Discussions and conclusions

In the marine realm, besides the lack of reliable historical records about what pristine systems may have looked like (Bulleri et al. 2007), assessment and interpretation of ecological impacts is compromised by the tendency of current scientific and management practices to consider the effects of individual threats in isolation. Attempts to address the increasing diversity and intensity of anthropogenic stressors should first recognize that human activities are acting at multiple temporal and spatial scales, making it particularly difficult to quantify past historical changes and present human effects and to predict future changes in biodiversity (McLeod et al. 2005).

Effects of multiple stressors can lead to the loss of resilience and an increased risk of regime shifts, which are often long lasting and difficult to reverse (Hughes 1994; Hughes et al. 2003; Hughes et al. 2005; Casini et al. 2009). Regime shifts are also currently difficult to predict (de Young et al. 2008), but implications in highly impacted ecosystems are clear: they result in homogenization of communities and ecosystems due to reductions in foodweb complexity, diversity within functional groups and biogenic habitat structure, as well as decreases in the size of organisms. Localized human perturbations combined with new threats such as climate change, invasive species and ocean acidification all contribute to generating new regimes of disturbances that are expected to greatly affect the stability and productivity of marine coastal ecosystems.

Despite the occurrence of the date mussel fisheries in the Mediterranean and its huge and significant impact on subtidal communities (Fraschetti et al. 2001; Guidetti et al. 2003; Guidetti and Boero 2004; Guidetti et al. 2004), knowledge about patterns of recovery of disturbed assemblages affected by this impact is still scarce. Date mussel fishery is not the only source of disturbance in Apulia. Very few experimental studies on marine communities had tried to understand how multiple stressors operating at different spatial scales affect the recovery of disturbed assemblages (Hughes and Connell 1999; Gardner 2005; Crain et al. 2008). Natural coastal systems are constantly affected by different source of disturbance so preserving the resilience of ecosystems should be an essential component of all conservation strategies, since disturbances most of times cannot be prevented (Knowlton 2004). One of the most important challenges of experimental ecology is to take into account the complexity of the phenomena involved in marine environment and try to quantify the cumulative effects of the interaction of these processes, including human threats.

Worm and Lotze (2006) tried to assess the interactive effects of eutrophication and grazing, considering these two processes as crucial in explaining community dynamics and recovery patterns of the rocky subtidal subject to anthropogenic pressures. Grazers are important in controlling algal bloom, but they cannot always definitively counter the effects of eutrophication. McClanahan et al. (2003), as other authors, conducted experiments to quantify which were the driving factors in coral reefs responsible of a shift from assemblages at high diversity to assemblages at low diversity and dominated by algae. They concluded that responsible of the shift is the interaction between the intake of nutrients and the grazing respondible of the control of algal communities and the structure and function of corals.

In the Marine research Station of Solbergstrand, Bokn et al. (2002) examined the response of rocky shore ecosystems to increased nutrient availability in eight land-based mesocosms. The growth rate of the periphyton and fast-growing macroalgae communities was stimulated by nutrient enrichment, while the response was less evident among perennial fucoids. Their results showed that the total system metabolism tended to increase slightly, but not significantly, with increased nutrient loading.

Jara et al. (2006) tried to assess the interactive effects of disturbance and nutrient enrichment at two sites on the coast of Brazil with widly different trophic condition (1 oligotrophic and 1 eutrophic). Their results revealed site-specific diversity-driving processes in the absence of disturbance. Nutrient enrichment increased total species richness and algal species richness in particular, but only at the oligotrophic site. Such interactive effects of disturbance and productivity on diversity confirm the general predictions of the Intermediate Disturbance Hipotesis Models (Sousa 1979). This study indicates that interactive effects of ‘bottom-up’ and ‘top-down’ processes may explain more of the variation in community diversity than the separate models of disturbance–diversity and productivity–diversity relationships.

Onother important factorial field experiment was conducted by Korpinen et al (2007). They manipulated nutrient levels and herbivory at two sublittoral depths and measured macroalgal colonization and the following young assemblage during the growing season. At the community level, grazing reduced algal colonization, though the effect varied with depth and its interaction with nutrient availability varied in time. In shallow water, for example, the total density of macroalgae increased in response to nutrient enrichment, but the ability of grazers to reduce macroalgal density also increased with the nutrient enrichment, and thus, the community could not escape from the top-down control. Their conclusions support the concept that temporal and spatial variability in both top-down and bottom-up control and in their interaction, especially along the depth gradient, may be crucially important for increasing diversity and for the successional dynamic in a rocky sublittoral environment. Guerry (2008) used rocky intertidal macroalgal communities to examine the effects of limpet grazing and nutrient enrichment on algal diversity throughout two years of succession revealing that grazing effects varied in time. During the first year, grazing effects were context-dependent with limpets resulting in lower species richness, especially at the highest level of limpet density. However, at the highest level of limpet density, high enrichment counteracted the negative effect of limpets such that diversity was similar to that in treatments with lower limpet densities. In the second year, grazing generally decreased richness values, regardless of enrichment.

Also at temperate latitudes, sea urchins grazing can be considered a small-scale process crucial in shaping rocky assemblages. It can directly influence colonization patterns of many algal species. Specifically in Southern Apulia, grazing is considered the major responsible to maintain barrens of rocky substrates impacted by the destructive date mussel fishery (Boero et al. 1996; Fanelli et al. 1994; Guidetti et al. 2004). In our experiment, we have quantified the separate and combine effect of grazing and nutrient enrichment in shaping recovery trajectories of the hard substrate damaged by the date mussel fishery. To our knowledge this was the first experiment trying to quantify the relative importance of the two factors in the subtidal on experimental plots of this size.

The no take zone of the Marine Protected Area of Porto Cesareo, chosen as study site, represents an ideal experimental context, where barren extension cover the 80% of the protected location. Notwithstanding the present level of enforcement, the severely impacted rocky plateau still does not show signs of recovery of the benthic assemblages. This condition supported our decision to develop the experiment in this location, quantifying the recovery trajectories of already disturbed assemblages.

Enhanced nutrients availability regulates primary productivity and species composition in aquatic ecosystems (Worm et al. 2000) favour phytoplankton and annual bloom forming algae. In many cases, nutrients supply is spatially and temporally variable, but occurs on large scale when derives from land run-off and wind-induced mixing and upwelling phenomena. Primary producers have evolved different strategies to exploit heterogeneity in nutrient supply. Microalgae and filamentous macroalgae have a relatively high surface area to volume ratio that results in rapid nutrient uptake and fast growth (Hein et al. 1995), but low nutrient storage capacity (2 to 8 days for filamentous algae). Perennial, canopy-forming macroalgae have thick, corticated thalli, and low surface area to volume ratios. They have slower nutrient uptake and growth rates, but higher nutrient storage capacities compared with phytoplankton and filamentous algae (weeks to several months , Pedersen and Borum 1996).

Our results suggest that grazing and nutrient enrichment could interact on biodiversity recovery and that oligotrophic conditions can be particularly critical in case of habitat destruction and fragmentation, as in our case.

Under non-enriched conditions and in presence of initially disturbed assemblages, herbivores exert a crucial role on biodiversity patterns, strongly limiting the potential of recovery of benthic assemblages. In such disturbed systems, recovery fails because of the presence of grazers. These results are partially strengthened with those already obtained from previous works that studied the effect of natural disturbance (Sala and Graham 2002; Guidetti et al. 2003).

Nutrient enrichment enhances the resilience of the system with the substantial disappearance of the barren. However, assemblages were still mostly characterized by algal turf when combined with grazing activity. Recolonization by macroalgae occurred only when grazing activity is removed. In particular, in those plots where grazing was removed and nutrients were enhanced, there was a gradual increase over time of the number of taxa, which implies a change in the structural diversity of the benthic assemblages. In contrast, where only the pressure of grazing was eliminated, the number of taxa generally did not exceed the maximum value obtained addicting nutrients and removing sea urchins. If we consider results of percentage cover, in some cases the maximum value (100%) had been reached, but, for example after one month from the beginning of the experiment, this value was determined by a low diversity assemblage constituted by unicellular algae and seaweed mucilaginous, often opportunistic species able to maximize the use of resources in the environment.

Results shows that differences between the assemblages under different driver effects strictly reflect the functional role of some algal species. The use of functional groups across different taxa that coexist in the same habitat surely provides the possibility to better understand and quantify community dynamics.

These results are supported by the functional composition of the assemblages observed. After one month from the beginning of the experiment, in the enriched plots, assemblages were characterized by ephemeral algae called STEA. This is a fairly common result, occurring during the first step of recolonization of a disturbed substrate, followed by a successive colonization and by an increase of structural and functional complexity. In presence of grazers, colonization processes do not go further the presence of algal turf. Recolonization by macroalgae (such as *Anadyomene stellata* and Dyctiotales) occurred only when grazing activity is removed. Under these conditions, after ten months from the beginning of the experiment, the cover of turf is reduced to very low values, while turf calcified algae (TCA like *Padina pavonica*) and turf non calcified algae (TnCA) like *Dyctiota dichotoma* and *Laurencia* spp, become apparent revealing first sign of recolonization patterns. In these plots where grazing was removed and nutrients were enhanced, there was a gradual increase over time of the number of taxa, which implies a change in structural diversity of benthic assemblages. By contrast, where the pressure of grazing was eliminated, the increase in the number of taxa was less apparent. If we consider the percentage cover at plot scale, the 100% had been occasionally reached. However, after one month from the beginning of the experiment, this value was reached only by low diversity assemblages constituted by unicellular algae and mucilaginous seaweed, mostly opportunistic taxa maximizing resources in the environment.

These results are supported by the functional composition of the assemblages. In the enriched plots, after one month from the beginning of the experiments, assemblages are represented by ephemeral algae called STEA. This is a fairly common result during the first steps of recolonization of a disturbed substrate, followed by a successive colonization and by an increase in the structural and functional complexity.

Our results stress that one year could be enough to see evident signs of restorations in a disturbed assemblage. When recolonization is completely dependent on propagules arriving from outside the patch, recovery is expected to be generally slower than in situation where vegetative regeneration plays a substantive role in regeneration (Connell and Slatyer 1977; Sousa 2001).

Our results support the theory that, locally (from a few meters to kilometres), an increase in productivity through the addition of nutrients reduces the specific diversity (DiTommaso and Aarsen, 1989; Schindler 1990), according to the resources heterogeneity hypotesis (RHH Tilman, 1987; Rosenzweig and Abramsky, 1993; Gough et al. 2000). When the average quality of habitat increases, it should increase the spatial variability and the diversity of resources, and hence the productivity and the diversity. After a certain level, however, there is a reduction of the heterogeneity of resources and therefore of diversity, with the increase of competitive species that are favoured under these conditions (Hall et al. 2000). Recolonization processes are crucial in allowing the recovery of a strongly disturbed habitat, highly simplified, with low levels of primary and secondary productivity and low diversity of fish and benthic species.

At the beginning of the experiment, we expected that the control exerted by grazers in enriched conditions on the opportunistic species favoured by more favourable trophic conditions, would have allowed the successive establishment of macroalgal communities, increasing local diversity. This response could have been interpreted as a synergistic effect of interactive drivers (Crain et al. 2008). Our results instead, suggest that nutrient enrichment and sea urchins grazing act as antagonist stressors in influencing pattern of recolonization of disturbed habitats. Other studies showed that stressor pairs can have additive-cumulative effects, but almost as many had antagonistic or synergistic cumulative effects. Antagonistic stressors, in particular, create management challenges, as all or most stressors would need to be eliminated to see substantial recovery, except in those cases where the antagonism is driven by a dominant stressor (Folt et al. 1999), so that mitigation of that stressor alone would substantially improve the state of species or communities. Synergisms, in contrast, may respond quite favourably to removal of a single stressor as long as the system has not passed a threshold into an alternative state.

In conclusion, our study confirm that ocean management can no longer focus on single-sector issues that address individual stressors (Halpern et al. 2007), but must incorporate cumulative stressor effects (Crain 2008) and that restoration of our system can be a difficult task.

Field experiments have the potential to analyze causes and effects of multiple drivers at ecosystems (Binkley and Vitousek 1991; Worm et al. 2000a; Morris and Keough 2003; McClanan et al. 2003; Russell and Connell 2006). Our experimental study is likely to provide useful indications to formulate methodology to promote systems resilience and system recovery of assemblages both in oligotrophic and eutrophic conditions, supporting the natural resource management and the implementation of new conservation strategies of marine environment.

Our experimental study is likely to provide useful indications to promote systems resilience and system recovery of assemblages under different trophic conditions, supporting the management of natural resources and the implementation of new conservation strategies of marine environment.