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**EFFECTS OF HABITAT CHARACTERISTICS ON CRYPTIC FISH
ASSEMBLAGES**

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SANTIN SELVAGGIA.

The relative importance of habitat complexity to cryptobenthic fish

Abstract

Habitat structure is known to influence the abundance of fishes on temperate reefs. Biotic interactions play a major role in determining the distribution and abundance of species. The significance of these forces in affecting the abundance of fishes may hinge on the presence of organisms that either create or alter habitat. On temperate reefs, for example, macroalgae are considered autogenic ecosystem engineers because they control resource availability to other species through their physical structure and provide much of the structure used by fish.

On both coral and temperate reefs, small cryptic reef fishes may comprise up to half of the fish numbers and constitute a diverse community containing many specialized species. Small cryptic fishes (<100 mm total length) may be responsible for the passage of 57% of the energy flow and constitute ca. 35% of the overall reef fish biomass on coral reefs. These benthic fish exploit restricted habitats where food and shelter are obtained in, or in relation to, conditions of substrate complexity and/or restricted living space. A range of mechanisms has been proposed to account for the diversity and the abundance of small fishes: (1) lifehistory strategies that promote short generation times, (2) habitat associations and behaviour that reduce predation and (3) resource partitioning that allows small species to coexist with larger competitors. Despite their abundance and potential importance within reef systems, little is known of the community ecology of cryptic fishes. Specifically on habitat associations many theories suggested a not clear direction on this subject.

My research contributes to the development of marine fish ecology by addressing the effects of habitat characteristics upon distribution of cryptobenthic fish assemblages. My focus was on the important shallow, coastal ecosystems that often serve as nursery habitat for many fish and where different type of habitat is likely to both play important roles in

organism distribution and survival.

My research included three related studies: (1) identification of structuring forces on cryptic fish assemblages, such as physical and biological forcing; (2) macroalgae as potential tools for cryptic fish and identification of different habitat features that could explain cryptic fish assemblages distribution; (3) canopy formers loss: consequences on cryptic fish and relationship with benthos modifications.

I found that: (1) cryptic fish assemblages differ between landward and seaward sides of coastal breakwaters in Adriatic Sea. These differences are explained by 50% of the habitat characteristics on two sides, mainly due to presence of the *Codium fragile*, sand and oyster assemblages. Microhabitat structure influence cryptic fish assemblages. (2) Different habitat support different cryptic fish assemblages. High heterogeneity on benthic assemblages reflect different fish assemblages. Biogenic components that explain different and diverse cryptic fish assemblages are: anemone bed, mussel bed, macroalgal stands and *Cystoseira barbata*, as canopy formers. (3) Canopy forming loss is not relevant in structuring directly cryptic fish assemblages. A removal of canopy forming algae did not affect the structure of cryptic fish assemblages. Canopy formers algae on Conero cliff, does not seem to act as structuring force, probably due to its regressive status.

In conclusion, cryptic fish have been shown to have species-specific associations with habitat features relating to the biological and non biological components afforded by fish. Canopy formers algae do not explain cryptic fish assemblages distribution and the results of this study and information from the literature (both from the Mediterranean Sea and elsewhere) show that there are no univocal responses of fish assemblages. Further examinations on a non regressive status of *Cystoseira* canopy habitat are needed to define and evaluate the relationship between canopy formers and fish on Mediterranean sea

CHAPTER 1

Direct versus indirect effects of wave exposure as a structuring force on temperate cryptobenthic fish assemblages

Santin, S. and Willis, T.J.

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Abstract

The structure of cryptic reef fish assemblages was assessed on sheltered and exposed aspects of coastal breakwaters at two locations in the northwestern Adriatic Sea.

20 There were distinct differences between the two levels of exposure which were consistent between locations. Habitat characteristics, measured at scales of 10's cm, explained 50% of the variability in assemblage structure between exposures, whereas "exposure" alone (implying direct effects of wave energy on the fish) explained < 5% of the variation. The most important explanatory variables were the presence of macroalgae, sandy habitat, and oyster shell, the last of which increases the degree of small-scale complexity and provided nesting sites for blennies. We found little evidence to suggest that wave action had large direct effects on the fish assemblages, although this may be in part due to the relatively small degree of difference between "exposed" and "sheltered" samples under the calm conditions of a sea with a relatively short fetch. These results suggest that wave action acts mainly indirectly as a structuring force on cryptic reef fish communities, by altering the composition and/or the relative density of epibiota that influence the distribution of fish.

Thus, relative wave energy may provide a useful means of predicting fish assemblage structure only at large spatial scales. Microhabitat, composed of a combination of physical complexity and biological elements, always explained the greater part of variability at small (< 1m) spatial scales.

Introduction

On both coral and temperate reefs, small cryptic reef fishes may comprise up to half of the fish numbers and constitute a diverse community containing many specialized species (e.g. Macpherson 1994; Willis and Roberts 1996; Ackerman and Bellwood 2000; Depczynski and Bellwood 2003; La Mesa et al. 2004; Griffiths et al. 2006). Small cryptic fishes (< 100 mm total length) may be responsible for the passage of 57% of the energy flow and constitute ca. 35% of the overall reef fish biomass on coral reefs (Ackerman and Bellwood 2000). A range of mechanisms has been proposed to account for the diversity and the abundance of small fishes: 1) life-history strategies that promote short generation times, 2) habitat associations and behaviour that reduce predation and 3) resource partitioning that allows small species to coexist with larger competitors (Munday and Jones 1998). Despite their abundance and potential importance within reef systems, little is known of the community ecology of cryptic fishes, and in many cases this applies also to individual species.

Since cryptobenthic reef fishes live in close association with the substratum, the physical complexity of their habitat plays a central role in their abundance and distribution (Behrens 1987, Hixon and Beets 1993; Macpherson 1994; Willis and Anderson 2003, Depczynski and Bellwood 2004; La Mesa et al. 2004, 2006). Structural features of habitat may provide shelter for fishes from physical stress, restrain predators, and modify the availability of resources. However, the relative importance of the various functional explanations for a dependence on shelter has not been conclusively assessed (Steele 1999).

Although predation is frequently assumed to be an important factor controlling the

assemblage structure of small fishes, it has been rarely demonstrated at reef-wide scales (but see Jennings and Polunin 1997; Willis and Anderson 2003; Graham et al. 2003).

Cryptobenthic fishes in shallow waters spend a large proportion of their lives in circumscribed and typically fixed patches (e.g. Connell and Jones 1991). They tend to have small size, negative buoyancy resulting from the absence or reduction of the swim-bladder, and fin modifications which have evolved to minimize displacement by turbulence and wave action (Gibson 1982). These features imply that the direct effect of water movement is an important determinant of the distribution of these species

Wave exposure has been correlated with differences in the assemblage structure of larger demersal reef fishes (Friedlander et al. 2003; Fulton and Bellwood 2004; Fulton et al. 2005). It is, however, also a well-known determinant of the assemblage structure of habitat-forming invertebrate species in coastal habitats (e.g. Dayton 1971; Denny 1987, 1995; McQuaid and Lindsay 2000; Hammond and Griffiths 2004; Tuya and Haroun 2006), and hence alters the amount of shelter available to small fishes. Wilson et al. (2003) described how the relative amount of detritus in the epilithic algal matrix, a primary food source for small cryptic species, may vary among and within reef zones of differing wave exposures. Thus, the effects of varying wave exposure may be manifest on cryptic fish assemblages by direct means through mechanical stress, or indirect means, through modification of habitat structure and/or food supply. Recently, Depczynski and Bellwood (2005) intimated that average wave energy is the strongest structuring force in assemblages of cryptic tropical fishes, with small-scale habitat characteristics taking a secondary role. In this study, we examined the relative importance of exposure and small-scale (cm-10's cm) habitat on the structure of

temperate cryptic fish assemblages occurring on coastal breakwaters in the northwestern Adriatic Sea to determine if this conclusion holds in a temperate system.

Materials and methods

Study area

The study was done on breakwaters defending the shoreline of the Emilia-Romagna region, Italy, in the North Adriatic Sea. The shore is a heavily modified flat sandy beach of 100 more than 200 km extent. Study locations were located at Casal Borsetti (44°33' N, 12°16' E) and Cesenatico (44°11' N, 12°24' E) separated by ca. 65 km (Fig. 1). Breakwater construction began in the 1970s to prevent erosion, and is still ongoing. The structures are composed mainly of limestone blocks, which at Casal Borsetti are placed 30-80 m from the shore in depths of 0.5-2.0 m, and at Cesenatico are ca. 200 m from shore in depths of 1.5- 3.0 m. At both locations the breakwaters are high-crested structures that are always emmersed. Further information is available from Bacchiocchi and Airoidi (2003) and Bulleri and Airoidi (2005).

Sampling methods

Indicative measures of the relative exposure to wave action under calm summer conditions were obtained from the erosion rates of plaster-of-paris “clod cards” (Muus 1968, Jokiel and Morrissey 1993) placed inside and outside breakwaters at Cesenatico. Ten clod cards were deployed on each side of the barriers on 13 July 2002 for 72 h (A Sundelöf and L Airoidi, unpubl. data). The distributions of fishes and their microhabitats were determined using a structured factorial sampling design, and sampling was done between June and August 2005. At each location, fish and habitat features were sampled at 1-2 m depth at three different 120 breakwaters. Within each

breakwater, two exposures were investigated: landward and seaward, with $n = 3$ transects sampled haphazardly within each exposure at each breakwater. The seaward side is defined as “exposed”, and the landward aspect as “sheltered”. Sampling of sites was done in a haphazard order between locations to avoid temporal confounding. Visual surveys of fishes were done by snorkelling along a 5 X 1 m transect (by swimming out a measuring tape to avoid disturbing the substratum prior to conducting counts) and identifying and recording the number and position on the reef of each species of fish. All sampling was carried out in calm weather conditions and with horizontal water visibility >2 m. The percent cover of habitat was estimated by resurveying the fish transects using a 1 m² aluminium quadrat. Each of the five quadrats was further subdivided into four subquadrats of 0.25 m² to improve the accuracy of % cover estimates. For the analyses presented here, the five quadrats were pooled into a single estimate per transect to match the spatial scale of the fish density estimates. Habitat variables measured on the breakwaters consisted of two groups: structural features of the habitat (sand, fine sediment, broken shell, and bare rock) and epifaunal cover, which consisted of mussels *Mytilus galloprovincialis* Lamarck, barnacles *Balanus spp.*, “oysters” (comprising two species, *Ostrea edulis* Linnaeus and *Crassostrea gigas* (Thunberg), grouped because of uncertainty in field identification), serpulid worms, algal turf (a dense mat of filaments of *Polysiphonia* and juvenile *Ulva spp.*), *Ulva latevierens* (Areschoug), *Ulva intestinalis* (Linnaeus), *Codium fragile* (Sur.) Harriott spp. *tomentosoides* (van Goor) Silva, filamentous red algae (usually occurring as a mixture of species, see Bacchiocchi and Airoidi 2003), and coarsely branched algae (*sensu* Littler and Littler 1984). Data analyses To test differences in the abundances of individuals and number of species among Aspect (2 levels, fixed), breakwater (3 levels, random) and Location (2 levels, fixed), we did a

3-factor permutational multivariate analysis of variance (PERMANOVA, formerly NPMANOVA, Anderson 2001) based on Bray-Curtis dissimilarities (Bray and Curtis 1957). The fish counts did not vary greatly in scale between species, so a transformation was unnecessary and the dissimilarity matrix was calculated from raw count data. A test of homogeneity of multivariate dispersions within treatments was tested using PERMDISP (Anderson 2006). To visualise differences among assemblages, an unconstrained ordination was done using non-metric multidimensional scaling (nMDS) (Kruskal and Wish 1978). Unconstrained ordinations attempt to place the samples in two-dimensional space in their most parsimonious configuration, such that real variability (or relative dispersion) between groups can be seen. In contrast, a constrained ordination, called canonical analysis of principal coordinates or CAP (Anderson and Willis 2003; Anderson and Robinson 2003) was used to conduct a discriminant analysis by reference to the null hypotheses of no differences between locations or aspects. This method is a type of discriminant analysis that searches for the axes in multivariate space that maximise between-group differences. A canonical correlation analysis (also using CAP) was done to identify specific components of the fish assemblage and habitat that were correlated with between-treatment differences. There were 18 taxa in the fish dataset, and 14 categories in the habitat dataset. Relationships between the fish assemblages, exposure and habitat were assessed by using distance-based multivariate regression (McArdle and Anderson 2001). Since some habitat variables covaried with aspect or with each other, initial estimates of the relative importance of 15 explanatory variables (14 habitat variables plus exposure) were made using conditional (sequential) tests that fitted each variable in a step-wise fashion, such that the estimated variability for each of the variables was conditional on the variables already

in the model. An overall relative estimate of the importance of habitat (all 14 variables) versus exposure on the structure of the fish assemblages was estimated from a general partitioning of multivariate variability, also using distance-based multiple regression (McArdle and Anderson 2001). This was done by running two analyses: the fish data matrix on aspect, with the habitat matrix included as covariables, and the fish data on habitat, with the factor aspect as a covariable. The proportion of total variability explained for aspect and habitat alone were estimated from their respective sum-of-squares (SS) when used as covariables (since the regression estimates the effect of covariables independently of other terms in the model) and the proportion of variability explained by “aspect given the effects of habitat” was estimated from the regression SS when habitat variables were covariables, and vice versa. The overlap between the factors (ie variance explained by a combination of both aspect and habitat) was then simply the total proportion of variance for one factor, minus the proportion explained by that factor in the presence of the other as a covariable.

Results

Relative measures of water movement

The initial mean dry weights of clod cards on landward and seaward sides of the barriers were 188.14 g (\pm 3.48 SE, N = 20) and 188.60 g (\pm 4.87 SE, N = 11) respectively. After 72 hours of immersion, the landward cards had lost an average dry weight of 48.84 g (\pm 1.89 SE), and the seaward cards 128.58 g (\pm 5.77 SE). Note that 9 of the 20 seaward cards deployed were lost. This indicates that water movement was

ca. 2.6 times greater on the seaward (exposed) side relative to the landward side of the breakwaters.

Variability in fish assemblages

Cryptic fish assemblages differed significantly between the two locations, and also between seaward and landward sides of the breakwaters (Table 1). There was no significant interaction between the two factors, indicating that differences between the exposure levels were consistent at the two locations (Table 1). The random effect of breakwater was significant, as was the interaction of breakwater with exposure. Pairwise tests showed that this was caused by significant differences between breakwaters at Cesenatico on the exposed side only. This was brought about not because of greater variability between breakwaters, but because of less variability within breakwaters (average within-group dissimilarities of 33.4-37.1) relative to the other treatments (average within-group dissimilarities of 38.0-73.3). Tests of multivariate dispersion were not significant for either of the main effects or their interaction ($p > 0.3$ in all cases), so differences seen in the PERMANOVA can be safely attributed to differences in multivariate location, rather than between-treatment differences in the dispersion, or spread, of points in multivariate space. The significant differences seen in the PERMANOVA were not clear in the nMDS plot (Fig. 2), however the high stress value of this ordination (0.22) indicates that the true 10 relationships between points are not well represented in two dimensions (Clarke 1993). The CAP analyses, however, did clearly separate the two locations and the two aspects (Fig. 3). At Casal Borsetti, there was limited overlap between fish assemblages in the landward and seaward sides of the breakwaters, with one “exposed” sample grouping with the “sheltered” samples. At Cesenatico, there was no

overlap between exposed and sheltered groups (Fig. 3). These clear differences are supported by the “leave-one-out” allocation success of the discriminant analysis (Table 2; Lachenbruch and Mickey 1968, Anderson and Willis 2003). The higher variability in the exposed data at Casal Borsetti resulted in correspondingly increased misclassification error for the factor aspect in the discriminant analysis (Table 2). Differences between aspects and locations in the fish assemblage were due to a combination of between-treatment differences in relative density of common species, and the presence of rare species at either one location or aspect only. Although the most abundant species *Salaria pavo* (Risso) did not differ in density between locations, it was consistently more abundant on the sheltered side of the breakwaters (Fig. 4). *Lipophrys canevae* (Vinciguerra) and *Lipophrys dalmantinus* (Steindachner and Kolombatovic) were also more common on the sheltered side, and *Gobius bucchichi* (Steindachner) occurred only on the sheltered side (Fig. 4). Conversely, *Aidablennius sphynx* (Valenciennes), *Parablennius zvonimiri* (Kolombatovic) and *Gobius niger* (Linnaeus) were more abundant under exposed conditions, and *Parablennius gattorugine* (Linnaeus) was recorded only on the seaward side (Fig. 4). Differences between locations were partly explained by relative density differences of species that were more abundant at Cesenatico (e.g. *A. sphynx*, *L. canevae*, *L. dalmantinus* and *Parablennius pilicornis* (Cuvier); Fig. 4, Table 3) but also to the distribution of some rare species. For example, *Coryphoblennius galerita* (Linnaeus), *Gobius roulei* de Buen and *Gobius cobitis* Pallas occurred only at Cesenatico, whereas *Parablennius incognitus* (Bath) was seen only at Casal Borsetti (Table 3). Variability explained by habitat structure Permutational multivariate regression (McArdle and Anderson 2001) of variables fitted individually (independently of each other) indicated that 7 of the 15 environmental variables made statistically significant

contributions to explaining variability in the fish assemblage (Table 4a). Exposure alone explained 10% of the variability, and the presence of *Codium*, *Mytilus*, and sandy substratum also made significant contributions. However, many of the variables were correlated with each other. Therefore, a sequential model was built using forward selection (Table 4b). With this model, the number of significant explanatory variables was reduced to 5, and the proportion of variability explained by exposure alone dropped to < 6%. The quantity of sand on the reef was correlated with fish assemblages at Casal Borsetti, and that the cover of fine sediments, oysters, and *Codium* were correlated with fish assemblages at Cesenatico. In fact *Codium* occurred only on the landward side at Cesenatico, and the cover of oysters was also considerably higher there than at Casal Borsetti or either of the seaward locations (Fig. 5). Despite being the most dominant epifaunal taxon and exhibiting clear differences in % cover between exposed and sheltered aspects (Fig. 5), *M. galloprovincialis* did not appear to contribute significantly to differences in fish assemblages between exposures (Table 4b).

Partitioning of variability

Since there was no significant interaction between the effects of Aspect and Location on the fish assemblages (Table 1), the locations were pooled for the partitioning of variability between habitat variables and the effects of wave exposure. The total variation explained by the factors habitat and aspect was 60.02%, most of which (50.16%) was accounted for by habitat alone (Fig. 6). The effects of aspect (our proxy for wave exposure) accounted for no more than 10% of the total explained variation – and this only if the overlap between factors is included (Fig. 6).

Discussion

This study found marked differences in shallow subtidal cryptic fish assemblages between sheltered and exposed faces of coastal breakwaters. While this finding superficially agrees with those of other studies that have found correlations between reef fish assemblages and the degree of wave action (Friedlander et al. 2003; Fulton and Bellwood 2004; Guidetti 2004; Depczynski and Bellwood 2005), it also agrees with the general consensus that small benthic fishes are likely to have distributions limited by qualitative or quantitative components of habitat complexity (Behrens 1987; Hixon and Beets 1993; Caley and St John 1996; Willis and Anderson 2003; Depczynski and Bellwood 2004; La Mesa et al. 2004, 2006). Wave action affects not only fishes, but is a strong structuring force on other components of the biota (Dayton 1971; Denny 1987, 1995; McQuaid and Lindsay 2000; Hammond and Griffiths 2004; Tuya and Haroun 2006), and thus the effects of exposure and habitat may generally be expected to covary. To address this problem, we partitioned the variability in fish assemblages attributable to habitat or exposure, or both, and found that the greater part of the explainable variance was probably due to an indirect, rather than direct, effect of wave action. That is, the degree of water movement alters the structure of microhabitat (which we define in this context as variability in the epibiota and physical structure of the substratum at the scale of cm-10's cm) in exposed relative to sheltered 13 locations, which in turn has direct effects on the composition and relative abundance of species within the fish assemblage. A considerable portion (almost 40%) of the variation between exposed and sheltered treatments remained unexplained. This could be partly a function of the naturally high variability generally found in all visual counts of reef fishes (Edgar et al. 2004; Willis et al. 2006), but may be due to other, unmeasured, variables that may be related to the degree of water

movement. For example, at Casal Borsetti, sediment accretion on the landward side of the barriers resulted in very shallow depths at low tide relative to the seaward side, whereas this did not occur at Cesenatico, where breakwaters are ca. 250 m from the shoreline. Sedimentation and sediment resuspension is very high around these structures, but may be expected to be most marked at the shallower Casal Borsetti sites, where we did in fact record lower overall densities of fish (see Table 3). Although sedimentation is known to have large effects on rocky reef systems (Airoldi 2003), little is known of its direct effects on the distribution of fishes. It is possible that lowered water clarity has effects on the distribution of many fishes due to reduction of their visual field (Rilov et al. in press), or alternatively, varying turbidity may contribute to variability in the accuracy of visual census estimates of fish density. For cryptic fishes, visual methods are known to be inaccurate (Willis 2001) and in this study biases were assumed to be consistent for the purpose of between-treatment comparisons. Our overall conclusion contrasts somewhat with that of Depczynski and Bellwood (2005), who suggested that “microhabitat plays a consistent but secondary role” to water movement. There are two possible reasons for this. First, their study was done on island reefs subject to much higher levels of wave energy than this study conducted in an enclosed sea. Although subject to occasional violent storms, the Adriatic Sea is not subject to oceanic swells, and the average intensity and frequency of storm events is likely to be considerably less than found on the Great Barrier Reef. Thus, the difference in wave energy between our seaward and landward sides of breakwaters is much less than their exposed and sheltered reef sites. Second, we measured habitat variables at scales of cm to m over the same area where fish counts were done, whereas Depczynski and Bellwood (2005) stratified their counts within broader habitat categories (at scales of m), which is likely to have reduced the

power of “habitat” as a predictive variable relative to our finescale measurements. Whether wave action might be regarded as a primary or secondary structuring force thus depends on the spatial scale at which the question is posed: sampling at larger spatial scales will emphasise the effects of wave action, whereas small-scale studies will emphasise the effect of microhabitat – which is partially determined by wave action. The reasons for habitat dependence of fishes under differing exposure conditions are many and varied. Habitat features are influenced by wave action, but may also influence hydrodynamics at small spatial scales (Madsen et al. 2001). Since small cryptic fishes are generally poor swimmers and often possess morphological and/or behavioural adaptations to resist physical displacement (Gibson 1982), the availability of shelter may therefore be important not only from the perspective of predation risk (e.g. Hixon and Beets 1993; Caley and St John 1996; Steele 1999), but as areas of reduced water movement where smaller fishes expend less energy and experience less risk of physical injury (e.g. Fulton and Bellwood 2002). Exposure-mediated habitat changes may also influence the availability of prey or detrital sources of nutrition (Wilson et al. 2003), and alter the distribution of sessile species that have direct associations with certain fish species. For example, the goby *Gobius bucchichi* is known to be associated with the anemone *Anemonia viridis* (Penn) (Abel 1960; La Mesa et al. 2006) and in our study both species 15 were found only on the landward side of the breakwaters. Similarly, the availability of biologically engineered habitat types can limit the density of some fishes (Buchheim and 350 Hixon 1992). In our case spawning males of the blennies *Lipophrys canevae* and *L. dalmatinus* were frequently observed inhabiting small holes excavated by boring bivalves [probably *Lithophaga lithophaga* (Linné) or *Gastrochaena dubia* (Pennant)], which were also observed to be more abundant on the landward side of the

breakwaters (S. Santin pers. obs.). One important habitat characteristic for some fish species that, in this study, explained an unexpectedly low proportion of the spatial variability in the fish assemblage is the presence of living or dead oyster shell attached to the substratum. Oysters are considered “foundation species” (Bruno and Bertness 2001) that facilitate the distribution and abundance of a range of associated species. Oyster shell, both living and dead, contribute greatly to increasing small-scale rugosity and hence the available shelter to small benthic reef fishes, but also provide nesting sites for numerically dominant species such as *Salaria pavo*. The dominant habitat-forming species of the study area, *Mytilis galloprovincialis*, contributed little to explaining differences in fish densities, despite varying between exposed and sheltered aspects. This was probably because the dense beds of mussels have interstitial spaces of less than 1 cm, and for fishes of 5-20 cm length, form functionally low rugosity habitat. Multiple regression indicated that *Codium* was the most important variable to the breakwater fish assemblage. However this invasive alga was found at only one of the two sites sampled, so it cannot be determined if the strong between-site differences observed were due to the presence of *Codium*, or whether the presence of *Codium* was incidental to a between-location effect caused by other factors as discussed above. Although the cover 16 of macroalgae has been shown to have compositional effects on cryptobenthic fish assemblages (Levin and Hay 1996; Willis and Anderson 2003; La Mesa et al. 2006), manipulative experiments are required to determine whether the invasive species *Codium* does have important effects on fish assemblage structure. The Blenniidae were the most diverse group of cryptic fishes in the study area, as on other Mediterranean rocky reefs (Illich and Kotrschal 1990, Macpherson 1994; Lipej and Richter 1999; La Mesa et al. 2004, 2006). The apparent absence of the Gobiesocidae, Scorpaenidae and Tripterygiidae in

these breakwater assemblages is peculiar. Although it is possible that the first two families do occur in low densities but were not detected by visual census (Willis 2001), the Tripterygiidae have been detected on other artificial structures in the Mediterranean (Guidetti 2004; Pérez-Ruzafa et al. 2006), and also occur on natural reef on the eastern Adriatic coast, as do the Gobiesocidae and Scorpaenidae (Lipej et al. 2003). Either the abrupt changes in salinity and turbidity common in the northwestern Adriatic (Zavatarelli et al. 1998) make it unsuitable habitat for these groups, particularly in these shallow reefs, or important components of their biotic habitat are missing from the species poor epibiotic assemblage found on these breakwaters (Bacchiocchi and Airoidi 2003). Alternatively, the larvae of these Families may have lower dispersal ability than gobies or blennies, and are unable to arrive on the breakwaters because of their isolation from natural reef habitat. If larvae of the Tripterygiidae or Gobiesocidae have low temperature or salinity tolerances relative to the Gobiidae and Blennidae, their distribution may be limited by the marked stratification of western Adriatic water masses (Montanari et al. 2006), particularly during the spring and summer when recruitment occurs. The advantage of examining the effects of wave action on the biota of artificial structures is that contrasting hydrodynamic regimes can be found on identical substrata (in terms of the physical structure, if not the biota) only a few m apart. On natural reefs, whether in tropical or temperate environments, comparisons between “exposed” and “sheltered” sites are necessarily confounded to some extent by being generally located some 100’s m from each other. One disadvantage is that the hydrodynamics generally differ on breakwaters from that of natural reefs because of their shape: waves breaking over artificial barriers may exert strong radial forces on the “sheltered” side due to the absence of reef to dissipate wave energy (Losada et al. 2005). A second

disadvantage in this study is that cryptobenthic fishes can use the spaces between boulders to move through a breakwater from one side to the other, which may cause underestimates of the direct effect of wave action relative to habitat if fish move to the sheltered side during rough sea conditions. The current study was done under calm summer conditions, which reduces the likelihood of such misinterpretation. Nonetheless, there remains the possibility that a part of the variability that we attribute here to habitat is the result of fish movements between exposure levels. In summary, while fin morphology and swimming performance undoubtedly dictate the degree to which many free-swimming reef fishes can tolerate wave exposure (Fulton and Bellwood 2004; Fulton et al. 2005, but see Denny 2005), the distribution of benthic species appears to be also driven by the indirect effects of wave action on their habitat. In this study, the relative difference in wave exposure at “exposed” and “sheltered” sides of breakwaters in the Adriatic Sea was considerably less than that encountered at locations subject to oceanic fetch conditions, which decreased the likelihood that fishes are vulnerable to prolonged periods of high water movement. The low levels of water turbulence in the Adriatic Sea compared to higher fetch systems probably contribute to the relative dominance of habitat effects over direct wave exposure. Much light will be cast on this issue by the responses of cryptobenthic fish assemblages to manipulations of habitat in areas of relatively high versus low wave action (especially in regard to the caveat 18 above concerning movement of fish between exposures), and by repetition of this experiment in locations that vary in the intensity of physical disturbance. We predict that the relative importance of small-scale habitat characteristics to benthic fish assemblage structure will decrease as absolute exposure increases

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Table 1 Results of mixed-model permutational multivariate analysis of variance (PERMANOVA) testing for differences between exposures (exposed and sheltered, fixed), location (Casal Borsetti and Cesenatico, fixed) and breakwaters (random effect, nested within location) in cryptic reef fish assemblages on breakwaters.

Source	<i>df</i>	SS	MS	<i>F</i>	<i>P</i>
Exposure	1	6687.3	6687.3	2.97	0.0237
Location	1	9082.2	9082.2	3.27	0.0151
Exposure x Location	1	2340.7	2340.7	1.03	0.4230
Breakwater (Loc)	4	11095.1	2773.8	2.36	0.0010
Exposure x Breakwater (Loc)	4	9011.2	2252.8	1.92	0.0100
Residual	24	28198.8	1174.9		
Total	35	66415.2			

Table 2. Results of canonical analysis of principal coordinates (CAP) examining the effects of Location and Aspect on cryptobenthic fish assemblages on breakwaters.

Factor	<i>m</i>	%Var	Allocation success (%)			δ^2	<i>P</i>
			Group 1	Group 2	Total		
Location	5	82.53	83.33 (Casal Borsetti)	88.89 (Cesenatico)	86.11	0.650	0.0001
Aspect	6	89.38	66.67 (Exposed)	88.89 (Sheltered)	77.78	0.583	0.0002

%Var Percentage of the total variation explained by the first *m* principal coordinate axes, *Allocation*

success percentage of points correctly allocated into each group, δ^2 = squared canonical correlation

Table 3. Families, species and number of individuals and numerical contributions (%) of each species to the entire assemblage sampled at exposed and sheltered breakwaters (Exp= exposed; Shelt= sheltered) at each Locations (CB=Casal Borsetti; CS=Cesenatico).

Family	Species	Casal Borsetti		Cesenatico		Total (T)	
		Exp	Shelt	Exp	Shelt	T	% of T
Blenniidae	<i>Aidablennius sphynx</i>	4	2	12	0	18	3.50
	<i>Coryphoblennius galerita</i>	0	0	1	0	1	0.19
	<i>Lipophrys adriaticus</i>	1	1	10	4	16	3.11
	<i>Lipophrys pavo</i>	25	44	31	43	143	27.82
	<i>Lipophrys trigloides</i>	1	0	4	2	7	1.36
	<i>Lipophrys canevae</i>	0	3	6	17	26	5.06
	<i>Lipophrys dalmantinus</i>	1	3	8	19	31	6.03
	<i>Parablennius pilicornis</i>	19	6	44	61	130	25.29
	<i>Parablennius tentacularis</i>	4	4	6	3	17	3.31
	<i>Parablennius zvonimiri</i>	10	5	9	5	29	5.64
	<i>Parablennius incognitus</i>	4	2	0	0	6	1.17
	<i>Parablennius gattoruggine</i>	3	0	5	0	8	1.56
	<i>Parablennius sanguinolentus</i>	2	3	12	6	23	4.47
Gobiidae	<i>Gobius paganellus</i>	1	0	0	1	2	0.30
	<i>Gobius roulei</i>	0	0	0	5	5	0.97
	<i>Gobius cobitis</i>	0	0	1	0	1	0.19
	<i>Gobius niger</i>	13	5	20	3	41	7.98
	<i>Gobius bucchicchi</i>	0	5	0	5	10	1.95

Table 4 Results of multivariate regression of breakwater fish assemblages on environmental variables for (a) each variable individually (ignoring other variables), and (b) forward-selection of variables, where each variable added to the model is conditional on the variables already in the model.

Variable	<i>F</i>	<i>P</i>	Variability explained (%)	Cumulative variability (%)
(a) Variables fitted individually				
<i>Codium</i>	4.41	0.0002	11.47	
Sand	4.31	0.0002	11.25	
Exposure	3.81	0.0004	10.07	
<i>Mytilus</i>	3.42	0.0004	9.14	
Sediment	2.51	0.0148	6.89	
<i>Balanus</i>	2.51	0.0112	6.88	
Coarse branched algae	2.10	0.0232	5.82	
Oysters	1.89	0.0560	5.26	
Algal turf	1.73	0.0990	4.83	
<i>Ulva intestinalis</i>	1.70	0.0964	4.76	
Bare rock	1.50	0.1222	4.23	
Shelly sand	1.26	0.2442	3.57	
Filamentous red algae	0.63	0.7856	1.82	
Serpulids	0.46	0.9156	1.32	
<i>Ulva latevierens</i>	0.34	0.9370	1.00	
(b) Variables fitted sequentially				
<i>Codium</i>	4.41	0.0002	11.47	11.47
Sand	4.66	0.0002	10.95	22.42
<i>Balanus</i>	2.86	0.0088	6.37	28.79
Exposure	2.55	0.0142	5.42	34.21
<i>Ulva intestinalis</i>	2.59	0.0158	5.23	39.44
Coarse branched algae	1.74	0.1156	3.42	42.86
Oysters	1.53	0.1606	2.97	45.83
Algal turf	1.48	0.1748	2.82	48.65
Filamentous red algae	1.15	0.3222	2.18	50.83
Bare rock	1.07	0.3672	2.02	52.85
Sediment	1.02	0.4276	1.94	54.79
Serpulids	0.96	0.4154	1.82	56.61
<i>Ulva latevierens</i>	0.79	0.5740	1.51	58.13
<i>Mytilus</i>	0.67	0.6472	1.30	59.43
Shelly sand	0.40	0.8412	0.80	60.23

Figure captions

Fig. 1 Map of the northern Adriatic Sea, showing the position of the two study locations, Casal Borsetti and Cesenatico.

Fig. 2 Non-metric multidimensional scaling (MDS) plot of assemblages of cryptic fish in each combination of exposed and sheltered aspects at two locations.

Fig. 3 Two-dimensional scatter plot of the canonical axes for Location and Aspect.

Fig. 4 Mean densities of species responsible for differences between aspects and/or locations.

Fig. 5 Mean % cover of oysters (*Ostrea edulis* and *Crassostrea gigas*) and *Mytilus galloprovincialis* in exposed and sheltered aspects at the two locations.

Fig. 6 Stacked bar plot illustrating the partitioning of variance attributable to habitat alone, aspect alone, and the overlap between the two factors.

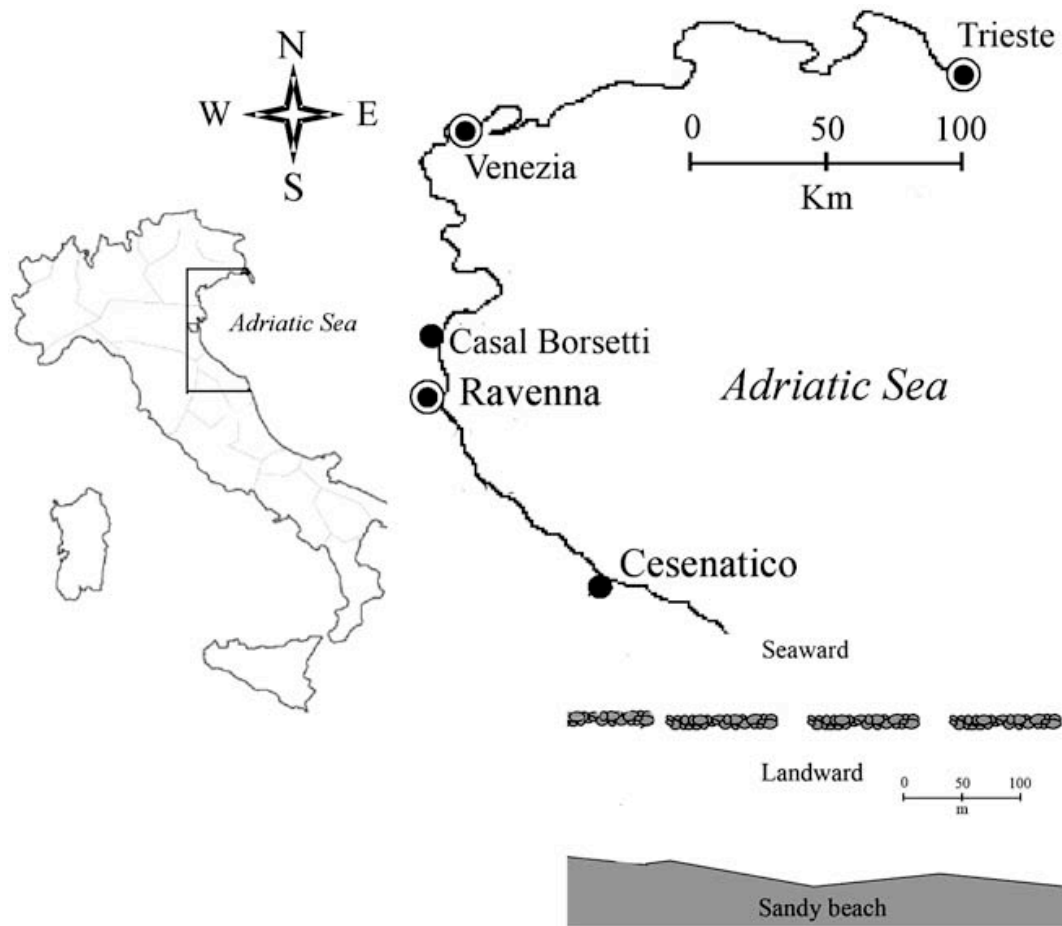
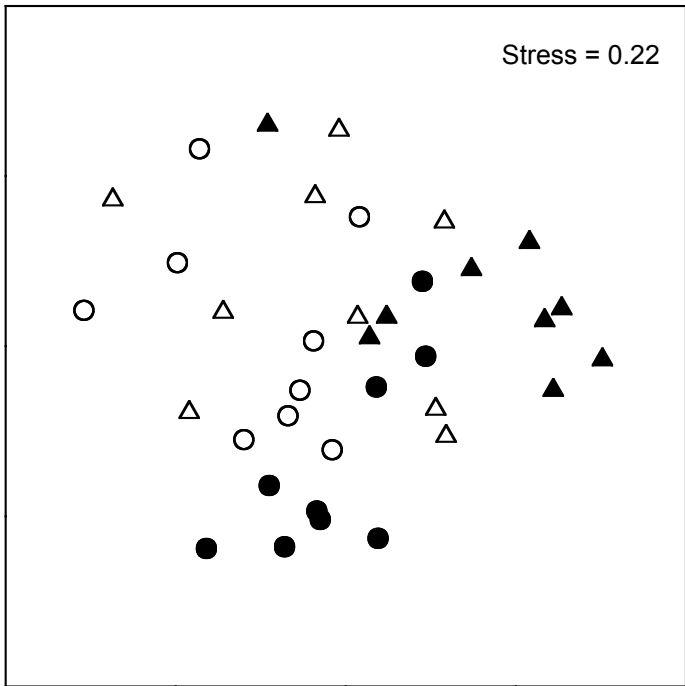


Figure 1



- △ Casal Borsetti, exposed
- ▲ Casal Borsetti, sheltered
- Cesenatico, exposed
- Cesenatico, sheltered

Figure 2

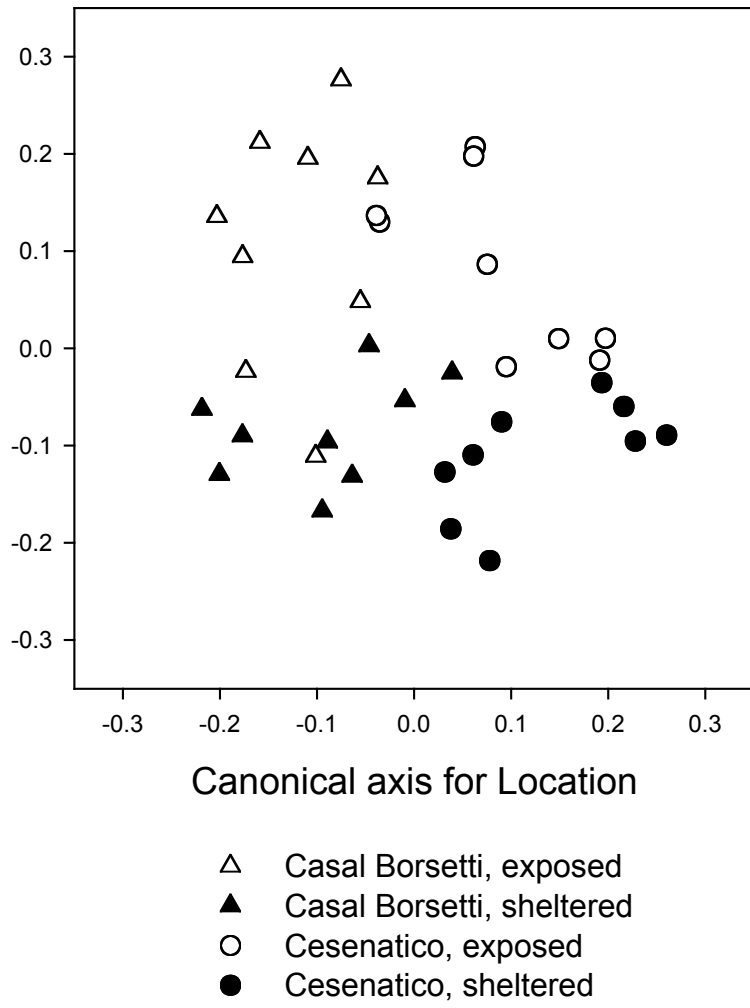


Figure 3

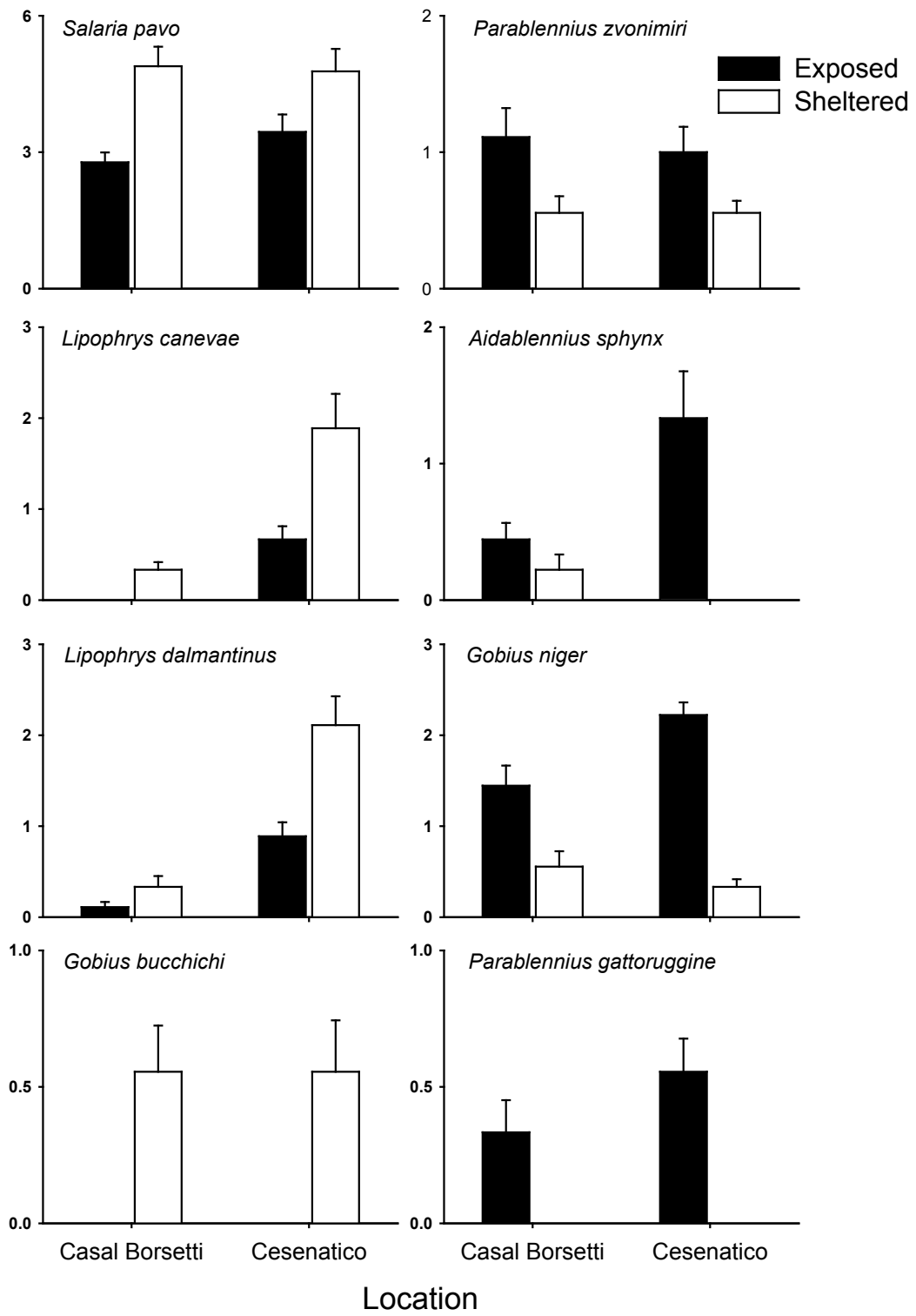


Figure 4

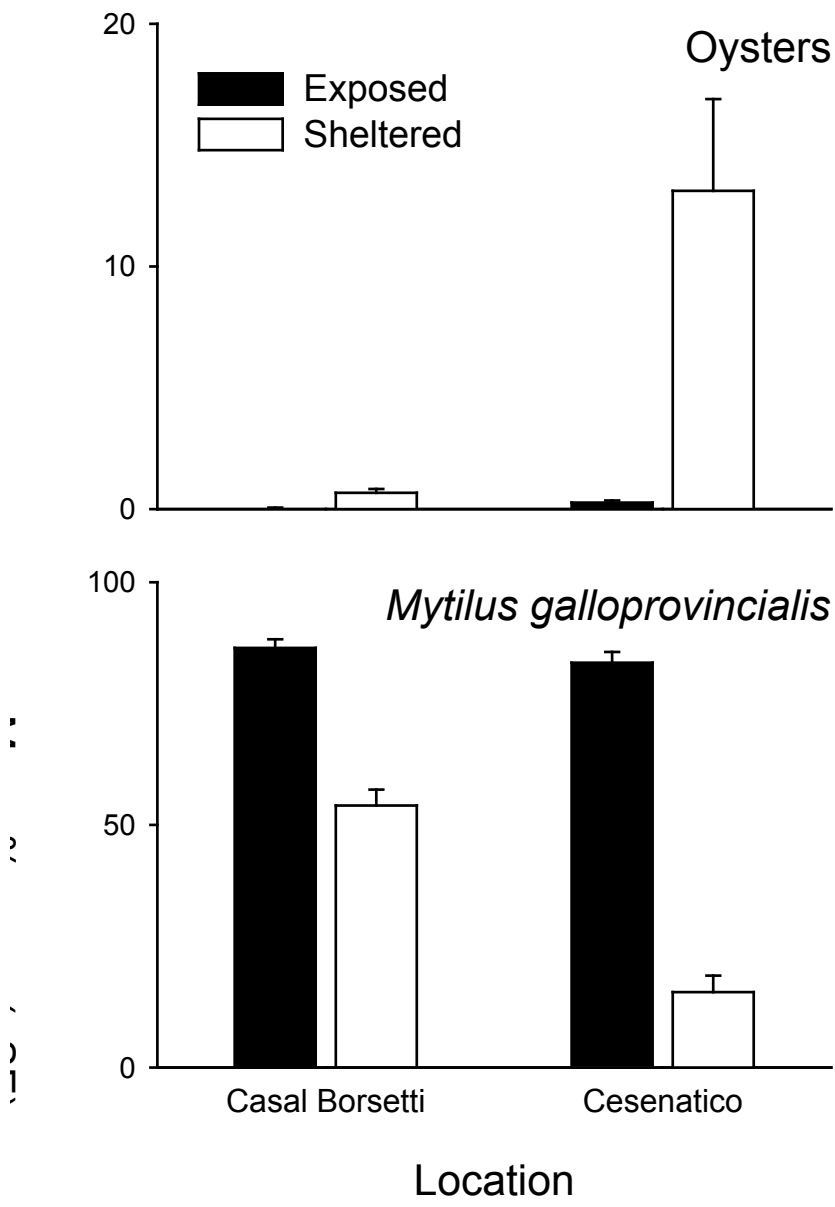


Figure 5

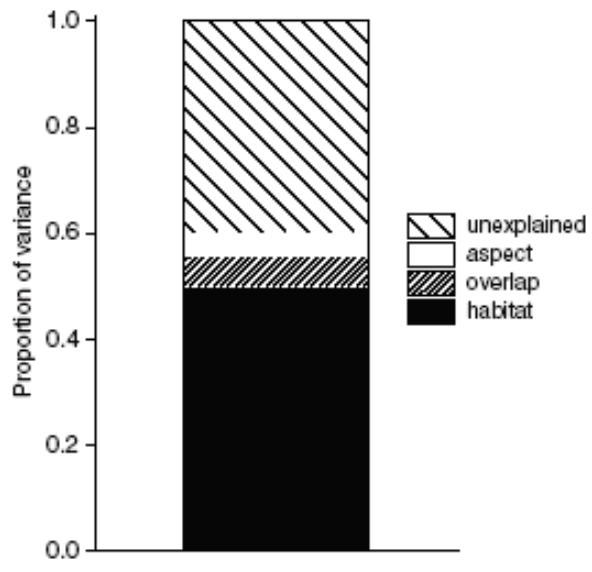


Figure 6

CHAPTER 2

Canopy-forming algae versus other habitat features: which relationship stands on for cryptic fish?

Santin, S.

Abstract

The importance of canopy-formers macroalgae to cryptobenthic fish assemblages was investigated in Adriatic Sea (Italy, Eastern Mediterranean). Fish were assessed by underwater visual census in habitat where canopy-formers macroalgae were present (C+) and absent (C-). Multivariate analysis showed that cryptic fish assemblages have a weak relationship with canopy presence and species richness and number of species were unaffected by canopy presence. On Conero cliff there is an high heterogeneity and others habitat features were identified along the shoreline; they were: Mytilus bed, Anemonia bed, Macroalgae bed and Cystoseira presence. Multivariate analysis evidence that on each habitat feature investigated a diverse cryptic fish assemblages was present, and some fish were correlated with habitat. For example Gobiids were most abundant in habitats devoid of macroalgae vegetation where anemonia barrens are present. Results were discussed in the light of both ecological mechanisms underlying coexistence of closely related species and the current knowledge of Mediterranean cryptic fish.

Introduction

On temperate rocky reef macroalgae provide much of the structure used by fish. Macroalgae are considered autogenic ecosystem engineers because they control resource availability to other species through their physical structure (Jones et al., 1994, 1997; Crooks, 2002; (Thibaut et al. 2005). The ecological importance of cryptobenthic fish, as energy mediators (Depczynski & Bellwood 2005), justifies an increased effort aimed at a deeper understanding of this component of the rocky coast fish assemblages. They are 'small bodied fish (<10 cm) that exploit restricted habitats where food and shelter are obtained in, or in relation to, conditions of substrate complexity and/or restricted living space, with a physical barrier likely to be interposed between the small fish and sympatric predators' (cf. Miller, 1979). Macroalgae add spatial complexity to the substratum creating a 3-dimensional habitat that increases species richness and diversity relative to unvegetated habitats (Miller 1979, Schmidt & Scheibling 2007). The genus of brown algae *Cystoseira* C. Agardh (Fucales, Phaeophyceae) is distributed worldwide, but most species are found in the Mediterranean and the adjoining Atlantic (Oliveras and Gomez, 1989; Amico, 1995). Mediterranean species inhabit a wide array of benthic ecosystems and they often dominate the canopy (Ballesteros, 1990a; Ballesteros et al. 1998; Ruitton et al. 2000; Bulleri et al. 2002; Chemello & Milazzo 2002). Most *Cystoseira* species have, however, vanished from large areas of the northwestern Mediterranean in the recent decades (Thibaut et al. 2005) and four of them are currently so rare that they have been classified as endangered by the Barcelona Convention., (Antonioli et al. 1999). *Cystoseira* disappearance has also been reported recently from parts of the Tyrrhenian and Ionian seas (Serio et al. 2006). In particular, Fucales are relatively large seaweed in relation to the average size of Mediterranean algae (Cardona et al., 2007). The algal assemblages can influence fish assemblages through food availability and viceversa: fish assemblages affect algal assemblages through grazing (Sala & Boudouresque

1997). Because most fish are linked to the algal assemblage of a reef area through the food chain, either directly (for herbivorous fish) or indirectly (for carnivorous fish), it could also be hypothesized that the observed difference in fish species composition between substrata is a reflection of the differences in available food resources. This is peculiar when fish are strictly connected to the substratum as are cryptobenthic fish. Thus, understanding how fishes respond to macroalgae abundance and species composition, as well as how this response changes with scale, may be critical for understanding the dynamics of cryptic reef fish populations.

We test the hypothesis that (1) canopy forming algae are relevant to cryptobenthic fish and (2) there are different habitat features that could explain fish assemblage distribution.

Materials and methods

Study area.

This sampling was carried out on 2006 and 2007 along Conero cliff (43°32'52, 22" N; 13°37'38, 74 E) (Fig.1). The underwater rocky habitats were highly heterogeneous and partially derive from the disintegration of the calcareous cliff that border the coastline. Limestone reefs have many cracks, pits, crevices and fissures and they are deposited on a sand bed

Cystoseira as canopy-forming.

To test if canopy presence can affect cryptic fish assemblages we selected randomly 4 locations where Cystoseira's canopy are distributed (Barriera, Rotaia, Sassi Neri, Scogli Lunghi) divided by hundreds of meters. On Barriera and Rotaia we attribute the level "Canopy presence" (hereafter defined as "Canopy +") and on the others "Canopy absence" (here after defined as "Canopy -").

Cryptic fish assemblages were sampled by means of visual census techniques. At each sampling location, 4 replicates of 5 m transects were haphazardly laid during daylight hours. The abundance of each cryptic fish species within 0,5 m of either transect's side was recorded on waterproof paper by a SCUBA diver, according to standard procedures. A nested design was applied to assess the effect of canopy-forming algae (Present and Absent) in 4 different Locations on the abundance of cryptobenthic fish. On each Locations 3 replicates were done.

Other habitat features.

Since Cystoseira was not more present on the Location reported by literature (such as Sassi Neri) an additional survey was necessary to define the real/ actual distribution of

this alga. To test if there are any other habitat feature that could explain the fish distribution we selected 4 Locations where all type of habitat were randomly distributed. To evaluate which habitat can be representative in our study site we perform an habitat mapping with three 50 m length transects, parallel to the shore, separated by 2 m ca. Main habitat features along each transects were annotated. Within all habitat features we selected 4 different habitats that are mainly distributed along this coast. Colosio et al (unpublished) defined 4 different habitat features present along this coast. They were Mussels bed, Macroalgae, Anemonia bed, Cystoseira spp. Once identified Cystoseira's bed extension and the principal habitat features, an orthogonal design was applied to assess the effect of different habitat feature (4 level, fixed) in 4 different Locations on the abundance of cryptobenthic fish. On each Locations 4 replicates were done. Sampling was done by means of visual census with a modified point fixed technique. The patch dimension was established in 2 X 2 m, in relation to the mean dimension of the habitat patch size (n=4).

Statistical analysis

Hypotheses were tested using multivariate and univariate procedures. To test for differences in the canopy presence we selected non-parametric approaches (Anderson (Anderson 2001), (Anderson & Millar 2004), and applied a mixed analysis technique by combining the semi-parametric, distance based, Permutational Multivariate Analysis of Variance (PERMANOVA, Anderson 2004), and non-metric multidimensional scaling (MDS) ordination (PRIMER software; Clarke & Warwick 1994). In both cases, data were untransformed and analyses were based on Bray-Curtis dissimilarities. PERMANOVA incorporated the following factors: (1) 'Canopy' (fixed factor with 2 levels: present versus absent), (2) 'Locations' (fixed

factor with 4 levels nested within 'Canopy'. Pairwise a posteriori comparisons were executed using 9,999 permutations. To visualize multivariate patterns, non-metric MDS ordinations were carried out.

To test other habitat feature PERMANOVA incorporated the following factors: (1) 'Habitat' (fixed factor with 4 levels: Cystoseira, Mussel bed, Macroalgae and Anemonia), (2) 'Locations' (fixed factor with 4 levels, orthogonal). When appropriate, pairwise a posteriori comparisons were executed using 9,999 permutations. An ordination cryptic fish assemblages were made by principal coordinate analysis (PCO, (Anderson & Willis 2003). To visualize multivariate patterns on PCO plot a projection biplot of species, with a raw Pearson correlations value > 0.65 , were superimposed onto it. The PCO axes were superimposed onto the corresponding PCO ordination, to visualize the variables most responsible for the patterns of multivariate dispersion.

Results

Cystoseira as canopy-forming.

The present study evidence that along Conero Cliff cryptobenthic fish assemblages is not distributed in relation to Cystoseira presence and there's no relationship between macroalga presence and a specific cryptic assemblages. The MDS ordination (Fig 2) shows a distinct separation of the fish assemblages on all different Localities investigated. Sassi Neri results well distinct from all the others (Barriera, Rotaia and Scogli lunghi) and there's no specific separation on fish assemblages within Canopy presence. The high stress value of this ordination (0.06) indicates that the true relationships between points are not well represented in two dimensions (Clarke

1993). In a multivariate space, cryptic fish assemblages results significantly different between Locations (tab 1.), as stressed by MDS ordination, but no differences were found within factor 'Canopy'. Test of multivariate dispersion was not significant for the factor 'Canopy' ($P > 0.2$), so differences seen in the PERMANOVA can be safely attributed to differences in multivariate location;

A total of 16 species were observed in two locations, belonging to Blennidae, Gobiidae and Trypterigiidae family. On Barriera and Rotaia (C+) there's an higher total number of specimens ($p = 0,0001$) and a total number of species ($p = 0,0082$) respect on Sassi Neri and Scogli Lunghi (C-) (Fig. 3 and 4). Sassi Neri has the lower number of species and specimens. Above all 3 species contribute to 80 % of the assemblage censused and the prominent were, in decreasing order, *Parablennius zvonimiri* (33%), *Parablennius incognitus* (22%) and *Parablennius pilicornis* (15%).

Other habitat features

There's and high variability along Conero cliff and Cystoseira stands are not present where indicated from previous works (Fig. 5)

PERMANOVA results evidence a cryptic fish assemblages significantly different between the 4 habitat investigated (Table 2). There is no significant interaction between the two factors, indicating that differences between the habitat levels were consistent at the 4 locations (Table 2). The random effect of Locations was not significant. Pairwise tests showed that this was caused by significant differences between the all the levels of the factor habitat. This was brought about not because of greater variability between Locations, but because of high variability within habitat (average within-group dissimilarities of 38.5–58.8).

PCO plot did separate 4 different habitats (Fig 5) and, specifically, cryptic fish associated with Anemonia bed are well divided by cryptic fish associated with Cystoseria. There's an overlap between points representing fish assemblages associated with Mitylus and Macroalgae bed.

Gobius cobitis and *G. bucchicchi* are correlated with Anemonia habitat; while *Parablennius zvonimiri* and *P. incognitos* are correlated with Cystoseira habitat.

Vector indicates the fish species that best correlated with the ordination of habitat characteristics (correlation with ordination axes $r > 0.5$). The vectors indicates the correlation with different groups of sample points and the length is proportional to the value of correlation.

Discussion

Along Conero cliff canopy presence seems not affect the cryptobenthic fish assemblages. On MDS plot there's a clear difference between assemblages present on Sassi Neri from all the others and, although Locations with C+ are separated, canopy presence seems not to influence the cryptic fish distribution. Within C- Locations, Sassi Neri assemblages is separated Scogli Lunghi assemblages, but this is between the two Localities with C+. Scogli Lunghi is reported (Quaderni IRPEM) as C+, but on preliminary survey, we weren't able to find any *Cystoseira* bed. Only few sparse and rare individuals of *Cystoseira* spp were present. This result suggest a recent disappearance of this macroalga.

PERMANOVA results underlines a difference between Locations and within Location with C+ and C-. Sassi Neri assemblages presents the lowest number of species and specimens. This Location is dominated by 'Anemonia barrens', even if *Cystoseira* presence have been present since 8 years ago (Airoldi, personal comment). Others authors report *Cystoseira* disappearance on Mediterranean sea, but no other data are available on study site. The habitat referred as 'Anemonia barens' has two important components: *Anemonia viridis* and sediment and it is considered depauperated habitat.

Whilst different authors underlay the importance of canopy presence for fish in the Mediterranean Sea, we have different results. *Cystoseira* canopy is not distributed as reported elsewhere (Quaderni IRPEM), and his scarce allocation could be supposed not to have enough influence on cryptic fish assemblages. Observations on cryptic fish assemblages nearby canopy presence evidence a differentiation not due, per se, to Canopy presence but to an high differentiation on Location investigated. Infact, habitat mapping support these ideas and stress a low presence of *Cystoseira* habitat

(only 14 % on the investigated shores) to an high variability between sites (Fig. 5). The high number of very small patch size, (Colosio et al., unpublished) hold a view of an high fragmentation of this habitat formers. All the others habitat formers identified support a different cryptic fish assemblage. Although most species were ubiquitous, being present in all habitat types, contrasting patterns in species abundance among habitats could be detected. These results suggest a certain level of spatial segregation within the cryptic fish assemblages. This is consistent with the results found by other studies, for example, on estuarine goby assemblages suggesting that within these fish guilds, spatial segregation could play an important role (Costello 1992, Wilkins & Myers 1992); Gill & Potter,1993; (Malavasi et al. 2005). In particular, PCO vector plot evidence the spatial association between the Anemonia habitat and the Gobiidae and their preference of sediment habitat, whereas mytilus bed seem to be afforded by the smaller species (*P. pilicornis* and *L. dalmatinus*). This suggests a certain degree of spatial segregation between the Gobiidae and Blennidae group. In particular, as revealed by PCO plot, within Blennidae group the species *P. zvonimiri* and *P. incognitus* strongly segregates from that formed by *P.pilicornis* and *L. dalmatinus*. According to (Gill & Potter 1993), patterns of spatial segregation are likely to reflect the life history characteristics of the species involved. In the case of the cryptic fish assemblage, it appears that the spatial segregation is consistent with the two contrasting life history features recognized by Miller (Miller 1984): the cryptobenthic, long lived, larger species (*P. gattorugine*) and the epibenthic, short-lived, smaller species (Lypophrys and Parablennius). This segregation correlates strongly with body size which, according with Tokeshi (1999), can be considered as 'one of the factors defining the niche of a species'. Body size differentiation within a local assemblage of closely related species may therefore help in promoting coexistence and mitigating

competition. Within Mediterranean cryptic fish, body size differentiation has probably arisen from common ancestors under contrasting environmental conditions. Once body size differences appear, they may promote species coexistence within a local assemblage through resource partitioning (Tokeshi 1999). Variation in habitat preference may originate from both phyletic heritage and the influence of selective forces related to recent ecological processes, as has been recognized for all phenotypic traits (Miles & Dunham 1993). In the Mediterranean, the spatial segregation between the large-sized and the small-sized species seems to reflect the phylogeny of the group, with the preference for vegetated and unvegetated habitats coinciding with habitat selection. Under the phylogenetic constraint related to the splitting of the two major clades within Blennidae family (Parablennius and Lypophrys) and their main habitat preferences, however, other ecological processes should act and contribute to the coexistence of species and to their present distribution in the environment. Within the clade of the large species (Parablennius), shift in timing of breeding may promote the reduction of the potential for interspecific competition, as for example for black goby and the grass goby (Sorice & Caputo 1999). Within the group of the smaller species, the present distribution could be determined not only by their intrinsic habitat choice, but also by the competitive/predation pressure due to the presence of larger species in unvegetated habitat, as *Anemonia* barren. Macroalgae are traditionally considered to be optimal habitats in terms of protection and refuge for small fishes (Rooker et al. 1998, Hindell et al. 2000): the results presented here seem to contradict this statement for the smaller and this contradiction could be explained by different ecological reasons. Smaller gobies do not show morphological adaptations to macroalgae: *G. bucchicchi*, for examples, have dorsal eyes, and this strongly indicates an epibenthic adaptation to open substrata. In addition, the colouration is cryptic for a

sandy-muddy substratum. By contrast, *P. zvonimiri* and the *P. incognitus* have dorsolateral eyes (Zander 1986, Hindell et al. 2000) and a completely different colouration, red-brown livrea, which potentially could promote survival in structured environments such as macroalgae or *Cystoseira* habitat.

These results suggest that further experiments are needed to better clarify the mechanisms leading coexistence between cryptic fish assemblages and different habitat features. Specifically, some hypothesis could be tested on site where canopy-forming macroalgae are not on disappearance or on a regressive status.

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Table 1 Results of permutational multivariate analysis of variance (PERMANOVA) testing for differences between ‘canopy’ (present and absent, fixed) and ‘location’ (Barriera, Rotaia, Sassi Neri and Scogli Lunghi, random, nested within ‘canopy’).

Source	df	SS	MS	Pseudo-F	P(perm)	Uperms
Canopy	1	4052.4	4052.4	0.7641	0.578	8949
Loc (Can)	2	10608	5303.8	5.2695	0.0001	8978
Res	8	8052	1006.5			
Total	11	22712				

Table 2 Results of permutational multivariate analysis of variance (PERMANOVA) testing for differences between ‘location’ (Barriera, Rotaia, Sassi Neri and Scogli Lunghi, random) and ‘habitat’(orthogonal).** unbalanced factor

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Location	3	4534.2	1511.4	0.9445	0.517	999
Habitat	3	49018	16339	23.406	0.001	997
LoxHa **	8	5584.6	698.07	0.43623	0.985	998
Res	45	72010	1600.2			

Table 3 Results of multivariate analysis of variance testing for differences between ‘canopy’ (present and absent, fixed) and ‘location’ (Barriera, Rotaia, Sassi Neri and Scogli Lunghi, random, nested within ‘canopy’) on total number of species and total number of specimen. Since there were too few permutations to obtain a meaningful P-value in a posteriori comparisons, 9999 random Montecarlo draws (P(MC) from the asymptotic permutation distribution were used (Anderson and Robinson 2004).

Source	df	N		S	
		Pseudo-F	P (MC)	Pseudo-F	P (MC)
Canopy	1	3,0109	0,2204	1,7337	0,3221
Loc (Can)	2	8,7555	0,0106	21,884	0,0007
Res	8				
Total	11				

Figure captions

Fig. 1 Map of the northern Adriatic Sea, showing the position of study location.

Fig. 2 Non-metric multidimensional scaling (MDS) plot of assemblages of cryptic fish in each combination of canopy present and absent at four locations (BA = Barriera; RO= Rotaia; SL = Scogli lunghi; SN =Sassi neri).

Fig. 3 Total number of species and specimens on each Location for the factor 'Canopy' (Canopy + and Canopy -).

Fig. 4 Total number of specimens on each Location for the factor 'Canopy' (Canopy + and Canopy -).

Fig. 5. Stacked-bar graph showing the % abundance of main habitat features measured along linear transects in the Localities investigated.

Fig. 6 PCO plot of assemblages of cryptic fish in each combination of different habitat features (C=Cystoseira spp.; M=Mussel bed; MA=Macroalgae; A=Anemonia) at four locations.



Figure 2

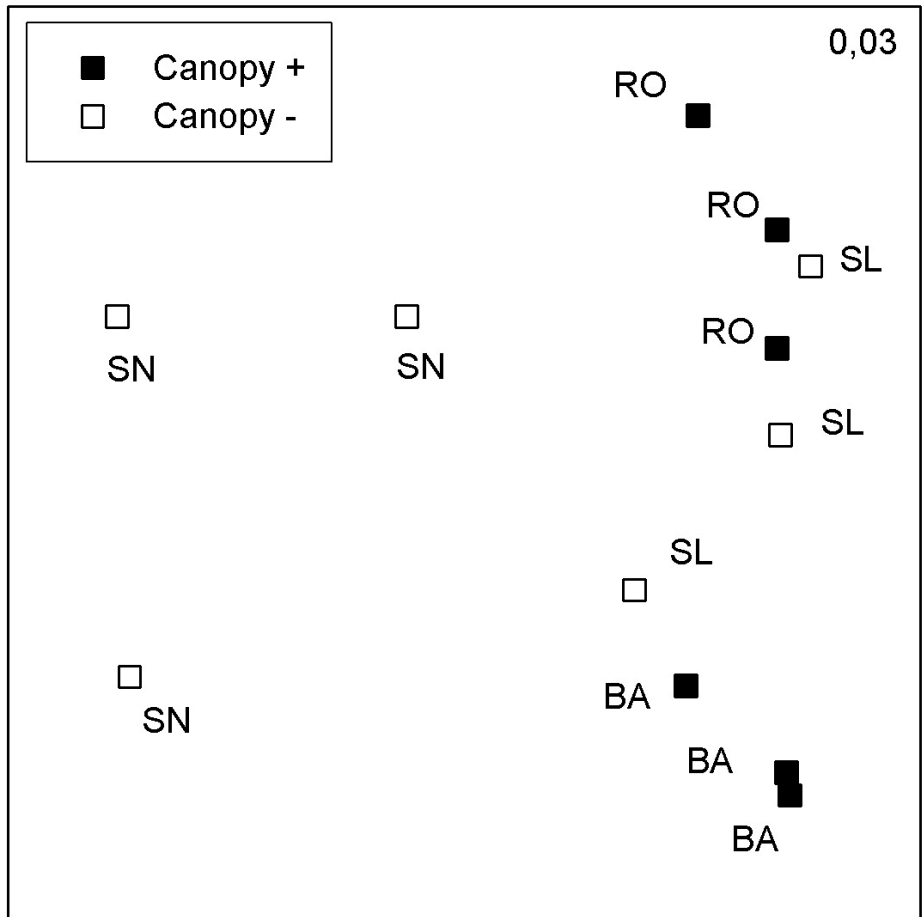


Figure 2

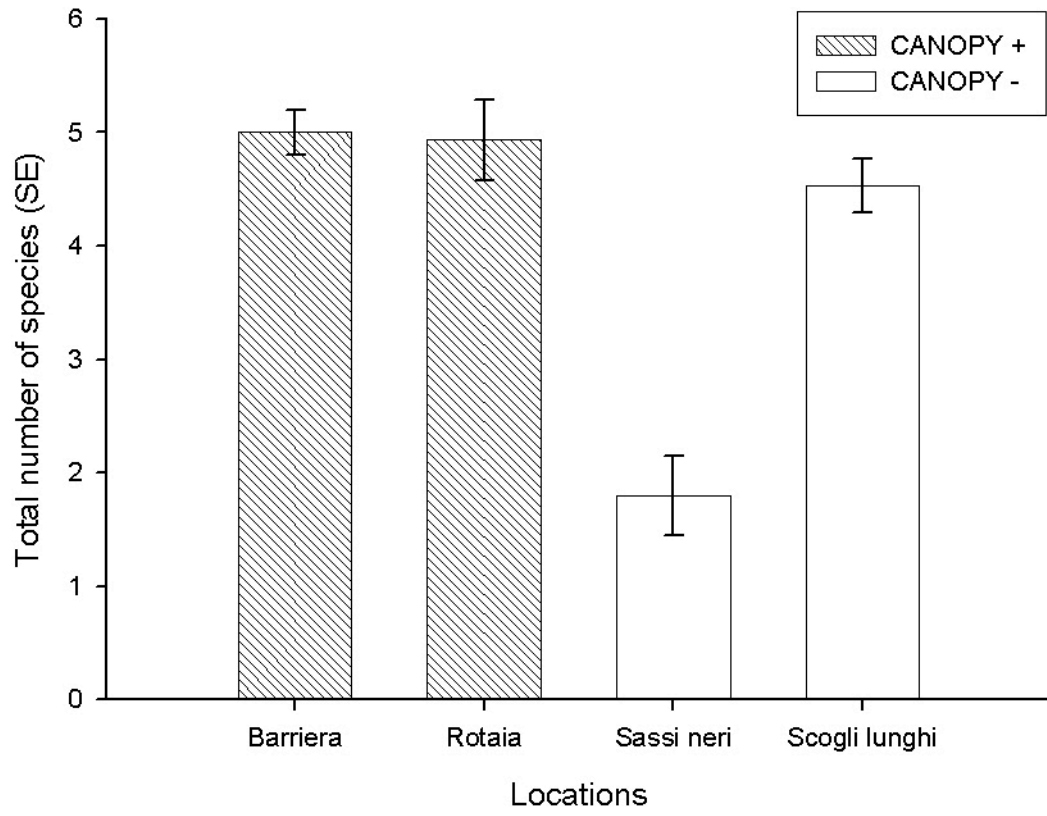


Figura 3

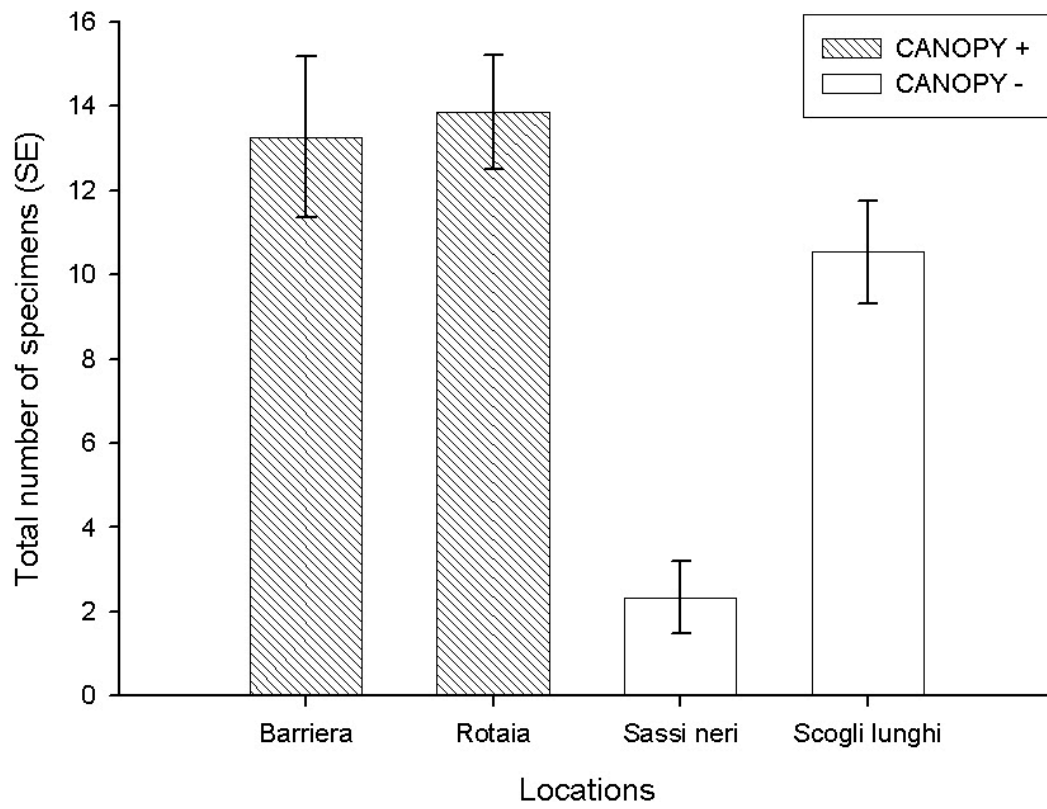


Figure 4

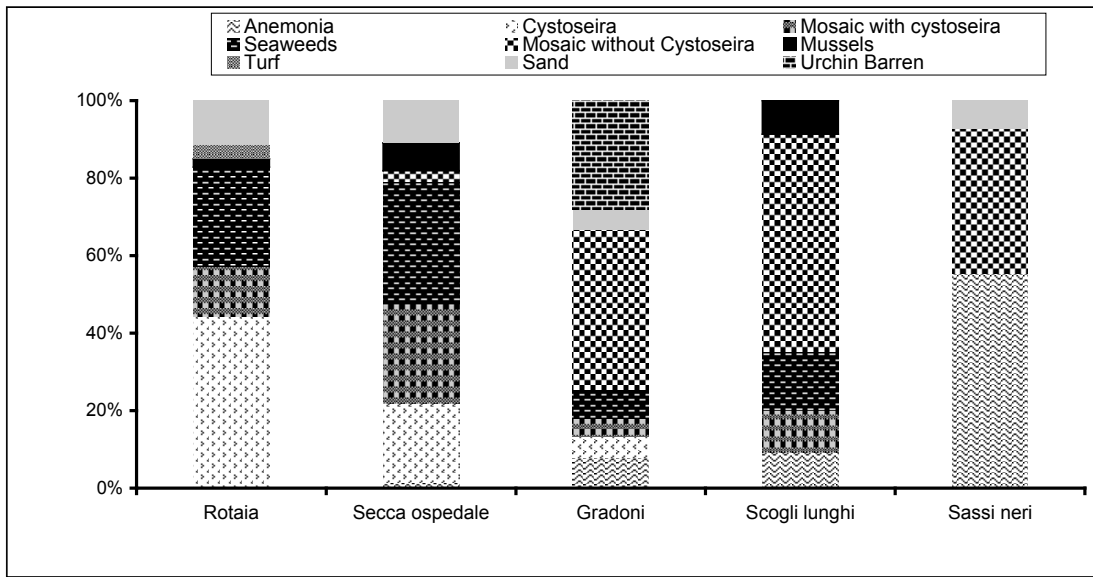


Figure 5

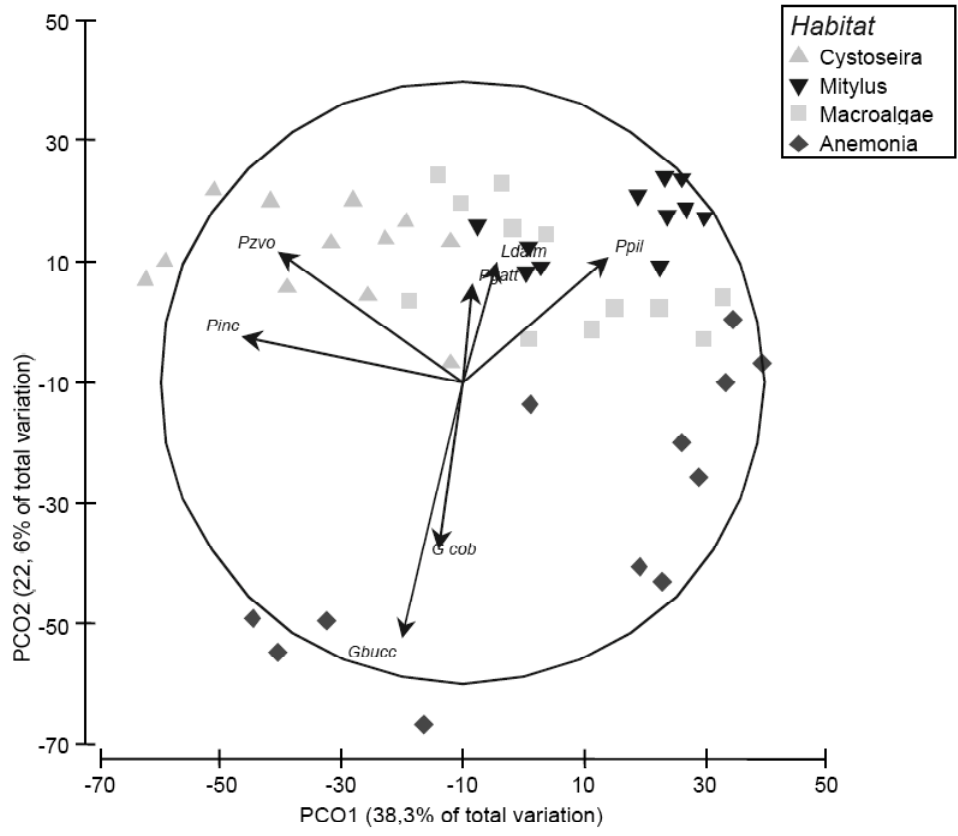


Figure 6

CHAPTER 3

Canopy forming-loss: which are consequences on cryptobenthic fishes?

Santin, S.

Abstract

Changes in crypto benthic fishes were monitored over 12 months in replicated control and cleared of *Cystoseira* spp. macroalgae experiment on Conero cliff (Nord Adriatic Sea). Fish were assessed by underwater visual census in habitat where canopy-formers macroalgae were manipulated (C-) and were naturally present (C+). Multivariate analysis showed that cryptic fish assemblages have a weak relationship with canopy presence and species richness and number of species were unaffected by canopy presence. Removal of canopy-forming macroalgae produced less change to fish assemblages than reported in studies elsewhere. The canopy forming macroalgae *Cystoseira* spp. is not generally indicative of high species richness of either macroalgae or vegetation-associated cryptic fishes. However, PCO analysis evidence that patches with *Cystoseira* vegetation removal housed a specific fish assemblage, such as presence of the Gobiidae specimen. This suggests that the present decline in *Cystoseira* spp is followed by a change in the communities of vegetation-associated invertebrates. This might in turn affect higher trophic levels, by changing food availability for predatory fish in shallow seaweed communities.

Introduction

Foundation species are generally dominant primary producers and/or habitat-forming species (such as kelps, corals, and mangroves), both in terms of abundance and community influence [sensu Dayton]. Their depletion by human activities, be it directly or through trophic cascades, can have tremendous effects on biodiversity at the community level (Coleman and Williams, 2002). Throughout Mediterranean Sea canopy forming macroalgae dominate community assemblages. The genus of brown algae *Cystoseira* C. Agardh (Fucales, Phaeophyceae) is distributed worldwide, but most species are found in the Mediterranean and the adjoining Atlantic (Amico, 1995). Mediterranean species inhabit a wide array of benthic ecosystems, from the upper infralittoral to the upper circalittoral (Go´mez et al., 2001), where they often dominate the canopy (Ballesteros et al., 1998; Ruitton et al., 2000; Bulleri et al., 2002; Chemello and Milazzo, 2002). Most *Cystoseira* species have, however, vanished from large areas of the northwestern Mediterranean in the recent decades (Thibaut et al., 2005) and four of them are currently so rare that they have been classified as endangered by the Barcelona Convention (Antonioli et al., 1999). *Cystoseira* disappearance has also been reported recently from parts of the Tyrrhenian and Ionian seas (Serio et al., 2006). The dominant canopy-forming algal species (*Cystoseira* spp.) in the northwest Mediterranean sheltered many other species of algae [>100 per 400 cm^2], invertebrates, and fish, and the loss of these habitat creating algae results in an order of magnitude loss of species richness and a decline in ecodeiversity (Sala & Knowlton, 2006). Moreover, the recruitment of many species of littoral fish occurs in shallow bottoms with abundant canopy-forming algae; hence the loss of the algae may also inhibit fish recruitment.

Loss of canopy algae typically results in recruitment of ephemeral algae which take advantage of the creation of unshaded space, followed by recruitment of longer lived species including the manipulated species itself (Benedetti-Cecchi & Cinelli 1992).

Manipulative studies aimed at assessing the role of seagrass canopies for fish have been carried out (see for instance Bell and Westoby 1986a,b,c; Connolly 1994a). Experimental manipulations on Mediterranean seagrasses have, however, been performed by Connolly and Butler (1996), to evaluate the effects of altering *Cymodocea nodosa* leaf height on small, motile invertebrates in Corsica (France). In the Mediterranean Sea, most authors have studied the fish fauna associated with *Posidonia oceanica* (Bell and Harmelin-Vivien 1982; Harmelin-Vivien and Francour 1992; Francour 1997; Guidetti 2000 and references cited therein), the most widespread seagrass endemic to the basin. Seagrass beds are often quoted as offering good protection from predation (Stephens, et al. 2006) due to within-patch structural complexity. Complexity benefits some smaller and cryptic fish by providing refugia, yet is detrimental to visual predators by concealing their prey (Edgar and Shaw, 1995). Cryptic fishes have complex ecological and behavioral interrelationships and spend a large proportion of their lives in circumscribed and typically fixed patches (e.g. Connell and Jones 1991).

The aim of this study is under the hypotheses that cryptic fish assemblage (species composition and relative abundances), species richness and fish density (of both the entire assemblages and individual species) are affected by canopy removal of *Cystoseira* spp. By comparing the utilization by cryptic fish of *Cystoseira* beds, changes in the ecological function of coastal areas with loss of macroalgae could be evaluated.

Materials and methods

Site description and experimental design

This study was conducted from August 2006 to October 2007 along a rocky coast south of Ancona (Fig. 1), on the north east of Italy, close to Conero cliff (43°32'52, 22'' N; 13°37'38, 74 E). Along these coast *Cystoseira* spp has presently a limited distribution, is absent from large areas and is the only canopy-forming species in shallow waters.

The most extensive habitats are rocky bottoms and seagrass meadows, although bare sandy areas exist in water deeper then 10 m, running parallel to the coast.

Previous studies (Santin, unpublished) reported macroalgal presence along Conero cliff in two site. Only in one location (Rotaia) *Cystoseira* bed was enough dense and wide to test our hypothesis. On this Location (hereafter called RO) we selected randomly and assigned either control or canopy removal treatments on transects. A factorial design was applied to assess the effect of canopy removal (C+ vs C-) and time (0, 1, 2, 3, 4) on the abundance of fish. Cryptic fish were censused along transects 5 m long and 1 m wide. For the removed patches, fish were thus sampled over a surface area slightly smaller in order to avoid the edges of habitats (following Connolly 1994a).

Eight patches (about 2 m²) were selected into the transect length and were surveyed during 1 month prior to algal canopy clearance (hereafter called time 0) and at intervals of 1, 3, 9 and 12 months after clearance (hereafter called, respectively time 1, 2, 3, 4). On each sampling occasion, 4 transects (except in time 3 and 4 due to bad sea weather conditions was not possible relocate clearance plot) was censused in 3–6-m water depth and the number on the reef of each species of fish was identified and recorded. We did 4 replicates for each time except on time 3 and 4 after clearance due to bad weather condition only 3 plots were relocated. All sampling was carried out in

calm weather conditions and with water visibility >3 m.

For each canopy clearings scissors were used, cutting back only fronds, in order to allow future-growing and attentions was paid not to damage the holdfasts of the alga.

Cleared and control patches were marked at their corners with epoxy putty (Subcoat Veneziani) for further identification.

Statistical analyses

Untransformed data of cryptic fish abundance was analysed using non-metric multidimensional scaling (nMDS) (Kruskal and Wish 1978), by means of the PRIMER© statistical package, to assess the differences in fish community structure among canopy and trough time. To test differences in the abundances of individuals and number of cryptic fish species among time (5 levels, fixed), and removal (2 levels, fixed), we did a 2-factor permutational multivariate analysis of variance (PERMANOVA, Anderson 2001) based on Bray-Curtis dissimilarities (Bray and Curtis 1957). For the analysis, 9,999 unrestricted random permutations of residuals were used to generate *P*-values (Anderson, 2001b). The fish counts did not vary greatly in scale between species and the dissimilarity matrix was calculated from raw count data. A test of homogeneity of multivariate dispersions within treatments was tested using PERMDISP (Anderson 2006). Pairwise a posteriori comparisons were executed using 9, 999 permutations (Anderson 2004). To test differences in the abundances of individuals and number of cryptic fish species on each time (5 levels, fixed), we did a 1-factor permutational multivariate analysis of variance (PERMANOVA, Anderson 2001a) based on Bray-Curtis dissimilarities (Bray and Curtis 1957). For the analysis, 9,999 unrestricted random permutations of residuals were used to generate *P*-values (Anderson, 2001b). Since there were too few permutations to obtain a meaningful *P*-value in a posteriori comparisons, 9999

random Montecarlo draws from the asymptotic permutation distribution were used (Anderson and Robinson 2004).

While unconstrained ordinations attempt to place the samples in two-dimensional space in their most parsimonious configuration, a constrained ordination, called canonical analysis of principal coordinates or CAP (Anderson and Willis 2003) was used to conduct a discriminant analysis by reference to the null hypotheses of no differences between removal or time. This method is a type of discriminant analysis that searches for the axes in multivariate space that maximise between-group differences.

An ordination of cryptic fish assemblages were made by principal coordinate analysis (PCO, Anderson and Willis, 2003) and to visualize multivariate patterns on PCO plot a projection biplot of species, with a raw Pearson correlations value > 0.65 , were superimposed onto it. The PCO axes were superimposed onto the corresponding PCO ordination, to visualize the species most responsible for the patterns of multivariate dispersion.

Results

There are no significant differences on cryptic fish assemblages in the nMDS plot (Fig 2), however the high stress value of these ordination (0.16) indicates that the true relationships between points are not well represented in two dimensions (Clarke 1993). The time trajectory of cryptic assemblages responses between the removal treatment can be visualized in the nMDS plots (Fig.3a and 3b). The fish assemblages in the canopy removal treatment (Fig. 3a) do not quickly diverged from the other in the multivariate space on time 0 and 1, but there's a clear separation on time 2. The fish assemblages in the canopy control treatment (Fig. 3b) showed variation and trajectory similar to canopy removal, with a marked separation in the multivariate space on time 2. On both plots the end points were remarkably similar to the start points.

PERMANOVA results showed differences in the factor time but not in the removal, there was no significant interaction between the two factors, indicating that are no differences between the removal levels (Table 2). Pairwise tests showed that this was caused by significant differences between time 0 and time 2 ($p= 0.0154$) and between time 0 and time 3 ($p=0.0381$), as underlined by MDS plots. Tests of multivariate dispersion were not significant for time ($p > 0.2$), so differences seen in the PERMANOVA can be safely attributed to differences in multivariate time, rather than between-treatment differences in the dispersion, or spread, of points in multivariate space. PERMANOVA results showed differences in the abundances of individuals and number of cryptic fish species on time (table 3).

The CAP analyses showed a significant effect of Time (Fig. 4) with a squared canonical correlation of $\partial^2 =0.562$ ($p= 0.0104$). The canonical axes corresponding to the 2 main effects separate the cryptic fish assemblages present on patch removed on

time 0 from time 2 and time 3. The relative distinctiveness of the assemblage is demonstrated by differences in the leave-one-out allocation success from the CAP analysis. Assemblages on time 4 were much more variable and harder to predict (i.e. had a greater misclassification error) than did assemblages on time 2 and time 3.

The PCO axes superimposed onto the corresponding PCO ordination (fig. 5) visualize the species that are most responsible for the patterns of multivariate dispersion. While there's a no clear separation between canopy and control, on patch whit C -, there' s a correlations with Gobiidae species (*G. cobitis* and *G. paganellus*) and with some Parablennius species (*P. rouxi*, *P. zvonimiri*, and *P. tentacularis*), while there's a no clear correlations on patch with C-.

Discussion

These results indicate that cryptic fish assemblage doesn't seem to have a clear distribution with canopy forming macroalgae on Conero cliff. MDS ordination indicate that the cryptic assemblage exhibited a weak response to canopy clearance . Trajectories of multivariate change through time evidence a separation among the initial phase of the removal experiment (time 0 and time 1), a transitional position, but well separated from all the others (time 2) and a compact group (time 3 and 4) closer to the initial phase. This trend is similar on removal and on control plot and seasonality could be supposed. This can be supported by PERMANOVA results that evidence a difference through time on the fish assemblage, confirmed by pairwise a posteriori comparison. Nevertheless, the diversity in the treated and control patches was not different. A great degree of seasonality in the abundance of cryptic fish assemblages was observed, probably due to the variation in temperature and, therefore, in the activity of these species. Even CAP results evidence a differentiation of multivariate assemblages on time 2 from all the others and again a support a seasonality, that could probably mask any Canopy effect.

On PCO plot, the preference of *G. cobitis* and *G. paganellus* for Canopy cleared substrata might just reflect their association with lighted environments, such as vertical or overhanging rocks, which are unsuitable for settlement of photophilic algae. In the absence of algal cover, their aptitude to rest on the bottom surface for very long time, along with a highly cryptic coloration, may compensate for the increased predation risk. Small benthic fishes may benefit from vegetation in terms of both feeding resources and refuge opportunities. As small crustacean-feeders *Parablennius* species can successfully rely on a variety of invertebrates usually associated with turf algae, that is present on patch cleared.

The structure of the macroalgae habitat serves a number of functions for fishes, and the heterogeneity of this structure provides opportunities for niche diversification (Stephens et al, 2006). Together, these factors account for the high

density and diversity of fishes associated with this habitat (Bond et al., 1999). Some of the more important functions provided by the reef/kelp or macroalgae habitat to the fish assemblage are shelter, food availability, and nesting sites for the fish assemblage.

Shelter is one of the most important functions provided by the reef/kelp habitat. Shelter is especially important to small species and to the young-of-year of many larger species but is also important to larger species that associate with the substrate.

The notion of “shelter” is actually complex and can work in a number of ways. Most obviously, hard structures can provide physical protection from predators. Here, crevices, burrows, and caves in rock and other solid structures can prevent a predator from gaining access to potential prey. Second, both hard and soft substrates can serve as objects behind which fish can hide, escaping visual detection by potential predators or prey. Closely related to the role of substrate in hiding fish, the substrate may provide a cryptic background that conceals a fish, even if it is not hidden behind an object.

Seagrass beds are often quoted as offering good protection from predation (Hindell et al., 2000) due to within-patch structural complexity. Complexity benefits some smaller and cryptic fish by providing refugia, yet is detrimental to visual predators by concealing their prey (Edgar and Shaw, 1995).

Although few studies involving experimental manipulations on seagrasses have so far been published, there is a large body of scientific literature supporting the model that denser seagrass habitats host fish assemblages that differ from those in unvegetated sands or sparser beds. For instance, Guidetti (2000) observed that species richness, total fish abundance and the densities of some individual species were significantly higher in *Posidonia oceanica* than on bare sand in two areas of the

Adriatic Sea. Bell and Westoby (1986c), by manipulating leaf height and density of two seagrass species (i.e. *Zostera capricorni* and *Posidonia australis*) in Botany Bay (NSW, Australia), observed that, at a local scale, species richness, total fish abundance and the densities of several individual species were not positively related to the structural complexity of seagrass meadows. Moreover, a fraction of the significant responses observed at a local scale were not repeated at a wider spatial scale (Bell and Westoby 1986b; but see Worthington et al. 1992), suggesting that factors other than the physical complexity of seagrasses are probably superimposed in impacting the associated fish assemblages. In other studies (Ebeling and Laur, 1985; Ebling et al., 1985; Carr, 1989; DeMartini and Roberts, 1990), changes in fish populations were detected following algal canopy clearance. Changes observed following manipulation may have resulted from direct associations between fishes and plants/macroalgae, both positive and negative, or may represent sampling artefacts caused by greater diver detectability of fishes in areas lacking dense algal cover (Edgar et al 2004). In addition to providing protection from predators (Guidetti, 2000), the presence of a dense plant canopy probably supports many fish populations in kelp forests through the productivity of associated food resources. Densities of small mesograzers, such as amphipods, polychaetes and gastropods, provide the bulk of the diets of the majority of small cryptobenthic fishes in vegetated habitats (Edgar and Shaw, 1995). Mesograzers can occur in tens of thousands per square meter amongst seaweeds (Edgar, 1983a,b), and are likely to be at least an order of magnitude more abundant in kelp forests than open barren grounds. Conversely, some fish species are negatively affected by the presence of dense kelp (Jones, 1992; McClanahan et al., 1999). Across southern Australia, fish communities associated with urchin barrens generally tend to be dominated by planktivorous and omnivorous

species, whereas carnivores that consume mesograzers and herbivores more typically predominate in kelp-dominated habitat (Edgar and Barrett, unpublished data).

Given the extent to which canopy loss affects many fish species, any consideration of ecosystem changes that accompany transformation of vegetated areas to urchin barrens or anemonia barrens should include an assessment of changes to associated fish populations, as well as the more readily documented changes to plants and invertebrates.

In conclusion, our data show that presence of the canopy forming macroalgae *Cystoseira spp.* is not generally indicative of high species richness of either macroalgae or vegetation-associated cryptic fishes. However, patches with *Cystoseira* vegetation removal housed a specific fish assemblage, such as presence of the Gobiidae specimen. This suggests that the present decline in *Cystoseira spp.* is followed by a change in the communities of vegetation-associated invertebrates. This might in turn affect higher trophic levels, by changing food availability for predatory fish in shallow seaweed communities.

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Table 3 PERMANOVA results on untransformed data, comparing assemblages between clearings and controls, between time (0, 1, 2, 3 and 4) since the initiation of the experiment. *P<0.05

Source	df	SS	MS	Pseudo-F
Removal	1	2355,7	2355,7	1,0211
Time	4	12247	3061,7	1,5983*
Removal x Time	4	9252,2	2313,1	1,2075
Res	26	49805	1915,6	
Total	35	73543		

Table 4 PERMANOVA of the effects of removal (fixed) and time (fixed and crossed with habitat) on the number of species and specimen accounting for differences in cryptic fish assemblages. *P<0.05, **P<0.01, ***P<0.001

Source	df	Number of species (N)		Number of specimen (S)	
		MS	Pseudo-F	MS	Pseudo-F
Removal	1	0,98039	0,14385	0,1201	7,14E-02
Time	4	11,545	5,7404***	1,9479	2,8267*
Re x Ti	4	6,8889	3,4252*	1,6979	2,464
Res	26	2,0112		0,6891	
Total	35				

Figure legends

Fig. 1 Map showing the study Location. (With courtesy to Ministero dell'Ambiente-Ispettorato Centrale per la difesa del mare)

Fig. 2 MDS plots showing changes in fish assemblages following canopy removal experiment.

Fig. 3a MDS plot showing cryptobenthic assemblages changes over time in patch not cleared of macroalgae are shown with arrows. Sampling occasions are 0: before; 1: 1 month post-disturbance; 2: 3 month postdisturbance; 4: 3 months post-disturbance; 5: 6 months post-disturbance.

Fig. 3b MDS plot showing cryptobenthic assemblages changes over time in patch cleared of macroalgae are shown with arrows. Sampling occasions are 0: before; 1: 1 month post-disturbance; 2: 3 month postdisturbance; 4: 3 months post-disturbance; 5: 6 months post-disturbance.

Fig. 4 Constrained CAP plots on the basis of Bray–Curtis dissimilarity measures calculated on untransformed data comparing assemblages in clearings among time (*triangle up* time 0, *triangle down* time 1, *square* time 2, *diamond* time 3, *circle* time 4).

Fig. 5 Plot of the first two principal coordinate axes (PCO, metric multidimensional scaling) showing ordination of sediment samples for removal treatment (C+ =Cystoseira naturally present; C-= Cystoseira removed). Analyses were based on Bray-Curtis distance on untransformed data matrix. Vectors indicate the species that best correlated with the ordination (correlation with ordination axes $r > 0.6.5$). The direction of vectors indicates the correlation with different groups of sample-points and the length is proportional to the value of correlation.

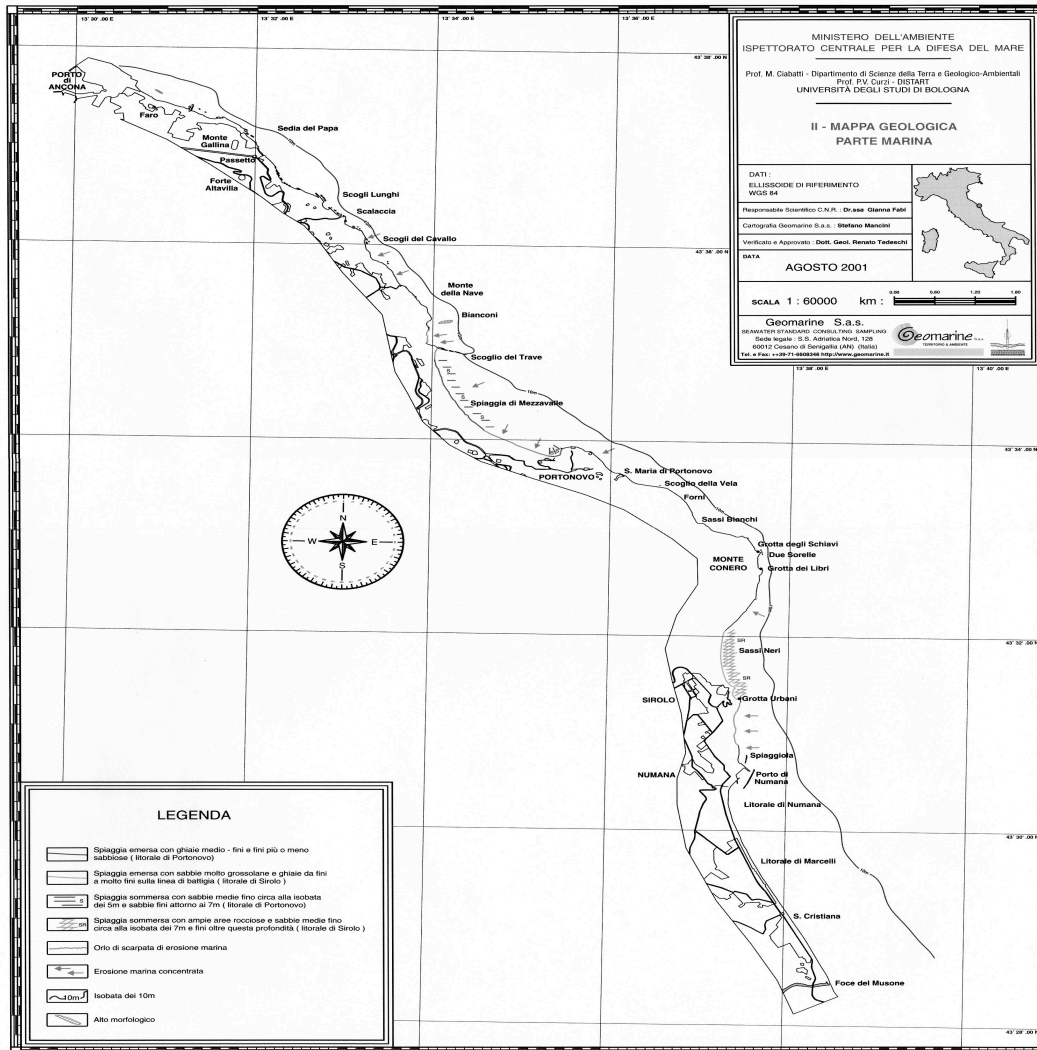


Figure 3

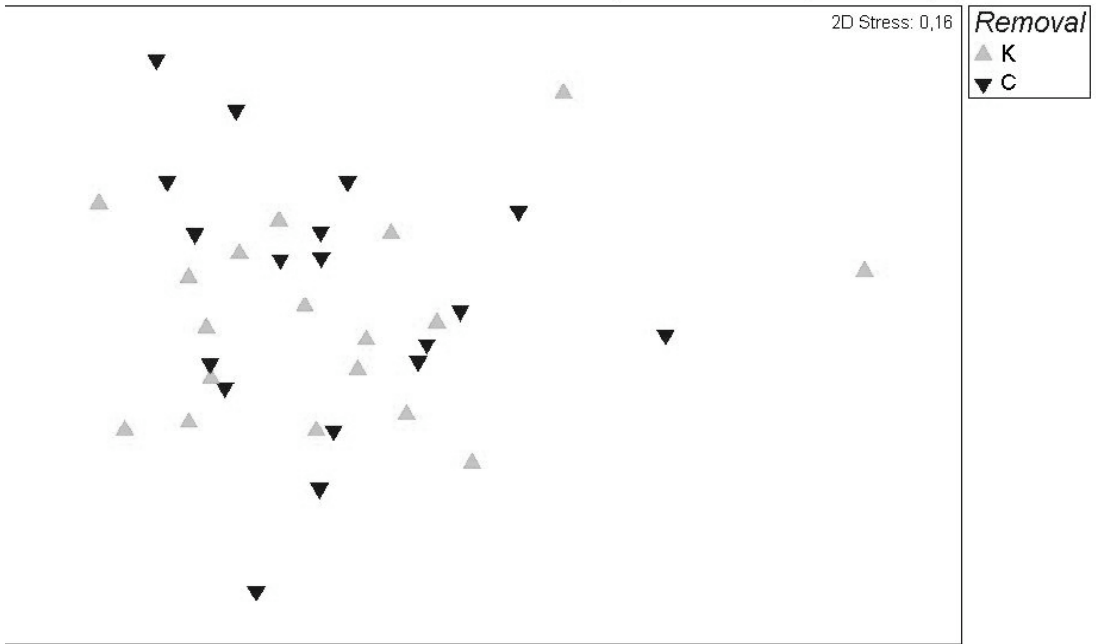


Figure 2

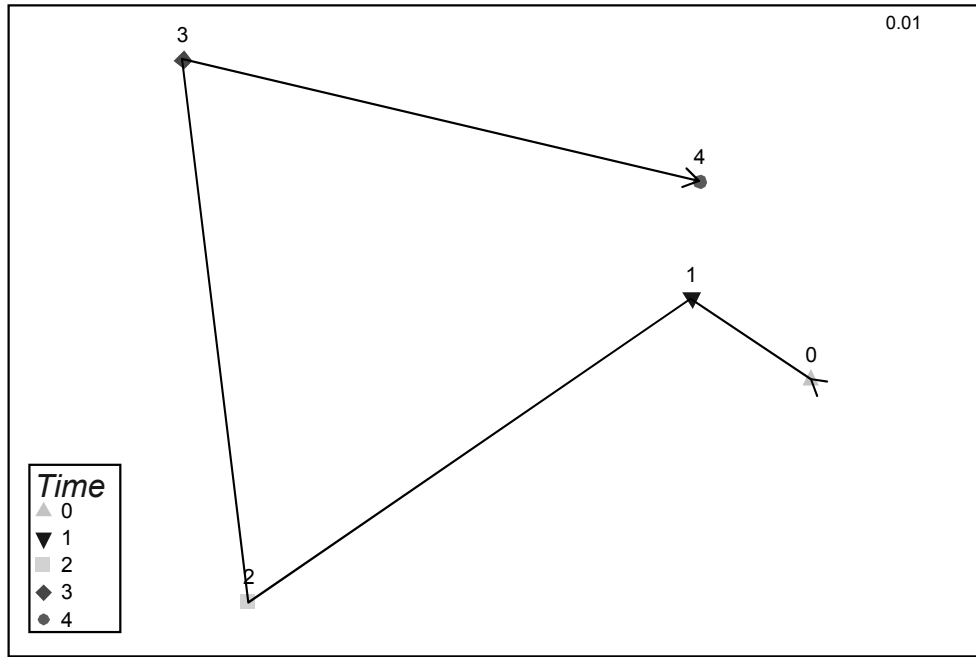


Figure 3a

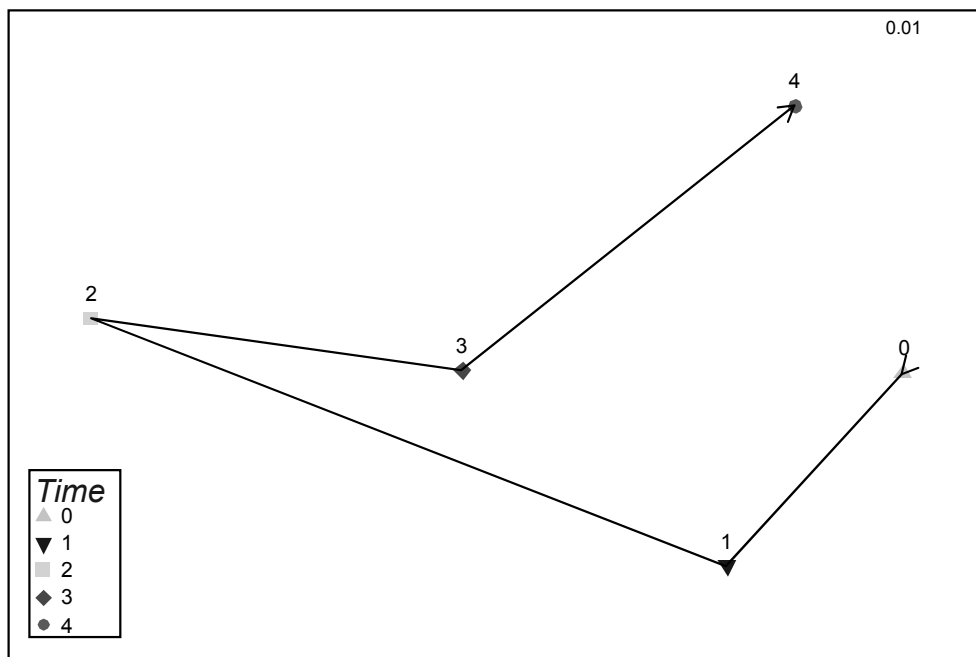


Figure 3b

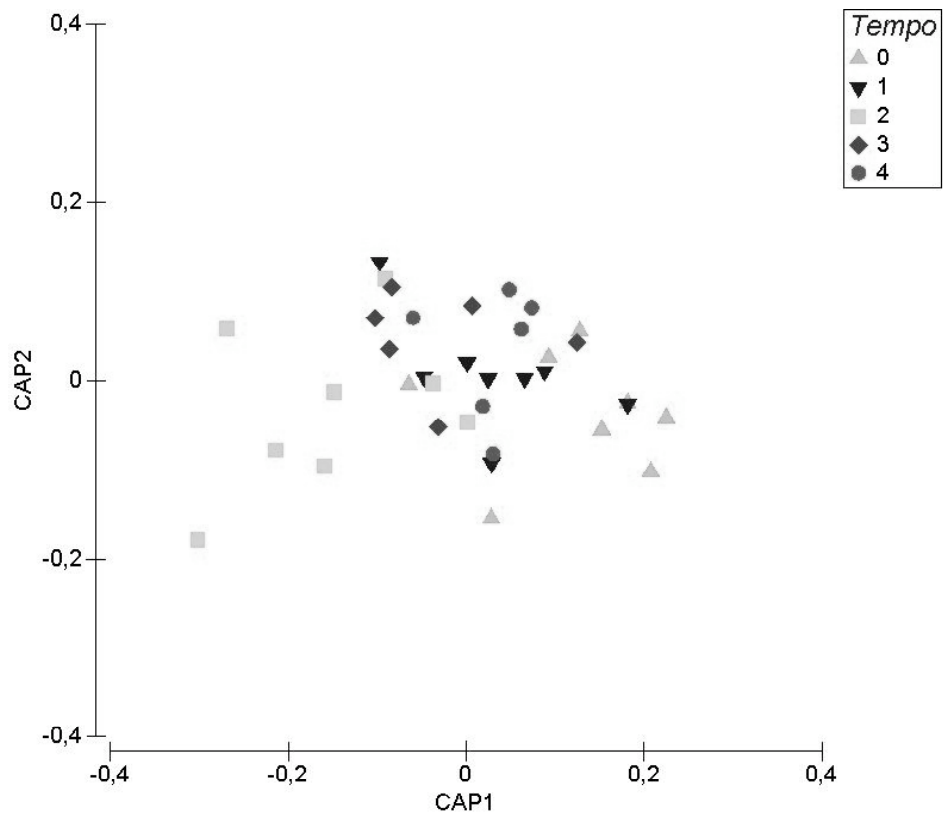


Figure 4

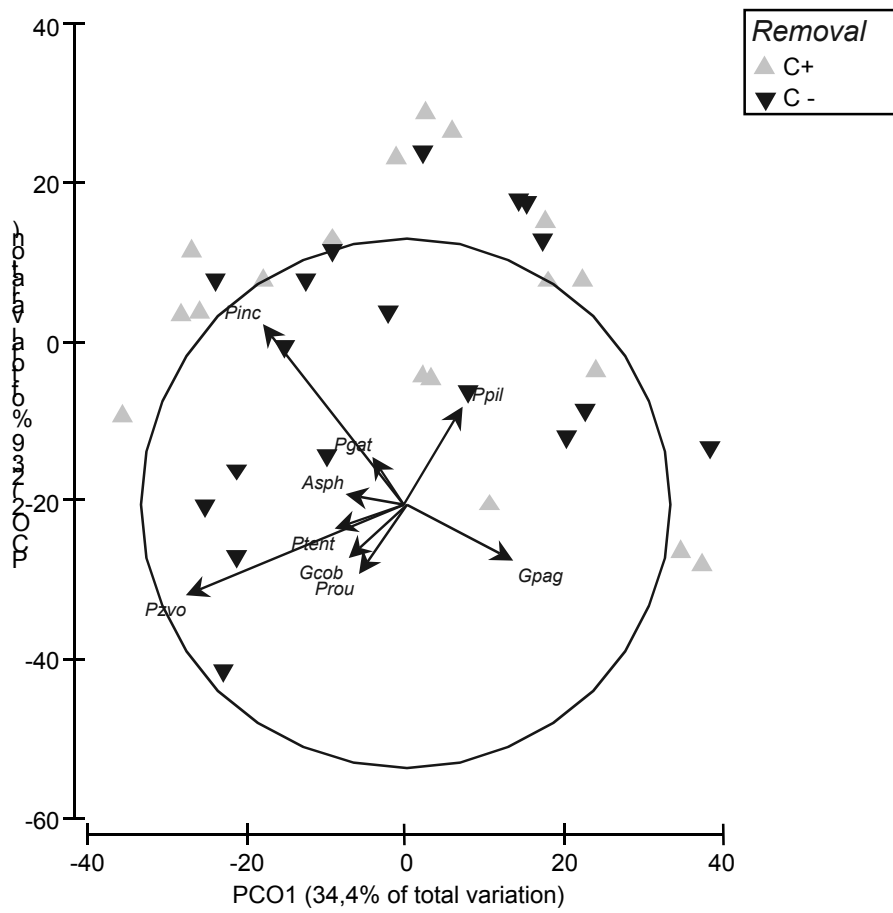


Figure 5