In fact, while disturbances may allow less competitive species to thrive, the seasonality of biological life cycles related, for

instance, to the availability of propagules or to the peaks of recruitment (Foster, 1975; Paine, 1977; Emerson and Zedler, 1978; Hawkins, 1981; Breitburg, 1985; Kennelly, 1987; Incera *et al.*, 2010) can drastically modulate the first stages of recovery after a disturbance and can influence subsequent colonization by mechanisms of facilitation or inhibition (Turner, 1983). Although some controversy still exists (Paine *et al.*, 1998), this modulation appears to be key for early colonists, but its importance reduces through time as, with the progress of recolonization and in the lack of further disturbance, domination is usually achieved by the same perennial species, driving the assemblages to a state possibly, similar to that existing before the disturbance (as predicted by the classical "climax" concept: Sousa, 1979a, 1985; Hawkins, 1981).

Nevertheless, disturbances are pervasive and critically responsible for maintaining the patchiness typical of intertidal systems. Intensity, frequency, size, location and time or seasonality (Kim and deWreede, 1996 and references therein) are among the main traits that have been examined aiming at describing and interpreting the effects of disturbance on natural assemblages. However, it is highly unlikely that any of these aspects occur independently of the others, which can drastically complicate the univocal interpretation of their own effects and the possibility to make generalizations (Paine *et al.*, 1998; Vaselli *et al.*, 2008; Helmuth *et al.*, 2011). In practice, all stressors naturally operate interactively and, as such, the study of single disturbances cannot allow meaningful predictions (Paine *et al.*, 1998; Bertocci *et al.*, 2010).

Moreover, human intervention on coastal systems is clearly increasing, reinforcing the need for the evaluation of the complex effects of compounded perturbations (e.g. Bertocci *et al.*, 2010), including those of direct human disturbances potentially interacting with extreme meteorological events related to global climate change (McGranahan *et al.,* 2007; IPCC 2007; IPCC report 2014 (Wong *et al.*, 2014)). Both IPCC Reports

2007 and 2014 agreed that a range of effects related to climate change are expected to severely increase the levels of stress to natural communities from coastal areas. These are mostly caused by the increased mean intensity and temporal variability of extreme events, such as wind and wave storms, ocean acidification and oceanic temperature that might exert their negative ecological consequences directly or exacerbating those of other anthropogenic disturbances (e.g. intense harvesting or fishing (Gray, 1997), pollution (Fraschetti *et al.*, 2005b), trampling (Araújo *et al.*, 2009)) already responsible for alterations of natural systems. This is of particular concern, for example, for organisms playing relevant ecological roles, such as habitat-forming species. These organisms, including algal canopies (Bertness *et al.*, 1999), corals (Stimson, 1985), seagrasses (Connolly, 1995), mussel beds (Seed, 1996) and ascidian colonies (Monteiro *et al.*, 2002), can modify habitats, increase spatial complexity and facilitate the presence of other species. Their buffering effect (Bertocci *et al.*, 2010, Oliveira *et al.*, 2014) can protect associated taxa from the effects of disturbances and, thus, maintain diverse understory assemblages that would likely be displaced once the habitat-former was eliminated (Dayton, 1975; Pimm, 1980).

It is, thus, not surprising, that the above described issues, associated to the increasing pressure of human population on coastal areas, made the investigation of compounded anthropogenic perturbations a main focus of ecological research. In such a context, a major difficulty is given by the fact that urbanization-related pressures can take several different forms. Sewage and industrial effluents (Balestri *et al.*, 2004), aquaculture, fish trawling, chemical pollution (Fraschetti *et al.*, 2005b), human frequentation (Guarnieri *et al.*, 2012), siltation (Newell *et al.*, 1998), structural modification (Bruun, 1994), recreational activities (Wynberg and Branch, 1994; Keough and Quinn 1998), introduction of exotic species (Cohen and Carlton, 1998) and overfishing (Gray, 1997) represent a non-exhaustive list of human pressures widely examined in the scientific

literature as being able, individually or through unpredictable interactions, to enhance the stress to which populations and assemblages may be already exposed naturally (Jones, 1975; Warwick and Clarke, 1993; Chapman *et al*., 1995).

In general, it could be hypothesized that biotic homogenization (i.e. a reduction of the β-diversity) would occur both with high and low intensities and/or frequencies of urbanization-related disturbances due, respectively, to the ability to survive only by a few very resistant species that could then become dominant, or to the ability of the best competitors to out-compete the weaker ones. Intensities falling between these two extremes would represent conditions allowing the existence of mosaics of diverse assemblages and patches of available resources, thus maintaining heterogeneous and species-rich systems. Such predictions were established by the classical Intermediate Disturbance Hypothesis (IDH: Connell, 1978). Results consistent with this theory have been found by various experimental studies conducted in terrestrial (Keeley *et al*., 1981; Denslow, 1985) and aquatic (Lubchenco, 1978; Sousa, 1979a, b; Paine and Levin, 1981; Dethier, 1984) environments and by mathematical models (Caswell and Cohen, 1991; Dial and Roughgarden, 1998). In contrast, findings from experiments involving freshwater invertebrates did not support IDH predictions (Reice, 1985; Robinson and Minshall, 1986; Resh *et al*., 1988; Lake, 1990; Death and Winterbourn, 1995), while the relationship between disturbance (storms) and the diversity of reef building corals was indicated as being largely variable, depending on factors such as the life-cycles and the morphology of the colonies, the depth, the ecological history of the site and the degree of anthropogenic stress (Rogers, 1993). Similarly, mathematical simulations indicated just limited changes of diversity in response to disturbances of variable intensity and temporal frequency and relatively greater effects of the sampling intensity and the structure of examined populations (Mackey and Currie, 2000). Regarding this last issue, Wootton (1998) pointed out that expectations consistent with the IDH (according to its original formulation)

may be supported by the analysis of theoretical systems including a single trophic level, while they would apply to most real systems involving multiple trophic levels. Furthermore, for species or functional groups characterized by high resilience against physical disturbance and invasions by non-native species, maximum values of dominance and minimum of diversity have been encountered at intermediate levels of intensity of disturbance, in clear contrast with IDH predictions (Petraitis, 1989).

Although the experiments included in the present thesis were not planned and designed so that their conclusions could be directly pooled together, all together can contribute to the knowledge of responses of intertidal organisms from north Portugal rocky shores and to highlight some general principles.

The consistency of the total number of taxa (a proxy for species richness) is a first issue evident from virtually all results. Interestingly, in particular, while the number of beaches ranged between 1 (the first published paper) and 4 (the third published paper) the total of number of identified taxa just varied between 33 and 45. Even though the adopted non-destructive sampling techniques might have been partially responsible for this due to the practical impossibility to obtain a fine taxonomical identification, this high degree of consistency suggests that the selection of those replicate shores for studies examining unconfounded effects of human disturbances was an adequate decision. In practice, the identity of sampled taxa was not always the same, but the most abundant species (or higher taxa) were generally the same, with main patterns of responses to disturbance mostly driven by variation in the relative distribution of abundances.

Another aspect resulting from almost all the preformed studies was the general positive relationship between the intensity or temporal variance of disturbance and biological heterogeneity. The increasing intensity of manipulated or actual disturbances tended to be associated to an increased heterogeneity of assemblages, maintained until a certain

resistance threshold was not exceeded. This was clearly observed, for example, when high mussel harvesting levels were combined with clustered disturbances, i.e. an experimental condition that could overcome the disturbance-buffering capacity of the mussel bed, causing a clear homogenization of plots. This result can be combined with those from the urbanization study as, there, reference ("pristine") shores were characterized by lower heterogeneity of assemblages compared to urban ones. At the latter, urbanization-related disturbance could be considered as of "intermediate" (*sensu* Connell, 1978) intensity able to promote biological heterogeneity.

The main challenge in the study of the heterogeneity of populations and assemblages is due to the difficulty to separate the actual natural and anthropogenic causes of variation over a range of spatial and temporal scales (Vanderklift and Lavery, 2000; Piazzi *et al*., 2004; Pardi *et al*., 2006; Balata *et al*., 2007, 2008) and under the largely variable life-traits of organisms.
In such a context, the urbanization project, which took into account both temporal and spatial scales of organisms as part of their possible response to anthropogenic pressures, provided interesting results consistent other authors' findings (e.g. Fraschetti *et al*., 2001, 2005c) and potentially relevant also to explain some of the variability documented in the last recovery study. Specifically, the evidence that most variability of intertidal organisms occurs at small and large, rather than intermediate, spatial scales is widespread in the literature and can be attributed to several factors and processes, as previously listed. These might have been relevant also for the inconsistencies between the beaches of Aguda and Marreco detected in the fourth paper. Processes like the local circulation of water and patterns of dispersal of larvae and propagules can vary over such spatial scales and over time and be potentially invoked to explain the present site-scale variability documented in the last study, but not in the previous ones. Independently of the specific mechanisms involved,

however, such large spatial and temporal variability suggests the need for properly including a range of spatial and temporal scales as both predictive and response variables in environmental studies (e.g. Underwood and Chapman, 1996; Benedetti-Cecchi *et al.*, 2003; Fraschetti *et al.*, 2005a; Bertocci *et al.*, 2012).

In terms of relationships between disturbance and biodiversity intended as richness of species (taxa), present findings, indicating changes in relative abundances of the same taxa as the main responses to anthropogenic disturbances, clearly do not support predictions from theories considering disturbance as a major driver of richness (Connell, 1978; Huston, 1979, 1994; Connell *et al.*, 1997). The peculiarity of the systems examined here could be mostly due to the large resistance and resilience capabilities of intertidal organisms, which are naturally exposed to drastically variable environmental conditions. Nevertheless, the documentation of significant effects of several types and traits of disturbance on such organisms makes studies such as those included in the present thesis ecologically relevant under present and predicted scenarios of multiple anthropogenic perturbations whose high intensity and frequency might likely maintain exposed assemblages in altered states independently of their recovery abilities (O'Connor and Crowe, 2005; Cardinale *et al.*, 2006; Stachowicz *et al.*, 2007; Maggi *et al.*, 2009; Bertocci *et al.*, 2010).

Conclusions

Natural and anthropogenic disturbances are increasingly common on coastal ecosystems and the current forecasts indicate a progressively larger impact over natural systems due to, among others, global modifications resulting from climate change and the increase of population living in littoral areas. The independent or combined effects of these factors may cause direct great modifications on natural populations and indirectly affect processes that are key for their patterns of distribution and diversity. The evaluation of these possible impacts on natural populations over a range of spatial and temporal scales and of their ability to sustain or recover from them is, therefore, of paramount importance in modern marine ecology.

When conceiving the experimental work of this thesis, a clear option was made to examine several different aspects of human and natural impacts. The selected objectives of evaluating current effects of the pressures associated with urbanization, testing the importance of foundation species in mediating present and anticipated natural and anthropogenic compounded perturbations and examining possible “memory effects” of past disturbances on patterns of recovery of intertidal populations was consistent with some of the main topics of recent ecological research. The broader goal was that of contributing to a better knowledge of the present health of intertidal habitats and how they could be expected to evolve under the forecasted alterations of the regime of disturbances. Special focus was given to the north Portuguese coast, but relevant implications were generalizable to other similar systems. These include the documentation (not new in general, but extended to a previously untested ecological context) of the crucial role of habitat-forming species in intertidal ecosystems and of the consequences of their reduction and/or loss, the relationship found between the intensity of disturbance and habitat heterogeneity, the evidence of the need to include adequate

temporal and spatial scales when assessing environmental impacts of natural systems and, finally, the confirmation of the high resilience capability of intertidal organisms, whose responses to disturbance would consist in modifications on their relative abundances rather than alterations in their identity and overall richness.

Throughout the whole project, several specific objectives were established (as illustrated in a previous section) as guidelines towards expected accomplishments and, once achieved, as proofs for the effective progress of the experimental work. Although necessarily, to some extent, subjective, the evaluation that the planned studies took place as desired and that the proposed goals were actually achieved was reinforced by the publication of the obtained results as several papers in leading journals in the field of marine biology or ecology.

The broad field of intertidal research, with the more specific assessment of the ecological role of disturbances, is a fascinating and productive area that provided seminal knowledge on some of the most fundamental concepts of marine and general ecology. Nevertheless, its conclusions are, in several cases, contradictory. This is likely owing to the large variability of natural systems, which makes difficult to tease apart the intended effects of the examined factors from those of concomitant processes. Present findings contributed, in some cases providing the first application of a particular experimental approach to previously untested combinations of predictive variables, to understand the ecological effects of compounded disturbances, an avenue of research that will further rise in interest and importance in the near future in parallel with the increasing human pressure on natural systems and the acknowledgement of the unsuitableness and uncertainty of extrapolations and predictions derived from studies of single disturbances.

Finally, the present research provides a seed for a range of next relevant investigations. These might include the application of similar theoretical

and experimental approaches to a wider range of ecological contexts and organisms, including, for example, testing hypotheses on the effects of multiple traits of other compounded disturbances (e.g. sediment inputs, temperature variations and acidification levels are all globally relevant factors feasible to experimental manipulations in field and/or laboratory studies) and focusing on other important habitat-forming species (e.g. kelp species, reef-forming polychaetes) that are exposed and sensitive to intense anthropogenic pressures.

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Appendix

Appendix A1. Collection of data on natural storm events and choice of experimental levels.

The adopted experimental procedures used to manipulate the temporal variability of mechanical disturbance were intended to simulate the physical stress intertidal assemblages are exposed to during extreme storms, directly through the impact of large waves or indirectly through the impact of solid materials carried by waves. Preliminary considerations and data collections were carried out with the aim of providing reasonably realistic treatments.

The total intensity of experimental disturbance, quantified by the total number of events of disturbance applied over the period of the experiment, had to be comparable to the frequency of natural storm events recorded at the study area. This goal was achieved by collecting data on natural extreme storms occurred along the west coast of Portugal available from *Instituto Hidrográfico* (IH) of the Portuguese Navy. Extreme storm events were defined as those associated with waves larger than 7 m (maximum height) and north-western winds (i.e. blowing from the sea to the land at the study area). A detailed check through published data (Booij et al. 1999, Costa et al. 2001, Esteves et al. 2010) and data obtained with courtesy of Ten. P. Maia Marques and Ten. R. Esteves from IH, we could identify a total of 19 extreme storms recorded between November 1996 and March 2008 (Tab. 1) by the Leixöes oceanographic buoy (41° 19’ 00” N, 08° 59’ 00” W, 83 m depth). This corresponded, on average, to a frequency of one event every about 7 months. Scaled over the duration of our experiment (18 months, which was selected as the maximum time achievable according to the available resources) this value would have corresponded, on average and after approximation to the closest unit, to 3 events of disturbance during the study. In order to take into account the increase in the frequency of extreme storms predicted by climate models (Raper 1993, Easterling et al. 2000, Benestad 2003, Meehl et al. 2000, 2007), such frequency was doubled to 6 experimental events of disturbance over 18 months.

Appendix

In order to test for effects of predicted changes in the variance of climate-related disturbance (Katz & Brown 1992, Hughes 2000, Muller & Stone 2001, Allen & Ingram 2002, Luterbacher et al. 2004), we then established a treatment where the six events of disturbance were regularly distributed over the period of the experiment, i.e. one event exactly every three months, and one (including two different sequences: Vaselli et al. 2008) where still six events were more heterogeneously distributed, being aggregated in shorter periods, separated by prolonged periods lacking experimental disturbance (e.g. Benedetti-Cecchi 2003, Bertocci et al. 2005). In both cases, the mean interval of time between pairs of consecutive events was three months, but the variance around this mean was 0 for the regular treatment and a positive value for the irregular treatment. In order to keep such positive value consistent with the variance of natural events, we first calculated the variance of the intervals of time between each consecutive pair of the 18 storm events recorded. The total obtained value (calculated in months) of variance was about 711 (with a mean interval of 6.1), corresponding to a mean variance of 711/18 = 39.5. The final step was then to create two different random sequences of 6 events of disturbance arranged over the experimental period of 18 months in such a way that the variance of their five intervals of time was comparable to the natural value calculated. Such two sequences were both characterized by a value of variance of the intervals of time between consecutive events of 38 (in months).

The same arrangements of events for each regular and irregular treatment were applied to each study site.

Storm date (start)	**Storm date (end)**	**Max height (m)**	**Direction**	**Duration (days)**

11-11-1996	13-11-1996	7.1	NW	2.6
11-02-1997	22-02-1997	7.0	NW	11.3
07-11-1997	12-11-1997	7.7	NW	5.1
17-11-1997	26-11-1997	7.5	WNW	9.3
02-04-1998	06-04-1998	7.6	NW	4.1
22-12-1998	31-12-1998	8.8	WNW	13.6
19-10-1999	26-10-1999	7.8	WNW	6.8
18-01-2002	24-01-2002	7.7	WNW	6.7
26-01-2002	07-02-2002	7.3	WNW	12.1
24-12-2002	28-12-2002	7.7	W	4.6
10-03-2003	12-03-2003	7.4	WNW	1.9
30-10-2003	02-11-2003	9.7	NW	2.8
18-01-2005	20-01-2005	8.1	NW	2.1
23-10-2006	24-10-2006	7.1	WNW	0.5
03-12-2006	10-12-2006	8.5	NW	7.3
08-02-2007	14-02-2007	7.4	WNW	5.5
09-12-2007	11-12-2007	7.6	NW	1.8
02-01-2008	07-01-2008	7.0	WNW	4.5
08-03-2008	13-03-2008	8.5	NW	4.6

Tab. 1. Extreme storms recorded at Leixöes (courtesy of R. Esteves from IH, modified).

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Appendix A2. Description of statistical analyses.

Response variables for the effects of experimental treatments were measures of variance in the abundance of individual taxa, in the total number of taxa and in the structure of assemblages over the period of the study.

For univariate analyses, variance components were calculated by means of one-way analyses of variance (ANOVAs) with Time as a factor (nine levels, corresponding to the nine dates of sampling), performed separately for each response variable in each experimental plot. This method produced estimates of temporal variance independent of sampling error (Searle et al. 1992). Negative values were considered as underestimates of null variances (Searle et al. 1992) and set to zero (Underwood 1997).

Multivariate pseudo-variance components (Anderson & Millar 2004) for the factor Time were estimated from a Bray-Curtis dissimilarity matrix (Bray & Curtis 1957) calculated for each

plot separately using multivariate analysis of variance based on permutations (PERMANOVA, Anderson 2001).

Variance and pseudo-variance components were finally analysed with a partially asymmetrical ANOVA, including the factors and levels described in detail in the text of the main paper. Before each ANOVA, the assumption of homogeneity of variances was checked through Cochran's test. In some cases, data were log-transformed to make variances homogenous. When this was not possible, such as for the total number of taxa only, data were left untransformed and ANOVA considered robust in case of non-significant results (Underwood 1997).

Univariate patterns (see appendix A4) were visualized by calculating the average value of each response variable over the three experimental plots sampled at each date. Multivariate patterns were visualized (see appendix A3) by means of a non-metric multidimensional scaling (nMDS) plot of centroids representing the average assemblage of the three plots sampled at each of nine dates over the course of the experiment. Centroids were calculated, for each plot and time of sampling, by generating a matrix based on Bray-Curtis dissimilarities (Bray & Curtis 1957) among the whole set of 351 observations (39 plots x 9 times of sampling) available at each study site. Since Bray-Curtis is a semi-metric index (Legendre & Anderson 1999), centroids could not be obtained by simply averaging these similarities (Anderson 2001). Therefore, principal coordinates (Gower 1966) were first calculated from the Bray-Curtis dissimilarity matrix. This procedure placed the observations into an Euclidean space in such a way that the distance between each pair of observations as resulting from principal coordinates was equivalent to the Bray-Curtis dissimilarity between the same observations obtained from the original variables. Centroids were calculated by arithmetically averaging the principal coordinates over the three plots assigned to each experimental treatment, separately for each date of sampling (McArdle & Anderson 2001).

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Appendix A3. nMDS plots of centroids of assemblages over the study (1 to 9: dates of sampling).

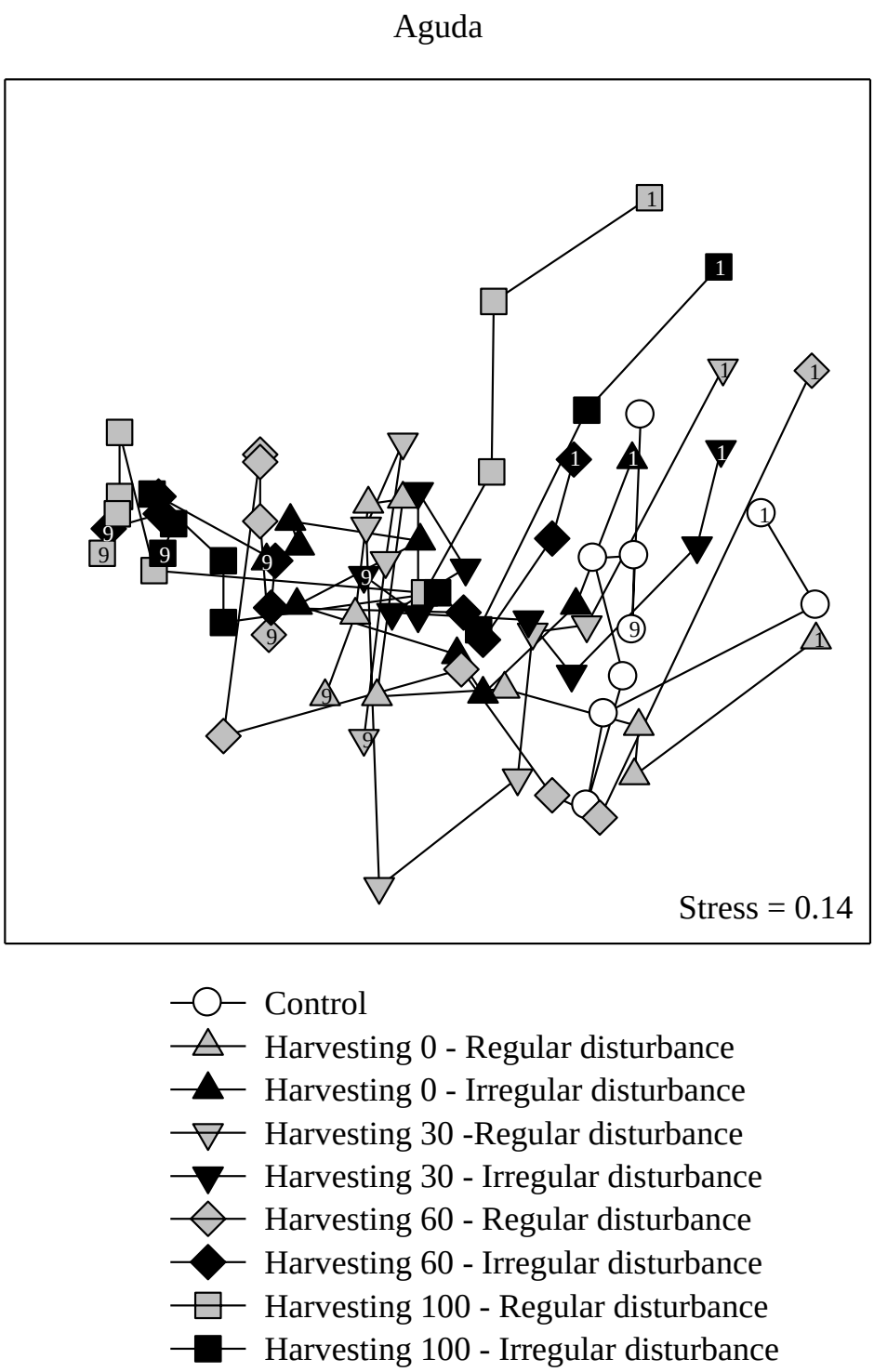


Fig. 1

Marreco

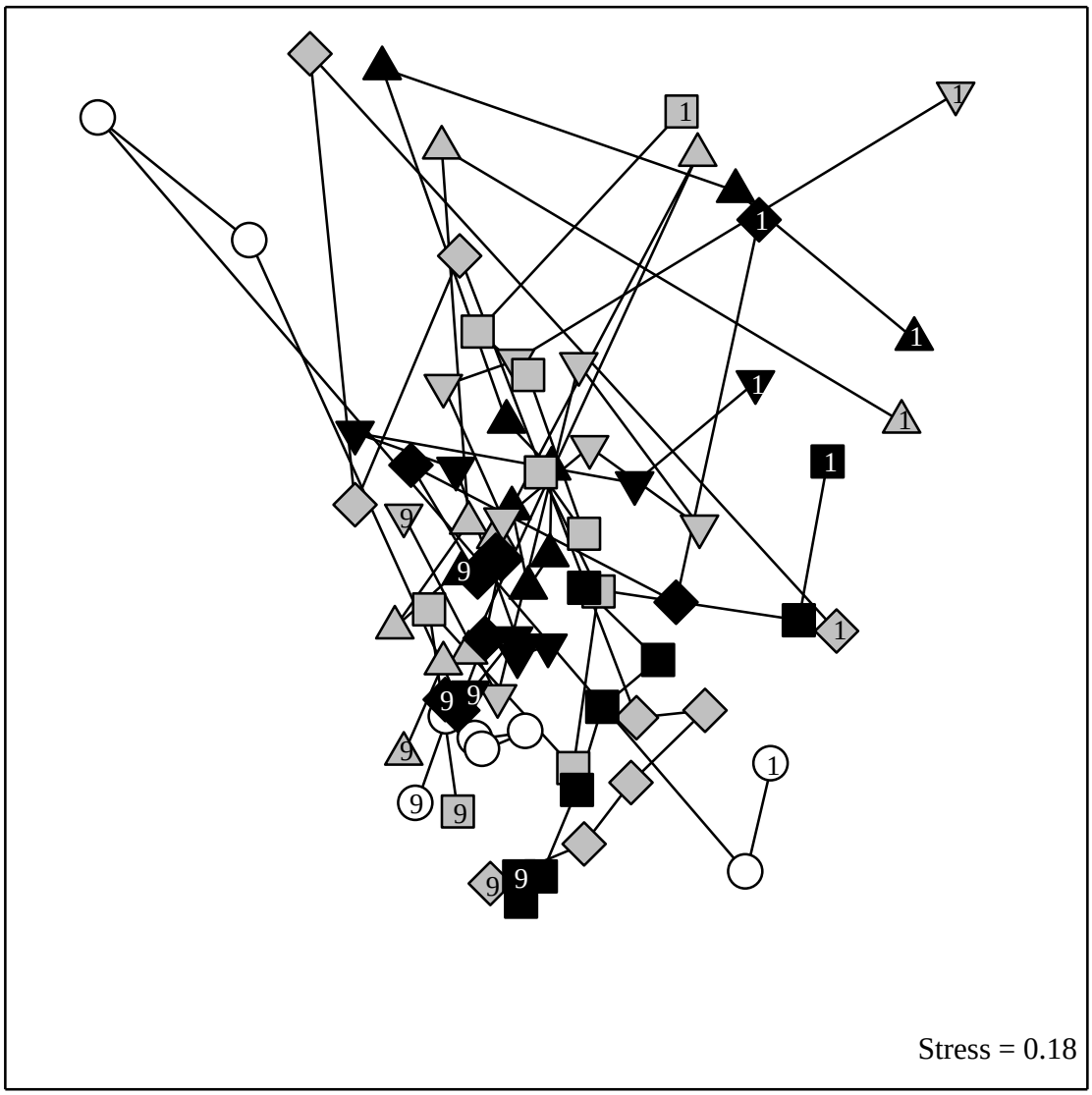
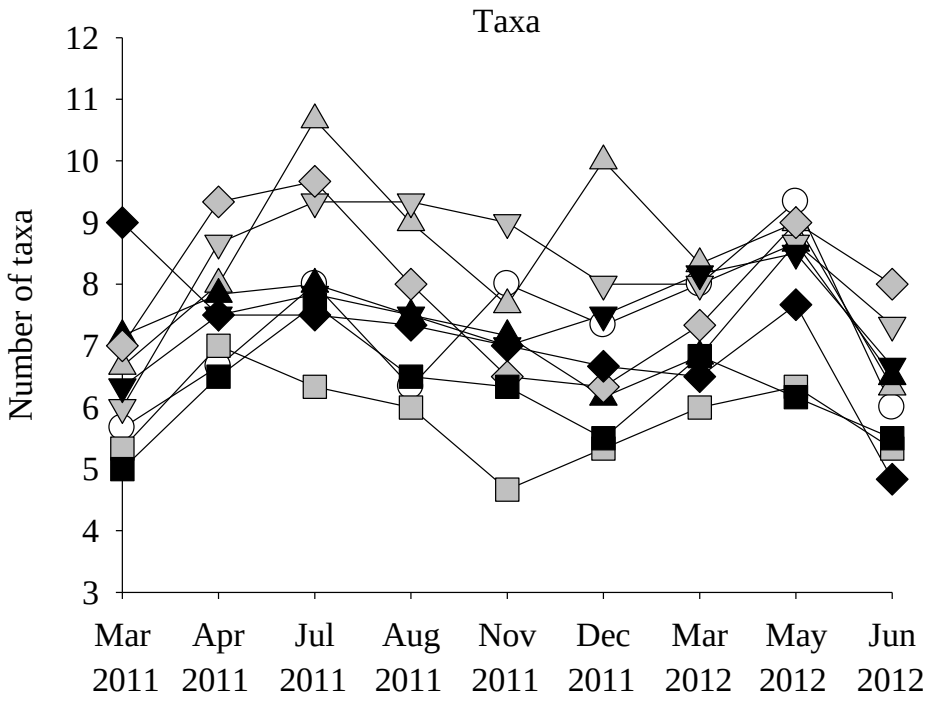


Fig. 2 (symbols as in Fig. 1).

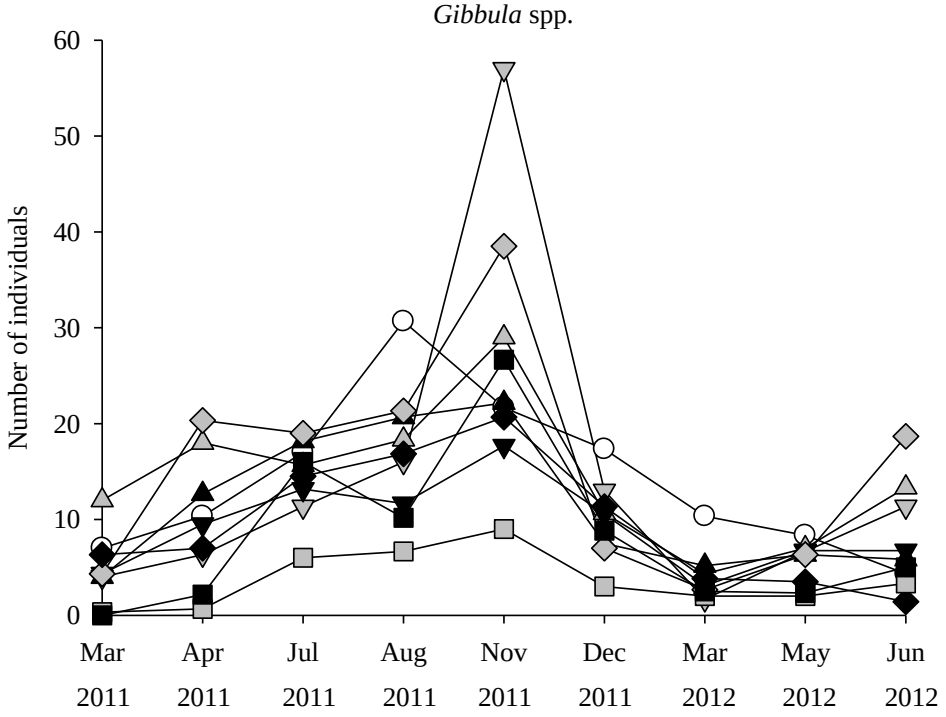
Appendix A4. Mean abundance and mean number of common taxa in control (n = 3) and manipulated plots (n = 3 for regular treatment; n = 6 for irregular treatment) over the study (9 dates of sampling). Symbols as in Appendix A3. Standard Error bars not reported for clarity of reading.

<u>Aguda</u>

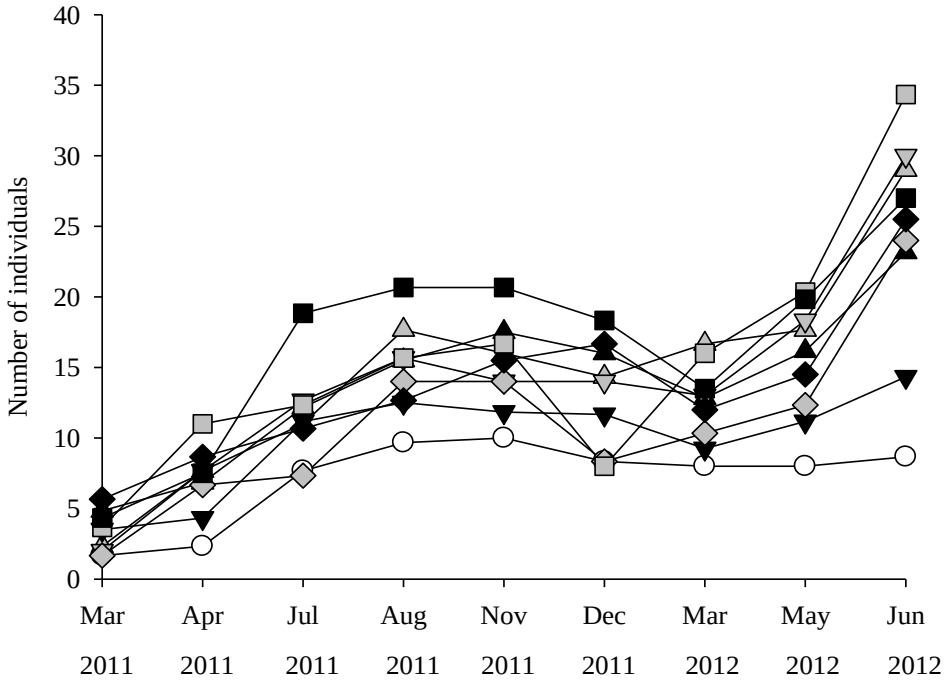


Appendix

Gibbula spp.

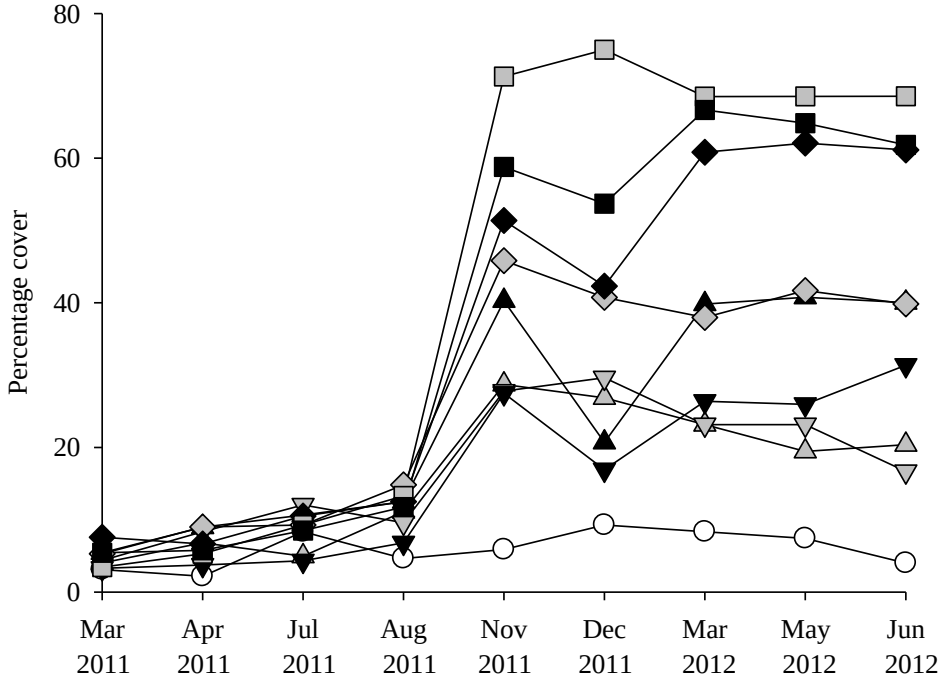


Patella spp.

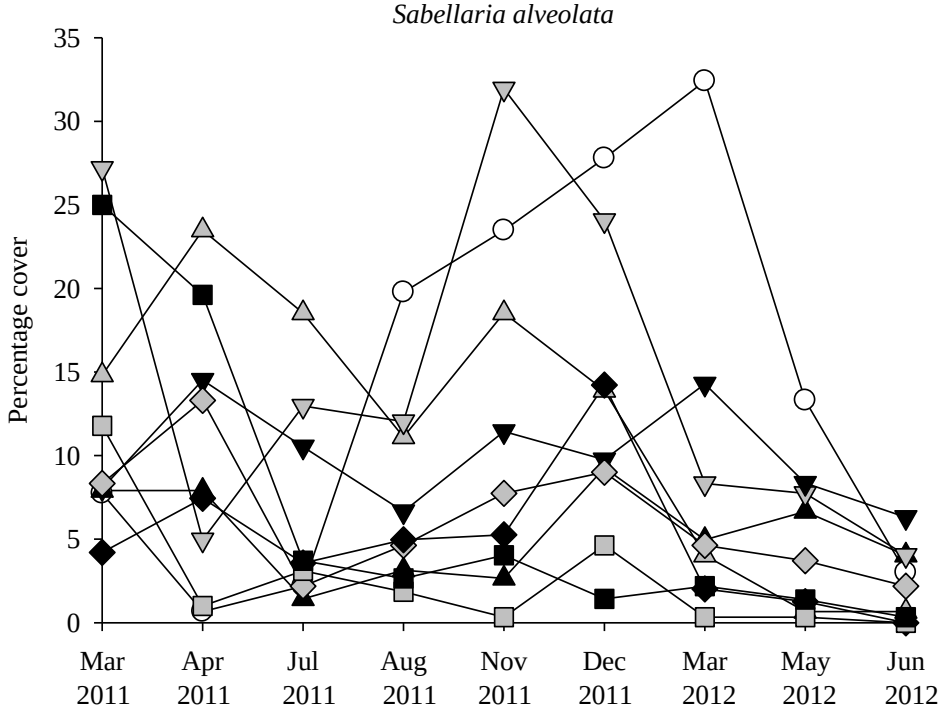


Appendix

Chthamalus stellatus

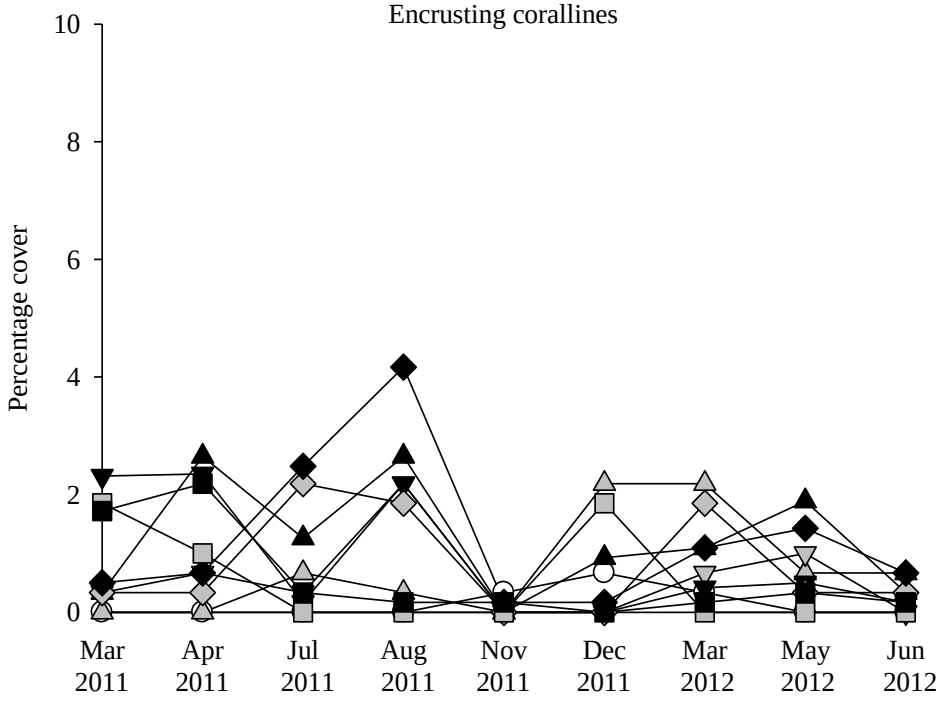


Sabellaria alveolata

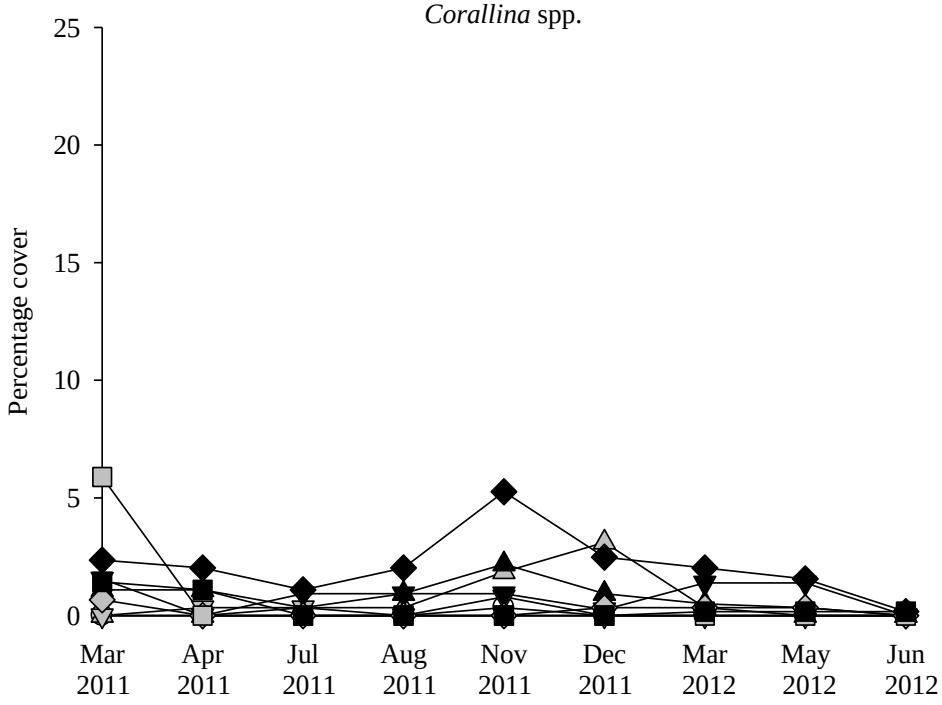


Appendix

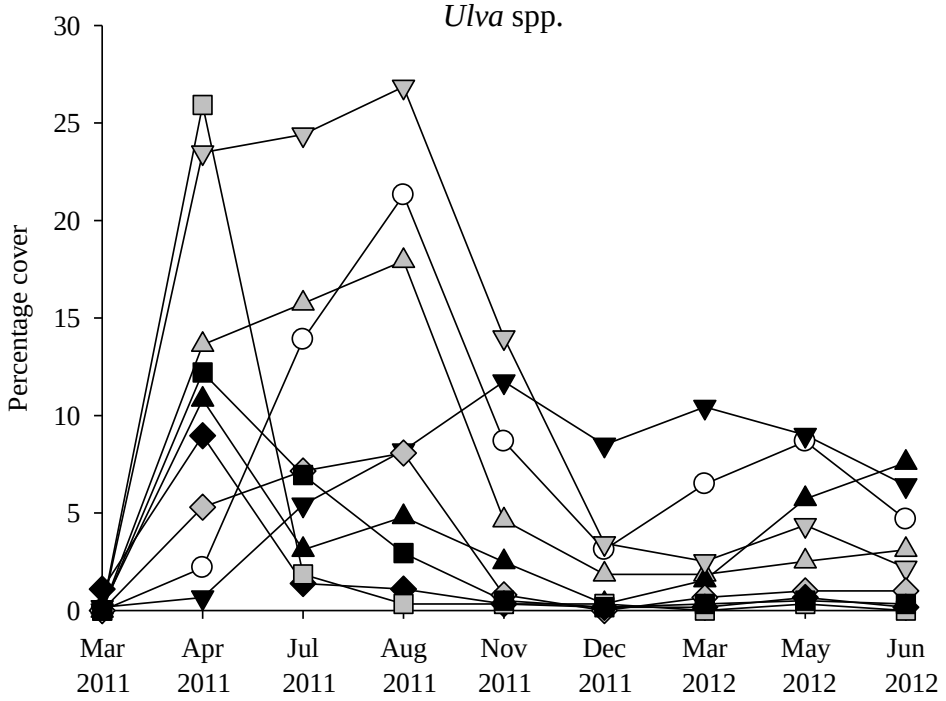
Encrusting corallines



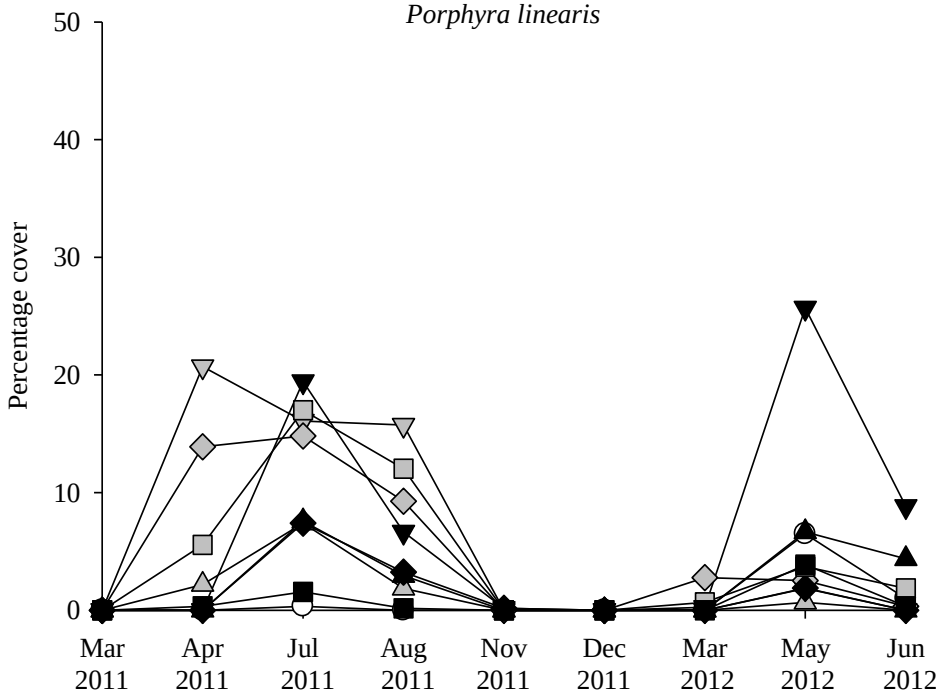
Corallina spp.



Ulva spp.

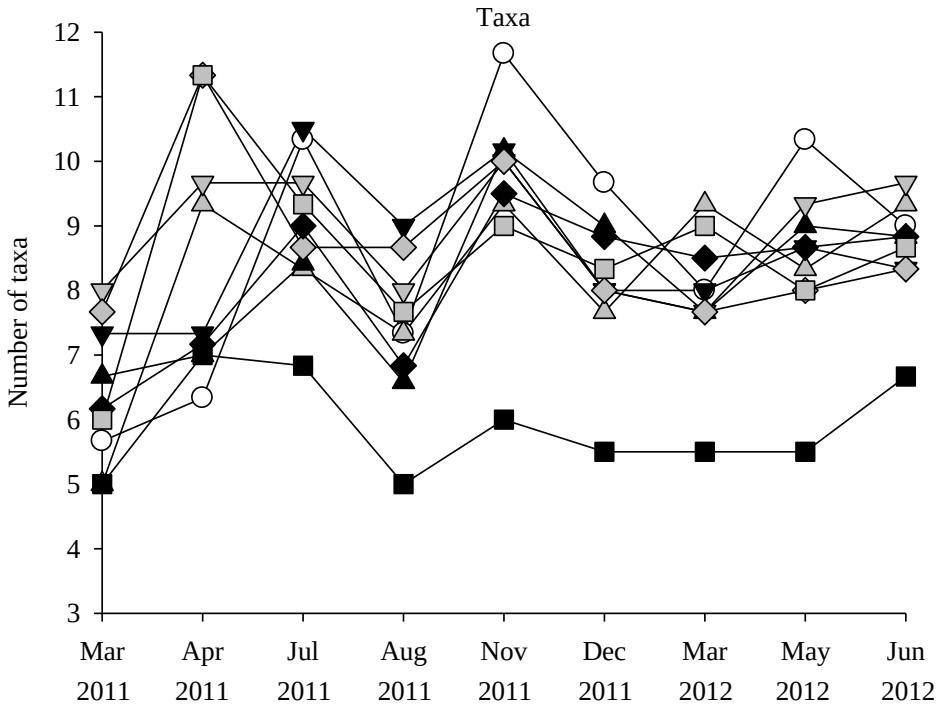


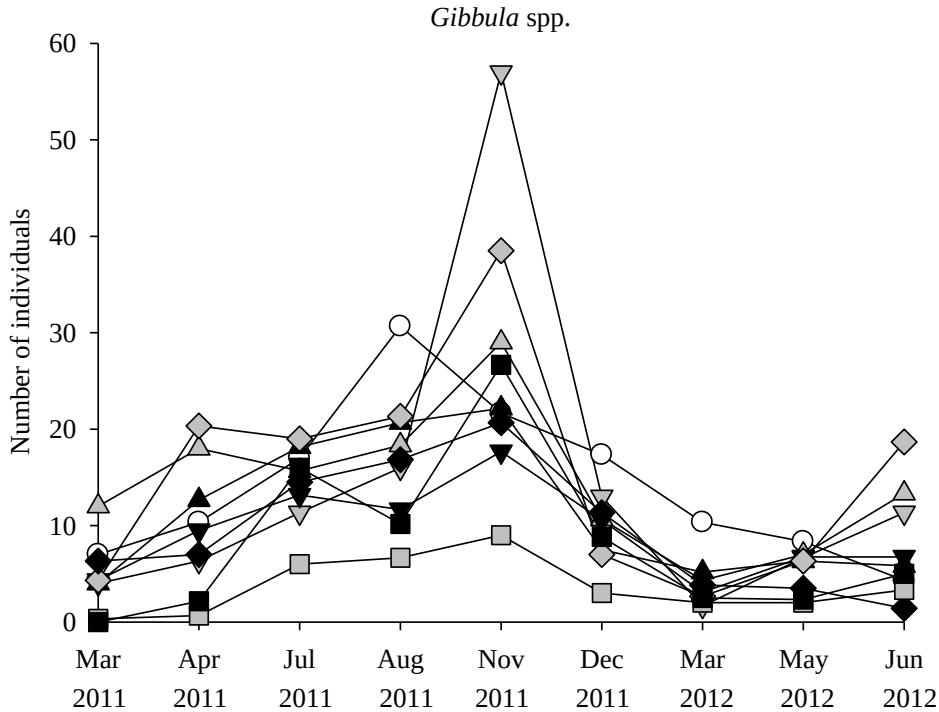
Porphyra linearis



Marreco

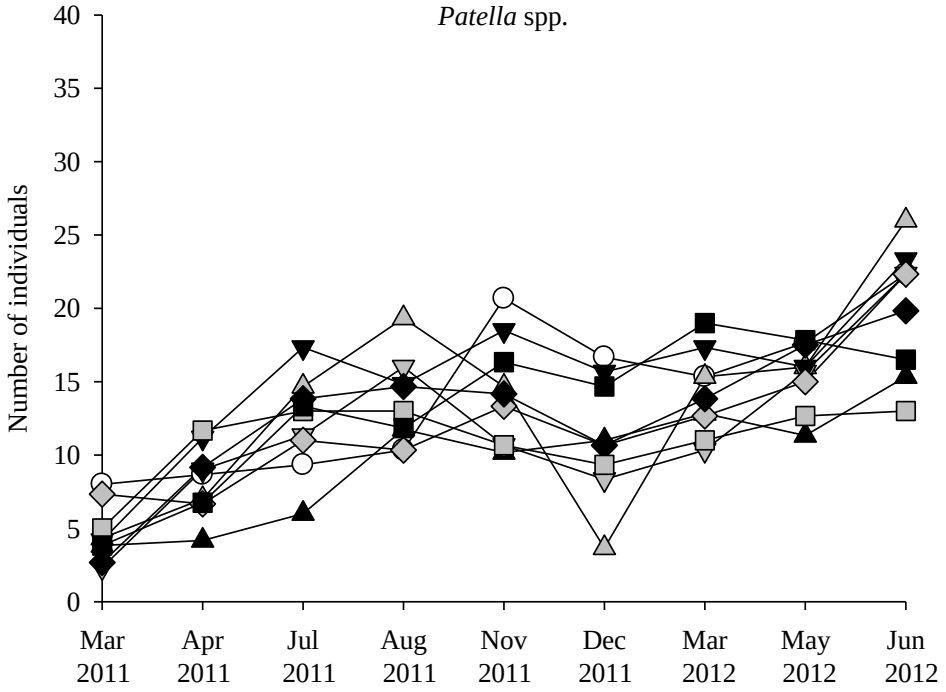
Appendix


Taxa
Number of taxa
12
11
10
9
8
7
6
5
4
3
Mar 2011
Apr 2011
Jul 2011
Aug 2011
Nov 2011
Dec 2011
Mar 2012
May 2012
Jun 2012

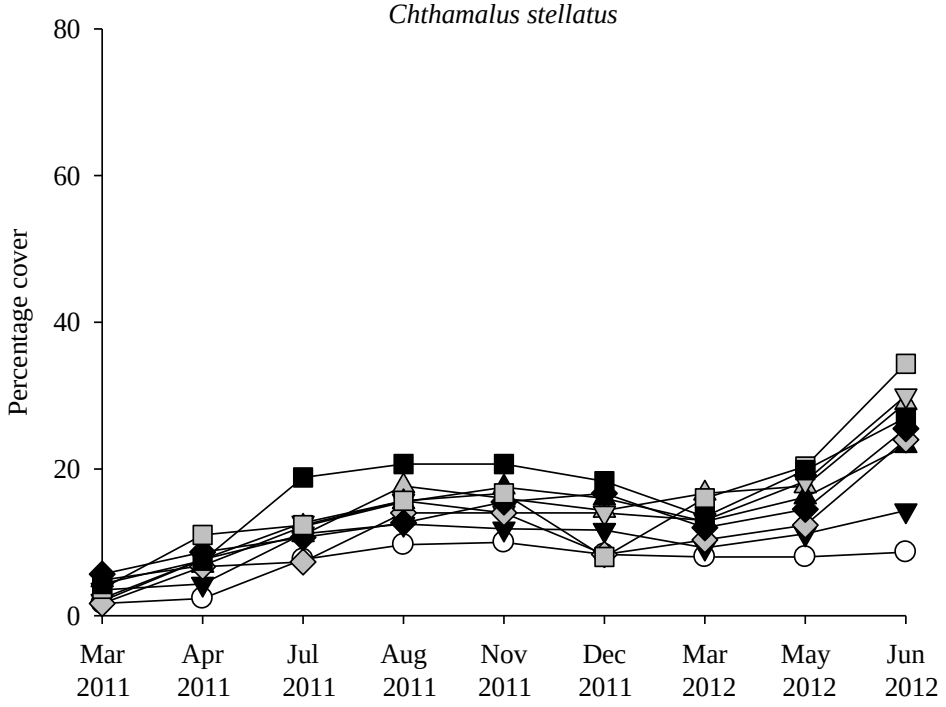

Gibbula spp.
Number of individuals
60
50
40
30
20
10
0
Mar 2011
Apr 2011
Jul 2011
Aug 2011
Nov 2011
Dec 2011
Mar 2012
May 2012
Jun 2012

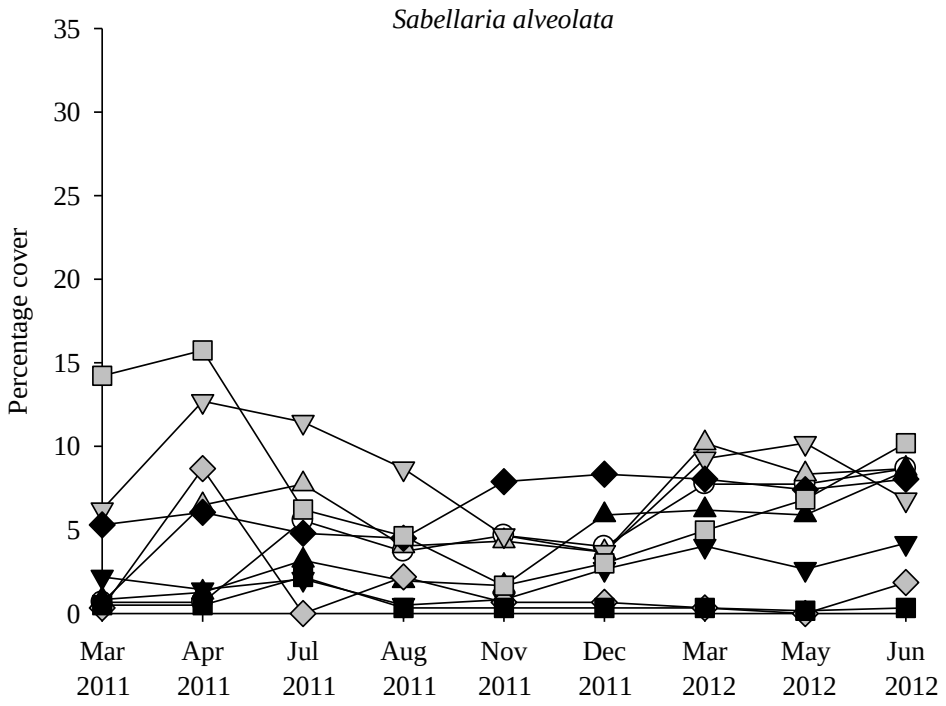
Appendix

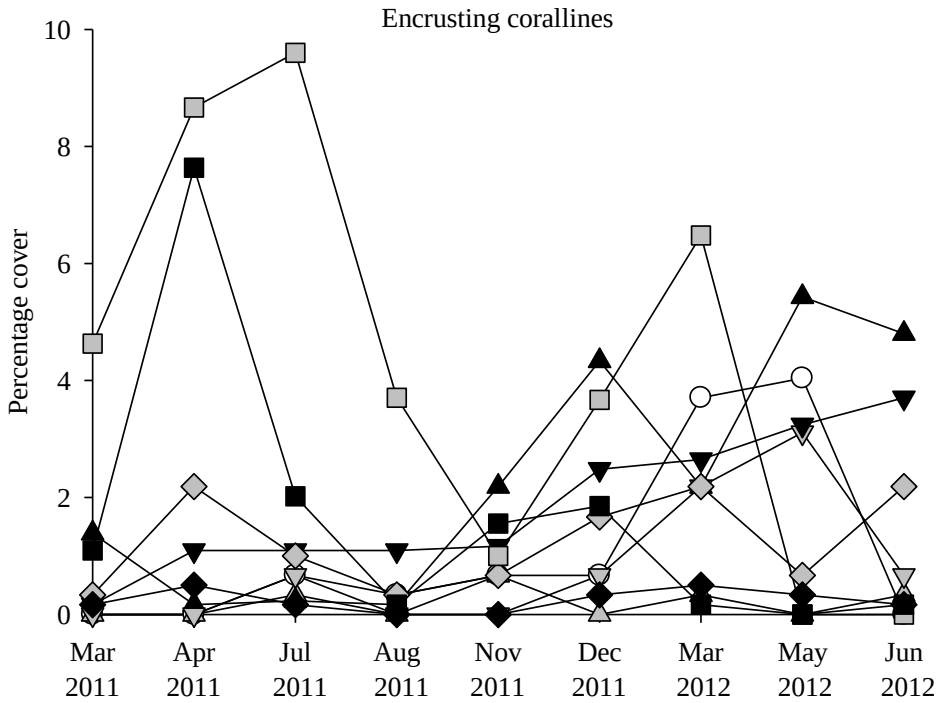
Patella spp.



Chthamalus stellatus

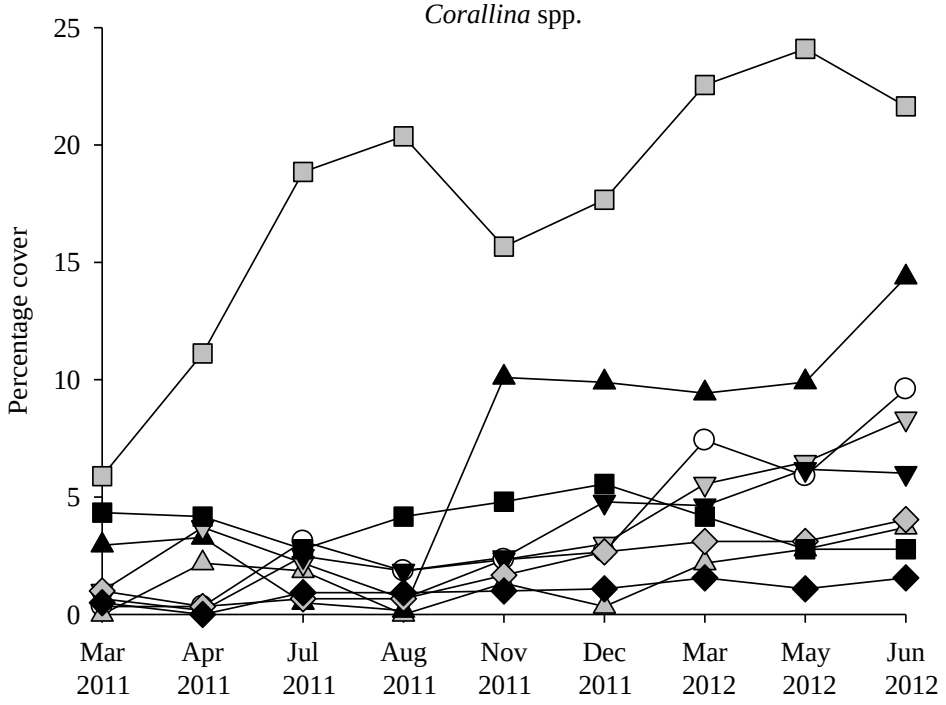



Sabellaria alveolata
Percentage cover
35
30
25
20
15
10
5
0
Mar 2011
Apr 2011
Jul 2011
Aug 2011
Nov 2011
Dec 2011
Mar 2012
May 2012
Jun 2012

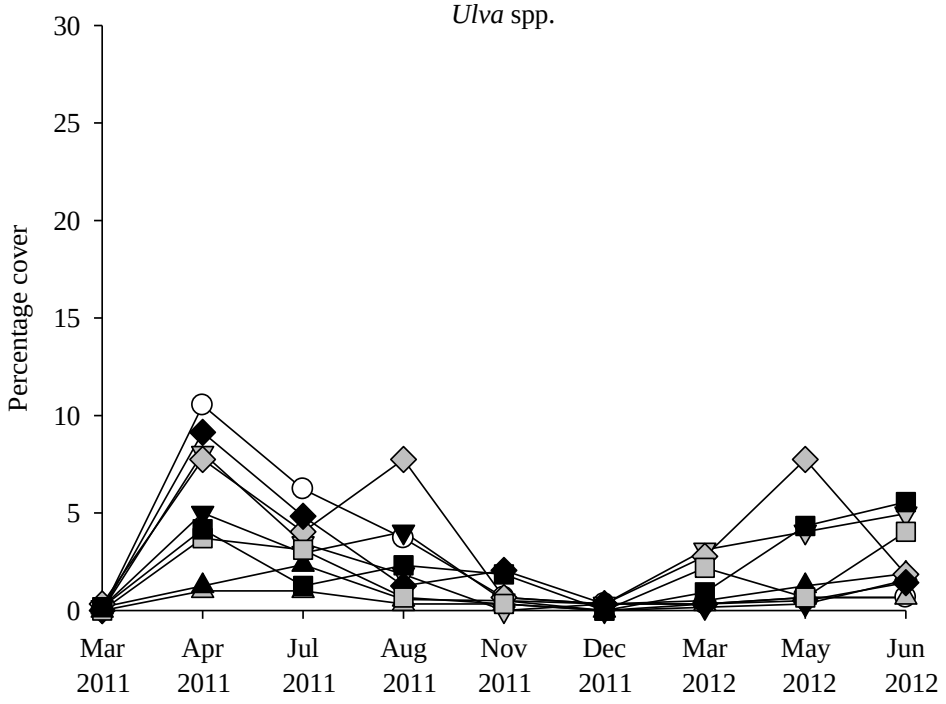

Encrusting corallines
Percentage cover
10
8
6
4
2
0
Mar 2011
Apr 2011
Jul 2011
Aug 2011
Nov 2011
Dec 2011
Mar 2012
May 2012
Jun 2012

Appendix

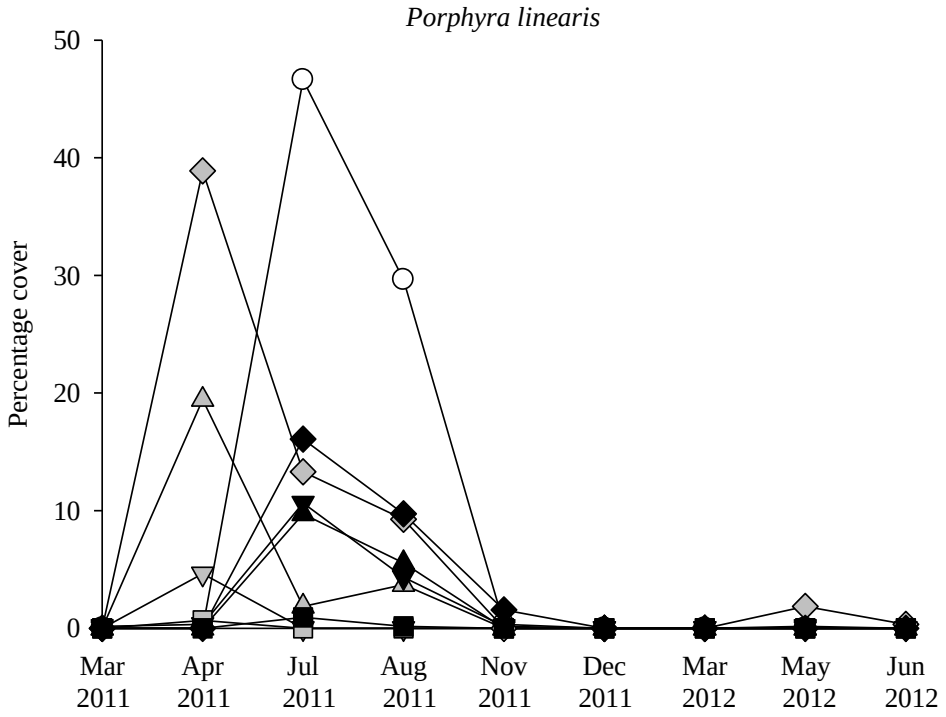
Corallina spp.



Ulva spp.



Appendix


Porphyra linearis
Percentage cover
50
40
30
20
10
0
Mar 2011
Apr 2011
Jul 2011
Aug 2011
Nov 2011
Dec 2011
Mar 2012
May 2012
Jun 2012

Appendix A5. Table 1. ANOVA on multivariate temporal variance in the structure of assemblages over the course of the experiment at each study site (ns = not significant, * p<0.05, ** p<0.01).

		Aguda		Marreco	
Source of variation	df	MS	*F*	MS	*F*
Among experimental levels	12	499731.8	3.17**	705479.4	2.98**
Control vs. treatments	1	29366.6	0.19	1399161.4	5.91 *
Among treatments	11	542492.3	3.44**	642417.5	2.71 *
Temporal variability = V	2	280136.8	1.78	19843.7	0.08
Low vs. High variability	1	559990.5	1977.39 *	33343.1	5.26
Among sequences = S	1	283.2	0.00	6344.3	0.03
Harvesting intensity = I	4	331342.0	2.10	1651310.4	6.97**
I x V	6	735519.3	4.66**	345495.5	1.46
I x Low vs. High variability	3	1330754.1	9.49 *	568514.0	4.64
I x S	3	140284.4	0.89	122477.1	0.52
Residual	26	157745.6		236925.8	
Total	38				
Cochran's *C* test	*C* = 0.289, ns				
Transformation	None				

Appendix A5. Table 2. ANOVA on measures of temporal variance in the abundance of the total number of taxa and dominant organisms over the course of the experiment at Aguda (ns = not significant, * p<0.05, ** p<0.01, *** p<0.001).

			Taxa		*Gibbula umbilicalis*		*Patella* spp.		*Chthamalus*. *stellatus*		*Sabellaria alveolata*	
Source of variation		df	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
Among experimental levels		12	1.13	0.75	27875.48	1.59	1.86	3.56**	493621.80	3.71**	33091.23	1.28
Control vs. treatments		1	0.01	0.01	4966.57	0.28	3.46	6.62 *	790173.68	5.94 *	75167.61	2.90
Among treatments		11	1.23	0.81	29790.43	1.69	1.71	3.28**	466662.54	3.51**	29266.11	1.13
Temporal variability	= V	2	1.11	0.73	17440.63	0.99	2.84	5.44 *	432088.71	3.25	34810.93	1.35
Low vs. High variability		10.20	0.1018462.68		0.83	1.61	0.40	208677.92	0.32	14497.87	0.26	
Among sequences	= S	1	2.02	1.33	22150.96	1.26	4.06	7.79**	655499.49	4.93 *	55123.99	2.13
Harvesting intensity	= I	4	0.68	0.45	14310.09	0.81	1.84	3.52 *	1170344.07	8.79***	38903.11	1.50
I x V		6	1.55	1.02	41647.20	2.37 *	1.27	2.44 *	126346.39	0.95	22599.33	0.87

Appendix

I x Low vs. High variability	3	0.15	0.05	71752.13	6.22	1.57	1.60	177915.17	2.38	30636.02	2.10
I x S	3	2.95	1.94	11542.27	0.66	0.98	1.88	74777.61	0.56	14562.65	0.56
Residual	26	1.52		17579.79		0.52		133086.24		25877.20	
Total	38										
Cochran's *C* test		*C* = 0.514, p<0.01		*C* = 0.348, ns		*C* = 0.310, ns		*C* = 0.207, ns		*C* = 0.310, ns	
Transformation		None		None		Ln(x+1)		None		None	

Appendix

Table 2, cont.

Source of variation	df	Encrusting corallines MS	*F*	*Corallina* spp. MS	*F*	*Ulva* spp. MS	*F*	*Porphyra linearis* MS	*F*
Among experimental levels	12	1.09	1.29	7.40	7.79 ***	3.15	0.48	6.18	1.76
Control vs. treatments	1	2.68	3.18	7.08	7.45 *	2.14	0.33	2.50	0.71
Among treatments	11	0.94	1.12	7.43	7.83 ***	3.25	0.50	6.52	1.86
Temporal variability = V	2	1.57	1.86	0.43	0.45	4.78	0.73	2.45	0.70
Low vs. High variability	11.15	0.58	0.84	53.19	6.82	2.49	4.90	16326.00	**
Among sequences = S	1	1.99	2.36	0.02	0.02	2.73	0.42	0.00	0.00
Harvesting intensity = I	4	0.41	0.49	14.20	14.95 ***	2.09	0.32	8.32	2.37
I x V	6	1.00	1.19	6.39	6.72 ***	3.31	0.51	6.97	1.98
I x Low vs. High variability	3	0.29	0.17	11.08	6.56	1.75	0.36	3.67	0.36

Appendix

I x S		3	1.71	2.03	1.69	1.78	4.88	0.75	10.27	2.92
Residual	26	0.84		0.95		6.51		3.51		
Total	38									
Cochran's *C* test		*C* = 0.257, ns			*C* = 0.444, ns			*C* = 0.163, ns		*C* = 0.209, ns
Transformation		Ln(x+1)			Ln(x+1)			Ln(x+1)		Ln(x+1)

Appendix A5. Table 3. ANOVA on measures of temporal variance in the abundance of the total number of taxa and dominant organisms over the course of the experiment at Marreco (ns = not significant, * p<0.05, ** p<0.01, *** p<0.001).

			Taxa		*Gibbula umbilicalis*		*Patella* spp.		*Chthamalus*. *stellatus*		*Sabellaria alveolata*	
Source of variation		df	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
Among experimental levels		12	2.97	1.10	9439.86	3.39**	2004.96	1.79	4.39	2.43 *	4.37	2.90 *
Control vs. treatments		1	6.80	2.52	3372.07	1.21	911.94	0.81	0.21	0.11	0.48	0.32
Among treatments		11	2.62	0.97	9991.48	3.59**	2104.33	1.88	4.78	2.64 *	4.72	3.14**
Temporal variability	= V	2	1.10	0.41	1383.27	0.50	922.69	0.82	0.18	0.10	3.94	2.62
Low vs. High variability		11.28	1.40	2467.37	8.25	7.64	0.00	0.06	0.20	6.76	5.96	
Among sequences	= S	1	0.92	0.34	299.18	0.11	1837.74	1.64	0.30	1.16	1.13	0.75
Harvesting intensity	= I	4	2.85	1.06	28694.90	10.30***	4873.85	4.35 *	16.61	9.19***	10.89	7.24**
I x V		6	3.01	1.11	3509.17	1.26	1113.44	0.99	0.39	0.22	1.90	1.26

Appendix

I x Low vs. High variability	3	2.52	0.72	6563.94	4.45 *	2108.81	17.86 *	0.32	0.68	2.76	2.67
I x S	3	3.51	1.30	454.39	0.16	118.07	0.11	0.47	0.26	1.03	0.69
Residual	26	2.70		2785.06		1119.39		1.81		1.51	
Total	38										
Cochran's *C* test		*C* = 0.317, ns		*C* = 0.347, ns		*C* = 0.308, ns		*C* = 0.204, ns		*C* = 0.372, ns	
Transformation		None		None		None		Ln(x+1)		Ln(x+1)	

Table 3, cont.

Source of variation		df	Encrusting corallines MS	*F*	*Corallina* spp. MS	*F*	*Ulva* spp. MS	*F*	*Porphyra linearis* MS	*F*
Among experimental levels		12	1.94	0.97	6.43	2.75 *	3.17	1.37	12.31	6.14***
Control vs. treatments		1	0.00	0.00	0.03	0.01	2.45	1.05	43.00	21.41***
Among treatments		11	2.12	1.05	7.01	3.00 *	3.24	1.39	9.53	4.75***
Temporal variability	= V	2	1.81	0.90	0.51	0.22	10.79	4.64 *	1.05	0.53
Low vs. High variability		10.66	0.22	0.52	1.07	1.16	0.06	1.19	1.29	
Among sequences	= S	1	2.97	1.47	0.49	0.21	20.42	8.79**	0.92	0.46
Harvesting intensity	= I	4	3.98	1.98	16.26	6.95**	3.20	1.38	12.77	6.36**
I x V		6	1.29	0.64	64.56	1.95	0.75	0.32	10.74	5.35**

Appendix

I x Low vs. High variability	3	0.84	0.48	8.93	48.20**	0.47	0.46	4.36	0.25
I x S	3	1.74	0.87	0.19	0.08	1.02	0.44	17.12	8.53***
Residual 26	2.01		2.34		2.32		2.01		
Total	38								
Cochran's *C* test		*C* = 0.315, ns		*C* = 0.206, ns		*C* = 0.166, ns		*C* = 0.306, ns	
Transformation		Ln(x+1)		Ln(x+1)		Ln(x+1)		Ln(x+1)	