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#### **Decapod Crustaceans associated with an artificial reef (Adriatic Sea)**

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#### **Abstract**

The aim of this study is to increase the knowledge on the distribution of decapod crustaceans associated with an artificial reef positioned on sandy-mud bottoms in the central Adriatic Sea. The reef is constituted of concrete modules assembled in pyramids and concrete poles. Hard and soft bottom samples were collected from 2001, just after reef construction, to 2005 (4 surveys per year). Regarding the soft seabed*,* three sites close to a pyramid, three inside the reef area at a distance of 10-15 m from the structures, and three 200 m outside the reef (control sites) were randomly sampled during each survey. At the same time, three pyramids (vertical and horizontal walls) and three poles were also investigated. After taxonomical analysis, decapod crustaceans were analysed using abundance and species richness. Sites and years were compared using a balanced, fixed effect, 2-way ANOVA and PERMANOVA. In addition, SIMPER analysis was performed to identify those species typifying each community inhabiting both the soft bottoms and the artificial substrates. The results showed that the artificial reef induced an increase in both abundance and diversity of the decapods of the natural habitat. In fact, man-made substrates may offer new available space for biological colonization and allow the settlement of new species usually living on hard bottoms, thus increasing the complexity of the original benthic communities.

**Keywords:** Artificial reef, artificial substrates, soft bottoms, decapod crustaceans, Adriatic Sea.

#### **Introduction**

Artificial reefs are man-made structures deliberately deployed in coastal waters to mimic certain characteristics of natural rocky habitats. They fall within the wider definition of "artificial habitats", including any object deliberately placed in the aquatic environment to protect, enhance and manage resources (UNEP MAP, 2005). Since the middle of the  $17<sup>th</sup>$  century artificial reefs of various shapes have been set up in Japan to attract fish and enhance local fisheries (Ino, 1974; Thierry, 1988). The modern concept of "artificial reef" was born in Japan in the following century and spread to the USA at the beginning of the 1800s. In Europe, this concept was adopted in the second half of the 1900s and artificial reefs have been deployed in many countries since then (Fabi *et al*., 2011).

Initially, artificial reefs were a tool used to promote fisheries through the aggregation of fish species in certain areas but later, thanks to the development of new technologies, their use has been extended to other applications both ecological and productive. For example, in the Mediterranean Sea, artificial reefs have been used for over 40 years mainly for fisheries management, involving protection of coastal areas or other sensitive habitats (which generally include spawning and nursery areas

for many commercial species) against illegal trawling, enhancement of small-scale fisheries, and reduction of conflicts among different fishing activities (Fabi & Spagnolo, 2011; Seaman *et al*., 2011).

Currently, about 200 artificial reefs are deployed in the Mediterranean Sea, and Italy was one of the first countries to make extensive use of them. More than 70 reefs have been placed in the Italian seas up to date. Most of them are medium- or large-scale reefs, while others are small-scale experimental reefs used for research (Fabi & Spagnolo, 2011).

The wide use of these structures necessarily requires the assessment of their effects on the marine environment, which is particularly sensitive, especially in coastal areas (Carter, 1988).

An important role played by artificial reefs regards their capability to represent scientific experimental grounds. Indeed, they may be used to investigate the modality of animals and plants colonization in time, given that the characteristics and deployment time of substrata are well known.

Several studies around the world have focused on the evolution of entire benthic communities settled on artificial substrates or living in the surrounding original seabed in order to evaluate the effects of these structures

on benthic ecosystems (Davis *et al*., 1982; Baynes & Szmant, 1989; Ambrose & Anderson, 1990; Posey & Ambrose, 1994; Bombase *et al*., 1997; Bortone *et al*., 1998; Fabi *et al*., 2002; Spagnolo *et al.,* 2004; Manoudis *et al*., 2005; Cheung *et al*., 2009; Fava *et al.,* 2010).

Artificial substrates, indeed, may induce either physical and/or biological changes in adjacent soft bottom habitats and related communities. For example, they may affect bottom currents and alter the sediment-size distribution thus favouring accumulation of organic material and, consequently, the presence of associated organisms (Ambrose & Anderson, 1990). Organic enrichment of sediments may also be caused by the activity of reefdwelling organisms. Moreover, greater prey availability may attract predators whose abundance may induce a decrease in infaunal densities around the reef (Davis *et al*., 1982).

Artificial reefs placed on soft seabed far from natural rocky areas allow the settlement of new species usually living on hard bottoms, thus increasing the complexity of the original benthic communities (Seaman & Sprague, 1991). Type of employed material, shape and height of modules along the water column as well as location of the reef are some of the factors that may influence the structure of the epibenthic population. For example, the horizontal walls of artificial reefs placed in front of a river mouth can be affected by intense sedimentation due to the river's inflow and will likely be covered by very fine mud; on the other hand, on the vertical surfaces, the hydrodynamics produce a continuous turn-over and reduce the accumulation of suspended material. As a consequence, the horizontal walls are colonized by hard-substrate species together with deposit- and filterfeeder organisms typical of soft bottom (e.g. gastropods and bivalves), while the vertical ones are characterized mainly by the settlement of filter-feeders such as bivalves, hydroids, and barnacles (Spagnolo *et al*., 2004).

In spite of wide knowledge on the overall benthic communities associated with artificial reefs, very few studies deal with specific groups constituting such communities and the interactions among them (Badalamenti & Riggio, 1986; Gravina *et al*., 1989; Somaschini *et al.,* 1997; Badalamenti *et al*., 2002; Marzialetti *et al*., 2009). Indeed, the analysis of the ecological processes leading to the development of specific organisms instead of others and of the linkages among the different groups is essential to plan new artificial reefs.

In order to contribute to filling this gap, this study refers to the temporal evolution and spatial distribution of the decapod crustaceans associated with an artificial reef located in the central Adriatic Sea. The decapod crustaceans represent one of the most dominant and diversified group of infaunal communities, constituting a key taxon linking lower and higher trophic levels (Wenner & Boesch, 1979; Cartes, 1998; Fanelli *et al*., 2007) and having a variety of ecological functions (De Juan & Cartes, 2011). They include organisms with varied feeding behaviour (e.g. from secondary carnivorous to deposit feeders) and belonging to different biocenoses.

This paper constitutes the first specific study focused on this taxon in Adriatic artificial reefs and one of the few in the Mediterranean Sea, and represents a step towards gaining a better understanding of the ecological role played by man-made structures as regards decapods living inside and outside the reef.

# **Material and Methods**

#### *Study area*

The artificial reef (Fig. 1) is located 3 nautical miles (nm) offshore from Porto Recanati (central Adriatic Sea) at a depth of 12.5 m in an area characterized by fine sediments with the presence of coarser fractions, without natural rocky outcrops or seagrasses.

This reef, deployed in spring 2001, covers an area of about 54.5 ha and consists of 222 pyramids (Py) positioned at a distance of 80 m from each other and of 444 poles (P).



*Fig. 1:* Location and drawing of Porto Recanati artificial reef.

Each pyramid is made of five cubic concrete blocks (2x2x2 m), four at the bottom and one at the top (height: 4 m), having rough surfaces to promote the settlement of sessile organisms and holes of different dimensions to provide shelter and habitat for various marine organisms. The concrete poles have a height of 4 m and are placed at regular intervals between the pyramids and along the reef perimeter.

### *Sampling strategy*

Soft- and hard-bottom macrozoobenthic assemblages were sampled from 2001, just after reef deployment, to 2005. In 2001, the poles were not sampled due to the lack of epibiotic organisms. Consequently, monitoring of these structures started in 2002. Four surveys were conducted each year, in the summer.

As regards the soft-bottom, three sites close to a pyramid (C), three inside the reef area 10-15 m from the structures (F), and three 200 m outside the reef (control sites: K) were randomly selected during each survey. Three samples (40x40 cm) were collected at each site using a suction sampler.

Three Py and three P randomly chosen were also investigated collecting three samples from the vertical walls (PyV), three from the horizontal (PyH) ones of each pyramid and three from each pole. The scraping technique (40x40 cm) was employed on PyV and P, while a suction sampler was used on PyH.

The collected material of both soft- and hard-bottom samples was sieved *in situ* through a 0.5 mm mesh and all organisms retained were preserved in 5% buffered formalin. In the laboratory, macrofauna was sorted using a stereomicroscope and a binocular microscope, identified to the lowest taxonomic level where possible and using standard nomenclature, quantified, and weighted.

#### *Data analysis*

Univariate analyses - Given that the poles were monitored starting from 2002, the statistical analysis regarding the man-made substrates (P and Py) were performed using the data collected in the period 2002-2005.

Decapods were analyzed using mean abundance (N; ind m<sup>-2</sup>) and mean species richness  $(S_m)$ . For both soft and hard bottom sampling sites, a statistical comparison of N and  $S_m$  between each site typologies (soft bottom: C, F and K; hard bottom: PyV, PyH, and P) was performed through a balanced, fixed effect, 2-way ANOVA (Lindman, 1992), using Site and Year as main factors. Prior to statistical analysis, normal distribution and homoscedasticity of variances were evaluated through Kolmogorov-Smirnov and Bartlett tests, respectively (Lindman, 1992). In the case of significant interactions, the effects were studied separately using a 1-way ANOVA. The Tukey HSD test (Lindman, 1992) was performed to make comparisons across all pairs of group means when

corresponding ANOVA tests were highly significant ( $p <$ 0.01) or significant ( $p < 0.05$ ).

Multivariate analyses – Both for soft and hard bottoms, differences in the composition and/or relative abundances of decapods between sites were tested by means of a 2-way permutation analysis of variance (PERMANOVA; 9,999 permutations of the raw data) using the same design as univariate ANOVA (Anderson, 2001; McArdle & Anderson, 2001). The test was based on the Bray-Curtis dissimilarity matrix calculated on logtransformed abundance data. In the case of significant interaction between the factors Year and Sites, pair-wise tests were applied.

The unconstrained Principal Coordinates (PCO) plot was used to confirm the PERMANOVA results and a projection biplot was drawn onto PCO axes to examine their relationship with the abundances of the main taxa (Anderson *et al*., 2008). Moreover, the multivariate multiple permutations test (SIMPER, Similarity Percentages, PRIMER; Clarke, 1993) was used to determine which species were responsible for the differentiation of the sites, for both soft and hard substrates.

# **Results**

*Soft bottom -* During the overall sampling period 229 taxa were found, mainly belonging to polychaetes (93), molluscs (65), crustaceans (51), and echinoderms (12).

Decapods (25 taxa; 49% of crustaceans) consisted mainly of Brachyura (16 taxa), mostly represented by *Liocarcinus maculatus, L. depurator, Brachynotus gemmellaroi, Derilambrus angulifrons,* and *Pilumnus hirtellus* (Table 1)*.* The other decapods were 4 Anomura (e.g. *Diogenes pugilator* and *Pisidia longimana*), 4 Caridea (e.g. *Alpheus dentipes* and *Athanas nitescens*), and 1 Gebiidea (*Upogebia tipica*).

At C sites some species typical of hard substrates, such as *A. nitescens*, *A. dentipes*, and *Eualus cranchii*, were recorded. At these sites abundance of decapods increased from 2001  $(2.08 \pm 1.22 \text{ ind m}^2)$  to 2003  $(9.99\pm2.80$  ind m<sup>-2</sup>) without consistent changes in the following years (Fig. 2). The F sites values were low over the years, ranging from  $1.04\pm0.36$  ind m<sup>-2</sup> (2001) to  $1.56 \pm 0.38$  ind m<sup>-2</sup> (2005) with the exception of 2004  $(9.90\pm3.73 \text{ ind m}^2)$ ; Fig. 2). Finally, at K sites abundance ranged between  $0.69 \pm 0.23$  ind m<sup>-2</sup> (2004) and  $3.13 \pm 0.47$ ind m-2 (2003).

ANOVA showed significant differences among sites, indicating that the highest values were recorded close to structures; moreover, 2004 resulted statistically different from 2001 and 2002 (Table 2).

Similarly to N,  $S_m$  resulted significantly higher at C sites compared to F and K sites (Table 2). However, a statistical interaction between the two factors was evidenced, due to the different pattern observed at each site. In particular, an increasing trend was observed at C sites from 2001  $(0.75\pm0.40)$  to 2004 (4.92 $\pm$ 0.56), when S<sub>m</sub> was significantly higher than in the previous years (Fig. 2; Table 2).

cant difference between these two years and year 2001 (Fig. 2; Table 2).

A similar pattern was also recorded at F sites, where  $S<sub>m</sub>$  was lowest in 2001 (0.67 $\pm$ 0.19) and highest in 2003  $(2.00\pm0.00)$  and 2004  $(2.00\pm0.56)$  with a highly signifi-

In contrast, at K sites,  $S_m$  remained practically constant in the first two years (2001: 2.00±0.43; 2002: 2.50±0.26) and significantly decreased until 2005 (0.75±0.33; Fig. 2; Table 2).

**Table 1.** Soft bottoms. List of Crustacea Decapoda recorded during the entire sampling period.  $K =$  control sites;  $F =$ sites far from a structure;  $C =$  sites close to a pyramid.

	2001		2002		2003		2004		2005						
	$\mathbf K$	$\mathbf{F}$	$\mathbf C$	$\mathbf K$	$\mathbf F$	$\mathbf C$	$\mathbf K$	$\mathbf{F}$	$\mathbf C$	$\mathbf K$	$\mathbf{F}$	$\mathbf C$	$\mathbf K$	$\mathbf{F}$	$\mathbf C$
Caridea															
Alpheus dentipes Guerin, 1832									X		X	X			X
Athanas nitescens (Leach, 1814)											$\mathbf X$	$\mathbf{X}$			X
Processa sp					$\mathbf X$		$\mathbf X$		$\mathbf X$						$\mathbf X$
Eualus cranchii (Leach, 1817 [in Leach, 1815-1875])										$\mathbf X$		$\mathbf X$			$\mathbf X$
Anomura															
Pisidia bluteli (Risso, 1816)									X						
Pisidia longimana (Risso, 1816)								$\mathbf X$	$\mathbf X$			$\mathbf X$		X	X
Anapagurus bicorniger A. Milne-Edwards & Bouvier, 1892											$\mathbf X$				
Diogenes pugilator (Roux, 1829)								X	$\mathbf X$		X	$\mathbf X$		$\mathbf X$	
<b>Brachyura</b>															
Brachynotus gemmellaroi (Rizza, 1839)	X		$\mathbf X$		X	$\mathbf X$			X		$\mathbf X$				
Liocarcinus depurator (Linnaeus, 1758)	X			$\mathbf X$	$\mathbf X$		$\mathbf X$	$\mathbf X$	X						
Liocarcinus maculatus (Risso, 1827)	X	$\mathbf X$		X						$\mathbf X$	$\mathbf X$	$\mathbf X$			
Liocarcinus vernalis (Risso, 1816)													$\mathbf X$		X
Pilumnus hirtellus (Linnaeus, 1761)				$\mathbf X$							X	$\mathbf X$			$\mathbf X$
Pilumnus sp						$\mathbf X$									
Xantho poressa (Olivi, 1792)												$\mathbf X$			
Xantho sp												$\mathbf X$			X
Corystes cassivelaunus (Pennant, 1777)													X		
Inachus comunissimus Rizza, 1839	$\mathbf X$					X					$\mathbf X$				
Inachus sp	$\mathbf X$		$\mathbf X$												
Derilambrus angulifrons (Latreille, 1825)			$\mathbf X$			X					X	$\mathbf X$			
Ebalia edwardsii Costa, 1838				$\mathbf X$		X									
Ebalia sp			$\mathbf X$												
Ethusa mascarone (Herbst, 1785)								$\mathbf X$							
Ilia nucleus (Linnaeus, 1758)									$\mathbf X$			$\mathbf X$			$\mathbf X$
Gebiidea															
Upogebia tipica (Nardo, 1869)											X				



*Fig. 2:* Soft bottoms. Mean decapod abundance (N; n. ind m<sup>-2</sup>  $\pm$  standard errors) and mean species richness (S<sub>m</sub>  $\pm$  standard errors) obtained during the overall sampling period (2001-2005). K = control sites; F = sites far from a structure;  $\tilde{C}$  = sites close to a pyramid.

2-way ANOVA				Soft bottom						
			$S_{m}$					$\mathbf N$		
	df(b, w)	$\rm{MS}$	$\mathbf F$	p-level			$\rm{MS}$	$\cal F$		p-level Tukey test
Site	(2, 163)	42.818	32.937	0.001	**		0.004	8.672	$0.001**$	$C>> K$ , F
Year	(4, 163)	10.394	7.995	0.001	**		0.001	2.967	$0.021*$	$'04$ > $'01$ , $'02$
Site x Year	(8, 163)	13.282	10.217	0.001	**		0.001	1.748	0.090	
1-way ANOVA			$S_{\scriptscriptstyle m}$							
	df(b,w)	MS	F	p-level		Tukey test				
Site	(2, 175)	45.344	22.153	$0.001**$	C>> K, F					
Year $(C)$	(4, 55)	25.898	12.263	$0.001**$				$0.05 \gg 0.01$ ; $0.4 \gg 0.01$ , $0.02$ , $0.03$ ; $0.03 \gg 0.01$		
Year $(F)$	(4, 55)	3.016	3.391	$0.014*$		$03.$ '04 $>>$ '01				
Year $(K)$	(4, 55)	7.566	8.213	$0.001**$				$02 >> 04$ , $05$ ; $02 > 03$ ; $01 >> 05$		
2-way ANOVA				<b>Hard bottom</b>						
		$S_{\scriptscriptstyle m}$				N				
	df(b, w)	MS	F	p-level		<b>MS</b>	F	p-level		
Site	(2, 132)	40.750	29.773	0.001	**	2.520	50.397	$0.001**$		
Year	(3, 132)	20.630	17.995 0.001		**	0.459	9.196	$0.001**$		
Site x Year	(6, 132)	16.491	12.049 0.001		**		0.182 3.647	$0.002**$		
1-way ANOVA			$S_m$					N		
	df(b, w)	<b>MS</b>	$\mathbf F$		p-level Tukey test		MS	$\mathbf F$		p-level Tukey test
Site	(2, 141)	40.750	16.253		$0.001**$ PyV >> P, PyH		2.520	39.158		$0.001** \frac{PyH}{>> P}$
Year $(P)$	(3, 44)	20.555	23.801	$0.001**$	$'05>> '03, '04, '02$ $'02>> '04; '03< '02$		0.057	4.165	$0.012*$	$'05$ $'04$ , $'02$
Year (PyV)	(3, 44)	10.972	5.081		$0.004**$ '03>> '04; '03> '02 0.159			3.080	$0.037*$	'03,'05>'02
Year (PyH)	(3, 44)	26.083	25.076	$0.001**$	$03, 04, 05 \gg 02; 0.607$ 05 > 04			7.198	$0.001**$	04>> 02; 05 > 02

**Table 2.** Results of ANalyses Of Variances (ANOVA) applied to abundance (N) and species richness (S<sub>m</sub>) for both hard and soft bottoms. \*; > = significant \*\*; >> = highly significant. K = control sites; F = sites far from a structure; C = sites close to a pyramid;  $PyV = vertical walls of pyramid; PyH = horizontal wall of pyramids; P = poles.$ 

PERMANOVA confirmed highly significant interaction among sites and years due to the different evolution of decapods at each site during the study period (Table 3).

In the PCO plot (52.9% of total variation; Fig. 3), the first axis separated the sites sampled in the first two years from the others. This was due to the presence everywhere of soft bottom species in 2001 and 2002, and of hard bottom and/or interstitial species, such as *A. nitescens*, *Xantho poressa*, *P. hirtellus* (Pallas *et al*., 2006) at F and C sites in the following years.

SIMPER analysis showed high dissimilarities among sites, ranging between 95.0% (F *vs* C sites) and 96.8% (K *vs* C sites; Table 4). In the first case this was due to the higher abundance of the major contributors (e.g. *D. pugilator*, *D. angulifrons*, and *P. longimana*) at C sites compared to F sites. The main contributors to the other dissimilarities (K *vs* C and K *vs* F) were *D. pugilator* (absent at K sites), *L. depurator* (more abundant at K sites), *Inachus comunissimus* and *L. maculatus* (less abundant at K sites).

*Hard bottom* – During the sampling period, 178 taxa were collected, 68 of which belonged to polychaetes, 53 to molluscs, and 42 to crustaceans.

Decapods (19 taxa) accounted for 45% of crustaceans and were represented by Caridea (e.g. *A. dentipes*, *A. nitescens*, *Eualus cranchii,* and *Lysmata seticaudata*), Anomura (e.g., *P. longimana* and *P. bluteli*), and Brachyura (e.g. *P. hirtellus* and *L. depurator*; Table 5).

Most of the species were typical of rocky substrates and only few taxa were characteristic of soft bottoms (e.g. *Anapagurus bicorniger*, and *D. pugilator*).

Two-way ANOVA highlighted a significant interaction between sites and years. One-way ANOVA evidenced significant differences between the two typologies of structures, with N values on PyV and PyH being higher than P values (Table 2).

At PyV, the lowest value of N (633.33±136.69 ind m-2) was recorded in 2002, resulting statistically different from the highest one obtained in 2003 and 2005  $(1,425.00\pm160.20 \text{ ind m}^2 \text{ and } 1,425.00\pm349.21 \text{ ind m}^2)$ respectively; Fig. 4 and Table 2). This high value was due to the abundance of a few species such as *A. dentipes* and *A. nitescens*.



*Fig. 3:* Soft bottoms. PCO ordination with projection of individual taxa onto the ordination axes. K = control sites; F = sites far from a structure;  $C =$  sites close to a pyramid.



*Fig. 4:* Hard bottoms. Mean decapod abundance (N; ind.  $m^2 \pm$  standard errors) and mean species richness (S  $\pm$  standard errors) obtained during the overall sampling period (2002-2005). PyV = vertical walls of pyramid; PyH = horizontal wall of pyramids; P = poles.

At PyH, N increased from  $266.67\pm72.13$  ind m<sup>-2</sup> (2002) to 2,725.00 $\pm$ 749.73 ind m<sup>-2</sup> (2004) and reduced in 2005 (1,400.00 $\pm$ 349.89 ind m<sup>-2</sup>), with significant differences between 2002 and the last two years (Fig. 4; Table 2). Also in this case the highest abundances were due mainly to *A. dentipes* and *A. nitescens*, representing about 90% and 61% respectively of the decapods in those periods.

At P, instead, N was relatively low in the first three years, ranging from  $56.33 \pm 13.72$  ind m<sup>-2</sup> (2002) to 91.00 $\pm$ 7.39 ind m<sup>-2</sup> (2003), and significantly increased up to  $354.33 \pm 154.89$  ind m<sup>-2</sup> in 2005 (Fig. 4; Table 2).

Similarly to abundance, two-way ANOVA highlighted a significant interaction between sites and years also for  $S_m$ . One-way ANOVA evidenced significant differences among the sites, with a higher number of species at PyV than PyH and P (Table 2).

At PyV,  $S_m$  showed some fluctuations ranging from 4.00 $\pm$ 0.49 (2004) to 6.17 $\pm$ 0.44 (2003; Fig. 4), with significant differences between the highest value and those recorded in 2002 (4.33±0.48) and in 2004 (Table 2). Instead, this index significantly increased from 2002  $(1.00\pm0.01)$  to 2005  $(4.50\pm0.29)$  at PyH (Fig. 4; Table 2).

Finally,  $S_m$  showed a different trend at P, character-

Table 3. Results of PERMANOVA analyses applied to log transformed abundance data for both hard and soft bottoms. \*; > = significant \*\*; >> = highly significant. K = control sites; F = sites far from a structure; C = sites close to a pyramid; PyV = vertical walls of pyramid;  $PyH = horizontal wall of pyramids$ ;  $P = poles$ .

2-way PERMANOVA			Soft bottom			
Factors	d. f.	<b>MS</b>	Pseudo-F	p(Perm)		
Site	$\sqrt{2}$	24770	8.1073	$0.001**$		
Year	4	26289	8.6045	$0.001**$		
Year x Site	8	20300	6.6443	$0.001**$		
	Pairwise tests					
	Factors	Results				
	Site		All combinations highly significant different			
	Year		All combinations highly significant different			
	Groups	$2001$ p(Perm)	2002 p (Perm)	2003 p (Perm)	2004 p (Perm)	2005 p (Perm)
	K, F	$0.001**$	$0.001**$	$0.001**$	$0.001**$	$0.012*$
	K, C	$0.003**$	$0.001**$	$0.001**$	$0.001**$	$0.009**$
	F, C	$0.004**$	$0.001**$	$0.001**$	$0.001**$	$0.002**$
2-way PERMANOVA			<b>Hard bottom</b>			
Factors	d. f.	<b>MS</b>	Pseudo-F	p(Perm)		
Site	2	44073	23.38	$0.001**$		
Year	3	7815.1	4.1458	$0.001**$		
Year x Site	6	6741.3	3.5762	$0.001**$		
	Pairwise tests					
	Factors	Results				
	Site		All combinations highly significant different			
	Year		$2002 \ge 2003$ ; $2004$ ; $2005 - 2003 \ge 2004$ ; $2005 - 2004 = 2005$			
	Groups	2002 p (Perm)	2003 p (Perm)	$2004 p$ (Perm)	2005 p (Perm)	
	P, PyV	$0.001**$	$0.001**$	$0.001**$	$0.001**$	
	P, PyH	$0.001**$	$0.002**$	$0.002**$	$0.003**$	
	PyV, PyH	$0.001**$	$0.037*$	$0.023*$	0.234	

ized by a significant decrease from 2002 (3.67±0.14) to  $2004$  ( $2.33\pm0.22$ ) and a significant increase in the last year (5.17±0.27; Fig. 4; Table 2).

PERMANOVA evidenced significant interaction between sites and years due to the different evolution of decapods at each site during the study period. The pairwise tests showed highly significant differences between the substrates over the years except for year 2005, when no differences were highlighted between PyH and PyV (Table 3). The PCO plot (64.0% of total variation; Fig. 5) showed the spatial distribution of the three substrates, with P far from the other two sites; this is due to the exclusive presence of *L. depurator* at P and to the greater abundance of other species, such as *A. nitescens* and *A. dentipes* at PyV and PyH.

These last two species represented the major contributors to the dissimilarities obtained by SIMPER analysis (Table 6). All the contributors showed low densities at P sites and high at PyH and/or PyV.

# **Discussion**

Detailed knowledge of the benthic communities colonizing an artificial reef is extremely important for assessing the actual effectiveness of man-made structures in enhancing certain ecological processes in the marine environment.

Specifically, this study represents the first attempt to describe the distribution of decapods on an artificial reef in the Adriatic Sea. Indeed, this taxonomic group plays a relevant role from an ecological point of view given that some species (i.e. *Athanas nitescens*, *Alpheus dentipes, Liocarcinus vernalis*) are the favourite or preferential prey items of a few reef-dwelling fish such as the brown meagre *Sciaena umbra*, annular seabream *Diplodus annularis*, striped seabream *Lithognathus mormyrus*, and comber *Serranus cabrilla*, inhabiting the artificial reefs deployed in the Italian seas (Fabi & Fiorentini, 1994; Ardizzone *et al.*, 1997; Fabi *et al.,* 1998; 2006; Relini *et al*., 2002).

**Table 4.** Soft bottoms. Summary of SIMPER analysis. Average abundances (Av. Abund.), Average dissimilarity (Av. Diss), % contribution (Contr.), and % cumulative contribution (Cum.) to average similarity are given for each species within the site groups. % average dissimilarities (Av. Diss.) between two site groups are also pointed out.  $K =$  control sites;  $F =$  sites far from a structure;  $C =$  sites close to a pyramid.

Soft bottom					
Groups K & F (Av. Diss. = 96.5 %)					
	Group K	Group F			
Species	Av.Abund	Av.Abund	Av.Diss	Contr. %	Cum.%
Liocarcinus depurator	1.74	0.52	20.07	20.80	20.80
Liocarcinus maculatus	0.65	1.39	9.33	9.67	30.48
Diogenes pugilator	$\boldsymbol{0}$	1.65	9.15	9.48	39.96
Pisidia longimana	$\theta$	0.30	8.87	9.19	49.15
Brachynotus gemmellaroi	0.35	0.35	7.61	7.89	57.04
Derilambrus angulifrons	$\boldsymbol{0}$	0.69	6.91	7.16	64.21
Anapagurus bicorniger	$\mathbf{0}$	0.35	5.56	5.76	69.97
Liocarcinus vernalis	0.37	$\theta$	5.40	5.59	75.56
Ebalia edwardsii	0.35	$\boldsymbol{0}$	4.55	4.71	80.28
Processa sp	0.17	0.17	4.23	4.39	84.66
Pilumnus hirtellus	0.17	0.35	3.91	4.05	88.71
Inachus comunissimus	0.17	0.17	3.54	3.67	92.38
Groups K & C (Av. Diss. = $96.8\%$ )					
	Group K	Group C			
Species	Av.Abund	Av.Abund	Av.Diss	Contr. %	Cum.%
Diogenes pugilator	$\theta$	5.08	14.75	15.24	15.24
Liocarcinus depurator	1.74	0.17	11.01	11.37	26.61
Inachus comunissimus	0.17	0.87	8.97	9.27	35.88
Liocarcinus vernalis	0.37	0.69	6.73	6.94	42.82
			6.27		
Pilumnus hirtellus	0.17	1.56		6.48	49.30
Brachynotus gemmellaroi	0.35	0.69	6.13	6.33	55.63
Derilambrus angulifrons	$\Omega$	1.48	5.66	5.84	61.47
Liocarcinus maculatus	0.65	0.43	5.41	5.58	67.05
Ilia nucleus	$\boldsymbol{0}$	1.52	4.48	4.63	71.68
Athanas nitescens	$\theta$	1.91	4.40	4.54	76.22
Ebalia edwardsii	0.35	0.17	3.98	4.11	80.34
Pisidia longimana	$\boldsymbol{0}$	1.39	3.67	3.49	84.13
Processa sp	0.17	0.69	2.91	3.01	87.13
Alpheus dentipes	$\boldsymbol{0}$	1.52	2.33	2.40	89.54
Eualus cranchi	0.17	0.35	2.16	2.23	91.76
Groups F & C (Av. Diss. = $95.0\%$ )					
Species	Group F	Group C			
	Av.Abund	Av.Abund	Av.Diss	Contr. %	Cum.%
Diogenes pugilator	1.65	5.08	18.86	19.85	19.85
Derilambrus angulifrons	0.69	1.48	8.91	9.37	29.22
Pisidia longimana	0.30	1.39	7.81	8.22	37.44
Inachus comunissimus	0.17	0.87	7.70	8.11	45.55
Pilumnus hirtellus	0.35	1.56	6.24	6.57	52.12
Athanas nitescens	1.04	1.91	5.97	6.28	58.40
Brachynotus gemmellaroi	0.35	0.69	5.46	5.74	64.15
Liocarcinus depurator	0.52	0.17	4.66	4.90	69.05
Ilia nucleus	$\boldsymbol{0}$	1.52	4.41	4.64	73.69
Liocarcinus vernalis	$\boldsymbol{0}$	0.69	3.65	3.84	77.53
Processa sp	0.17	0.69	3.50	3.68	81.21
Anapagurus bicorniger	0.35	$\overline{0}$	3.41	3.59	84.80
Alpheus dentipes	0.26	1.52	2.95	3.11	87.91
Liocarcinus maculatus	1.39	0.43	2.47	2.60	90.51

According to Davis *et al*. (1982), Fabi *et al*. (2002), and Cheung *et al*. (2009), the results obtained confirm the ecological role of an artificial reef in inducing qualitative and quantitative changes in benthic communities characterized by higher density and species richness close to the structures rather than far from them. Indeed, decapod species typical of both the natural soft bottom and hard substrates were recorded at C sites. Among the hard bottom species, *A. nitescens*, *A. dentipes,* and *Eualus cranchii*  are commonly observed on artificial modules (Bombace *et al*., 1997; Relini *et al*., 2002; Manoudis *et al*., 2005). A potential reason for their occurrence around artificial structures is linked to their carnivorous and/or detritivorous feeding (Falciai & Minervini, 1992; Gaudencio &



Table 5. Hard bottoms. List of Crustacea Decapoda recorded during the entire sampling period. PyV = vertical walls of pyramid;  $PyH = horizontal wall of pyramids; P = poles.$ 

**Table 6.** Hard bottoms. Summary of SIMPER analysis. Average abundances (Av. Abund.), Average dissimilarity (Av. Diss), % contribution (Contr.), and % cumulative contribution (Cum.) to the average similarity are given for each species within the site groups. % average dissimilarities (Av. Diss.) between two site groups are also pointed out. PyV = vertical walls of pyramid; PyH  $=$  horizontal wall of pyramids;  $P =$  poles.



Cabral, 2007). In fact, close to the pyramids, carnivorous organisms find high prey availability mainly represented by filter-feeders and detritivores that are attracted by the "organic snow" produced by the biological activities occurring on the artificial substrates and falling on the surrounding seabed. Moreover, some of these hard-bottom species, such as *A. nitescens*, show relatively little active swimming behaviour, which may lead them to search for shelter in cobbles and crevices near the pyramids (Pallas *et al*., 2006). Also, some soft bottom species recorded close to the structures were carnivores (e.g. *L. vernalis*; Gaudencio & Cabral, 2007) or scavengers (e.g. *Diogenes pugilator*; Dolbeth *et al*., 2006), and it is possible that they migrated from the surrounding area attracted by higher food availability. All these observations agree with the fact that any artificial structure placed on soft bottom produces discontinuity and provides a hard substrate that alters the initial state, thus creating the conditions for the establishment of new ecosystems (Terlizzi *et al*., 2008; Manoukian *et al*., 2010).

In contrast, F and K sites were mainly characterized by soft bottom species, such as *L. depurator, L. maculatus*, and *Brachynotus gemmellaroi*.

Abundance and mean species richness followed similar rising trends at C and F sites from 2001 to 2004, probably due to the increasing complexity of the artificial reef ecosystem, while outside the reef the indices showed a different pattern with lower values in the last two years.

These results, associated with those derived from the multivariate analysis, indicate that the artificial structures did not induced relevant changes in the distribution of decapods living in the surrounding soft seabed, both inside and outside the reef. This outcome would appear to be in contrast with the findings obtained by Fabi *et al*. (2002) who, in a study carried out at the Senigallia artificial reef (northern Adriatic Sea), found that in summer the structures affected the overall soft bottom benthic community up to a distance of about 10 m. However, it is noteworthy that the reef investigated by the above authors was around 10 years old while the Porto Recanati artificial reef is still a "young" reef where all the biological and ecological processes are likely to be ongoing. Moreover, the artificial structures constituting the Porto Recanati reef were placed at greater distance from each other compared to those forming the Senigallia reef (Fabi *et al*., 2002).

As expected, most of the decapod taxa recorded on the artificial substrates were typical of rocky bottoms. Besides food availability, these species were also attracted by the presence of mussels (*Mytilus galloprovincialis*), which settled on the artificial walls in 2002. As a consequence, a mussel facies developed, characterized by several cryptic, mobile species such as *P. hirtellus* and *Pisidia bluteli*. The development of huge populations of Mediterranean mussel at artificial reefs is well documented in the Adriatic Sea (Bombace *et al*., 1997; Spag-

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nolo *et al*., 2004) where the large amount of energy in the form of nitrates, phosphates and organic matter favours the development of sessile filter-feeders such as bivalves (Bombace *et al*., 1997; Spagnolo *et al*., 2004).

Mussels colonized the vertical walls of the pyramids first of all, probably due to the orientation and dimension of these surfaces that retained more effectively the pelagic larvae than horizontal walls and poles. Indeed, the poles were the last substrates to be colonized by a benthic community, which is likely the consequence of either their rounded shape and high hydrodinamism around them that rendered larval settlement more difficult. From a qualitative point of view, the vertical walls appeared more diversified showing a higher number of species compared to the other substrates. On the other hand, from a quantitative point of view, both vertical and horizontal walls showed higher densities than poles. Therefore, the orientation of pyramid walls affected the distribution of decapods in qualitative terms only.

In apparent contrast with the findings of this study, higher species richness was reported on the horizontal surfaces of concrete pyramids at an inshore artificial reef in the northern Adriatic Sea, as a consequence of the greater abundance of deposit- and suspension-feeders such as molluscs and polychaetes typical of soft bottom (Bombace *et al*., 1997; Spagnolo *et al*., 2004), while no relevant differences in the number of decapod species were observed. However, it is noteworthy that the dominance of certain trophic groups was linked to the thin layer of mud covering the horizontal walls due to the constant river inflow and the sedimentation of inorganic particles re-suspended during storm events (Bombace *et al*., 1997; Spagnolo *et al*., 2004).

These observations, associated with the results obtained in this study, highlight the importance of conducting specific studies on the different components of benthic communities colonizing artificial reefs, rather than on the overall benthic communities. In fact, specific studies could be useful either to gain a better understanding of the ecological role of these structures under different environmental conditions and to obtain useful information for maximizing the effectiveness of future installations.

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