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First release of hatchery juveniles of the dusky grouper *Epinephelus marginatus* (Lowe, 1834) (Serranidae: Teleostei) at artificial reefs in the Mediterranean: results from a pilot study

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SUMMARY: A pilot experiment of stock enhancement based on the release of hatchery-reared juveniles of dusky grouper, *Epinephelus marginatus*, was carried out at two artificial reefs located 0.9 km offshore the south-western Sicilian coast (Central Mediterranean). The fish assemblages inhabiting the reefs were characterized using underwater visual census (UVC) surveys. Despite some differences in fish species richness, diversity and abundance, both artificial reefs provide suitable feeding resources and shelter opportunities for the settlement of dusky grouper juveniles. A total of 95 dusky grouper juveniles, which were 3 and 4 years old, were released. No stress-related effects on fish swimming or behaviour were observed during the step by step transportation to the sea-bottom at 20 m depth. After release, several groupers showed very low reactivity; freezing and schooling were the most common behaviours. The sighting rate after one month of UVC surveys was 15.7% and 20% at the two artificial reefs. No effect of fish size at release on grouper survival was observed. Some groupers were not recorded on the pyramid of release but elsewhere in the same artificial reef, which demonstrated their ability to move around the reef. The extent of dispersal of the released juveniles largely exceeded the area encompassed by the reefs, which was shown by recaptures of tagged groupers by local fishermen up to 13 km from the release site. This result provides a first insight into the potential of stock enhancement of hatchery reared dusky grouper juveniles for marine ranching and conservation purposes.

Keywords: stock enhancement, hatchery-reared juveniles, *Epinephelus marginatus*, artificial reef, Mediterranean.

RESUMEN: Primera liberación de juveniles de mero *Epinephelus marginatus* (Lowe, 1834) (Serranidae: Teleostei) cultivados en criadero en arrecifes artificiales en el mar Mediterráneo: resultados de un estudio experimental. – Un experimento de repoblación, basado en la liberación de juveniles de mero *Epinephelus marginatus* cultivados en criadero, fue realizado en dos arrecifes artificiales situados 0.9 kilómetros fuera de las costas sicilianas sur-occidentales (Mediterráneo Central). La ictiofauna de los arrecifes fue caracterizada a través de inmersiones submarinas, utilizando la técnica de censo visual (UVC). A pesar de algunas diferencias en número de especies, diversidad y abundancia, ambos arrecifes artificiales proporcionaron recursos alimenticios y refugio adecuados para el establecimiento de juveniles de mero. En total se liberaron 95 meros juveniles de 3 y 4 años de edad. Ningún efecto relacionado al estrés fue observado en el comportamiento de natación de los peces durante el gradual transporte hasta 20 m de profundidad. Después de la liberación, varios meros mostraron una reactividad muy baja, siendo el comportamiento más común quedarse inmóviles y agregados. Después de un mes de censos visuales, las tasas de avistamientos obtenidas en los arrecifes artificiales fueron de 15.7% y 20%. No se observó relación entre el tamaño en el momento de la liberación y la supervivencia. Algunos peces no fueron observados cerca del punto de liberación sino en otra parte del mismo arrecife artificial, demostrando así su capacidad de moverse por los alrededores. Según lo indicado por los pescadores locales que recobraron meros marcados hasta 13 kilómetros del punto de liberación, el grado de la dispersión de los juveniles liberados superó ampliamente el área abarcada por los arrecifes. Este resultado proporcionan la primera evaluación del potencial de los juveniles de *E. marginatus* cultivados en criadero para actividades de cría en mar y conservación.

Palabras clave: repoblación, juveniles cultivados en criadero, *Epinephelus marginatus*, arrecife artificial, Mediterráneo.

INTRODUCTION

The use of stock enhancement as a fisheries management tool has been debated for over a century. The steady decline of fishery catches world-wide (FAO, 2000), coupled with recent advances in marine aquaculture technology, has renewed the interest in stock enhancement based on releasing hatchery-reared fish.

Many authors have indicated that stocking juvenile fish in natural habitats is a valuable addition to more conventional fishery management tools, such as decreasing fishing effort and environmental protection (McEachron *et al*., 1995; Leber *et al*., 1996). The potential of hatchery releases to restore depleted marine fish stocks has been shown in many countries. Some species that have been targeted by stock enhancement programmes are *Pagrus major* and *Paralichthys olivaceus* in Japan (Fushimi, 2001), *Gadus morhua* in Norway (Svåsand *et al*., 2000) and *Mugil cephalus* in the USA (Leber and Arce, 1996).

The major factors affecting stocking effectiveness have been demonstrated to be habitat and season, fish size and density at release, fish quality and release technique (Leber *et al*., 1998; Kristiansen, 1999; Svåsand *et al.*, 2000; Sánchez-Lamadrid, 2002). Indeed, several attempts to enhance wild stocks by means of fish releases failed because of unsuitable habitat and time of release (Bohnsack, 1996), poor knowledge of the biology and ecology of species (Masuda and Tsukamoto, 1998) and low fitness of hatchery-reared fishes due to behavioural and physiological deficits (Munro and Bell, 1997; Howell, 1994). Besides increasing production of commercial species, enhancement programmes have been implemented to aid recovery of endangered species (Brown and Day, 2002). Among the Mediterranean epinephelids, the dusky grouper *Epinephelus marginatus* (Lowe, 1834) is one of the species most heavily targeted by fishing activities. Due to its biological features and high vulnerability to overfishing, in the last decades this species has markedly decreased in number, especially in the western Mediterranean (IUCN, 2007). Fishery management for the dusky grouper, as well as for other groupers, is rather complicated due to their sequential hermaphroditism, mating system and their potential for sperm limitation (Bannerot *et al.*, 1987). Moreover, like many other epinephelids, dusky groupers aggregate to spawn during the summer period (Marino *et al*., 2001, Zabala *et al*., 1997), which is known to contribute to making grouper species prone to overexploitation (Sadovy *et al.*, 1994). Based on the substantial decline in total catches in many areas of its geographical range, *E. marginatus* has been classified as an endangered species since 1996 (Baillie and Groombridge, 1996) and recently (Baillie *et al*., 2004) revised and classified as "EN A2d", i.e. a taxon which is currently facing a very high risk of extinction in the wild, due to a suspected population size reduction of well over 50% over the last three generations, where the causes of the reduction have not ceased.

The application of fish size and/or bag limits, the spearfishing ban and the enforcement of an environmental regime within marine protected areas have been the only measures adopted so far for managing and conserving Mediterranean populations of dusky grouper (Harmelin and Robert, 2001).

In 2001, the Central Institute for Marine Research in Italy started a multidisciplinary research project aimed at producing dusky grouper juveniles for stock enhancement purposes and aquaculture. Reproduction and larval rearing techniques were set up and fifteen thousand juveniles were produced (Marino *et al*., 2002; Marino *et al*., 2003; Longobardi *et al*., 2004).

Culture conditions, however, may produce hatchery juveniles that show different characteristics to their wild counterparts and who may not be able to survive after release in the new environment (Munro and Bell, 1997; McEachron *et al*., 1998; Sánchez-Lamadrid, 2002). Malnutrition, due to the inability of hatchery juveniles to feed on live preys, has been identified as one of the main sources of mortality for hatchery fish following release (Brown and Day, 2002). Therefore, training trials using live prey were carried out to prepare hatchery grouper individuals before the release. The results demonstrated that pellet-reared, hatchery-produced dusky grouper juveniles have an innate capability to recognize and capture live prey and would be able to forage on natural prey once released into the wild (Marino *et al.*, 2007; Donadelli *et al*. 2007).

When hatchery fish are used for stock enhancement it is also important to remember that the release of cultured fish can modify ecosystems and the genetic constitution of the wild population. This implies that released fish should be as genetically similar as possible to wild fish (FAO, 1995). Genetic monitoring of stock enhancement and/or supportive breeding programs is highly recommended (Hansen *et al*., 2000), especially when the final aim of the project is the restoration of an endangered species. Therefore, a major concern was to ensure the genetic consistency between hatchery-reared grouper juveniles and the wild counterpart (De Innocentiis *et al*., 2006, 2007, 2008) to avoid genetic effects of hatchery fish on those in the wild (Munro and Bell, 1997). Moreover, release sites were identified not far from the place where breeders were captured, namely along the south western coast of Sicily (Marino *et al.*, 2007). In this area, artificial reefs deployed since the 80s play an important role in the integrated management of coastal resources (Riggio *et al*., 2000).

Production of healthy hatchery juveniles is also crucial in order to mitigate the risk of spreading disease into the wild and to increase the survival of the fish when they are released into a new environment. The health status of grouper juveniles was assessed by microbiological, parasitological and virological analyses before release (Marino *et al.*, 2007).

This paper presents the results of the first pilot stock enhancement program for dusky groupers in the Mediterranean Sea and provides evidence on the feasibility of releasing hatchery-produced dusky grouper juveniles at artificial reefs for conservation purposes. We evaluated the suitability of artificial reefs as release sites for grouper stock enhancement by analyzing the occurrence of potential preys and competitors (i.e. other predator fishes) at the reefs and the presence of wild dusky groupers within the reefs. Fish behaviour at the time of release and the effects of fish size and density at release on grouper survival were evaluated. The re-sighting rate of grouper juveniles in the artificial reefs during a post-release monitoring period was taken as a measure of the survival of released fish.

MATERIALS AND METHODS

Production of dusky grouper juveniles and quality assessment

The hatchery reared juveniles of *E. marginatus* used for stock enhancement were produced in 2001 and 2002 using larviculture techniques (Marino *et al*., 2002). Juveniles were stocked for 36 months in concrete tanks (50 m^3) under a natural photoperiod and temperature regime and fed with inert food at 0.6 to 2% body weigh according to water temperature.

Conditioning trials were carried out to prepare reared hatchery dusky grouper juveniles before the release (Marino *et al*., 2007). Inert food was gradually replaced with live prey of suitable size, mainly *Pagrus major* juveniles of 4-8 cm TL, for twenty days before release (Donadelli *et al*., 2007).

Genetic variation of wild and hatchery groupers was assayed in order to monitor the preservation of genetic diversity after release of hatchery grouper juveniles. DNA fingerprinting at microsatellite loci was performed to assess genetic variability of broodstocks and F1 juveniles in comparison to natural populations and to investigate genetic relationships between hatchery and wild specimens inhabiting the surroundings of the target area. Genetic tests showed that dusky grouper captive broodstocks and F1 juveniles met the genetic criteria required for sustainable stock enhancement actions (De Innocentiis *et al*., 2008).

The morphological quality of hatchery juveniles was assessed by means of x-rays and in toto staining to assess the presence of skeletal anomalies (Boglione *et al*., 2006). Juveniles showing some heavy cephalic anomalies, deviation of the vertebral axis and/or fin anomalies were excluded from the sample used for the stock enhancement action.

The health condition of hatchery grouper juveniles was assessed by microbiological, parasitological and virological analyses before they were released. Microbiological examinations performed on fresh tissues were negative for the most common pathogens, such as *Vibrio* spp., *Aeromonas* spp. and *Pseudomonas* spp. Virological analyses were carried out to search for *Nodavirus*, the causative agent of viral nervous-necrosis (VNN), which can cause heavy losses in wild and hatchery dusky groupers (Marino and Azzurro, 2001; Katharios *et al*., 2004), and in other grouper species (Chang, 2001). Enzyme-linked immunosorbent assay and polymerase chain reaction applied to selected samples both resulted negative. No parasites were detected in examined fish. The SPF status of juveniles ensured their use for restocking purposes.

Tagging operation

A preliminary tagging trial was performed in order to test the suitability of T-bar anchor tags for dusky grouper juveniles. Two groups of one hundred fishes (14 to 29 cm TL) were tagged with fine T-bar anchor tags (Hallprint Ltd., Golden Hill, Australia), inserted below the dorsal fin with a tag insertion gun

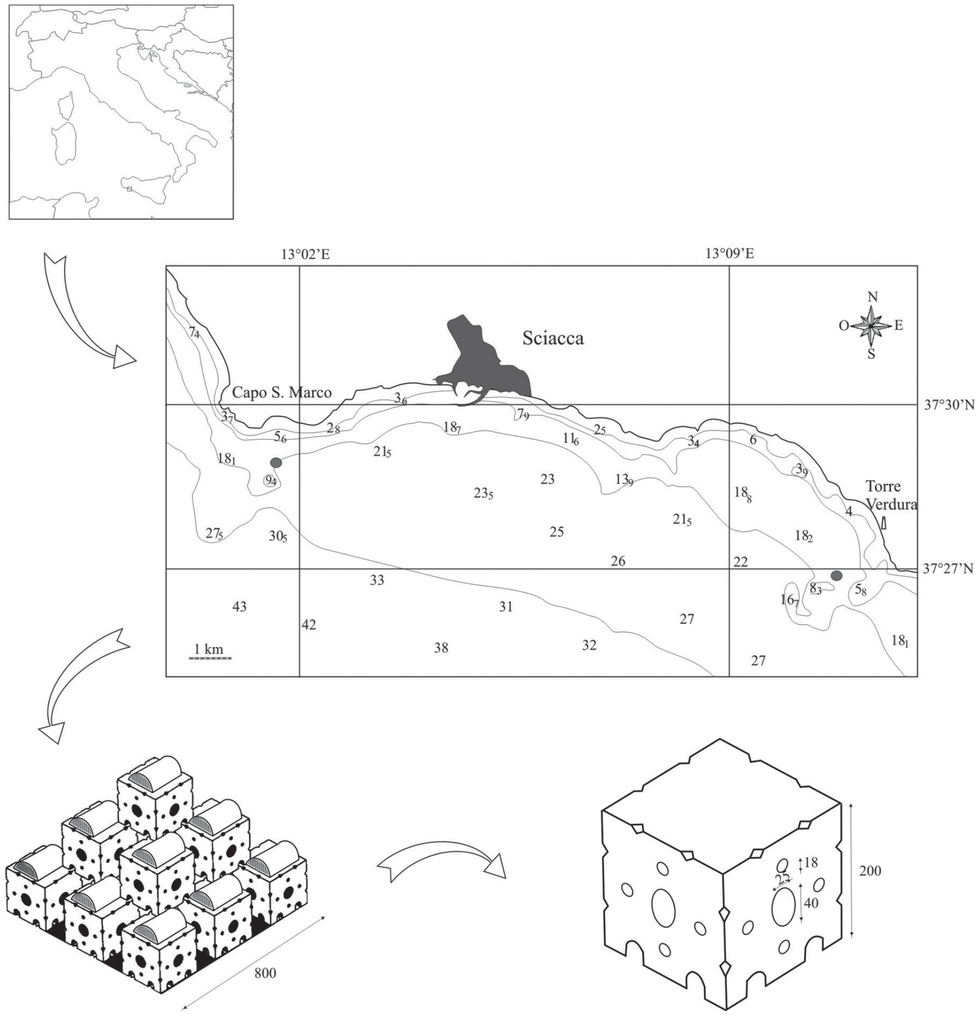

FIG. 1. – Study area and location of the artificial reefs (*filled circles*) off Capo S. Marco and Torre Verdura (Central Mediterranean Sea) with shapes and dimensions (cm) of the reef units.

(Avery Dennison 10312). One hundred groupers of comparable size were used as the control group. After 240 days, tag retention was very high (98% and 91%) and there were no significant differences in survival and growth between the tagged and control groups. Based on this result, T-bar anchor tags of different sizes and colours were used for tagging grouper juveniles to be released. From the time of the tagging operation until release at artificial reefs no fish mortality was observed.

Study sites

The artificial reefs are located off Capo S. Marco and Torre Verdura, 4.5 km west and 9 km east of Sciacca (south western coast of Sicily) respectively (Fig. 1). The artificial reefs are composed by an irregularly spaced assemblage of pyramids (6 pyramids at Capo S. Marco, 5 at Torre Verdura), located 0.9 km offshore at 20 m depth. Each three-layer pyramid is made of 14 cubic concrete blocks of 8 m^3 (Fig. 1). Some blocks have a perforated concrete cap on top. Each block has cavities of different shapes and sizes. The areas surrounding the reefs are characterized by a sandy to muddy bottom with patches of *Posidonia oceanica*.

Assessment of the fish assemblages at the artificial reefs

Data on fish assemblages in the artificial reefs of Capo S. Marco (CSM-AR) and Torre Verdura (TV-AR) were collected during visual census surveys carried out in July and September 2004 and July 2005. Sampling operations were conducted by means of SCUBA dives in the morning and with good sea-weather conditions. To quantitatively assess the fish species on the reefs we used the "spatial census tech-

Table 1. – Number (N) and characteristics (Mean ± SE) of tagged dusky groupers released at the artificial reefs of Capo S. Marco (CSM-AR) and Torre Verdura (TV-AR).

Reef	Pyramid	Fish				Tag	
		N	Size class	Total length (cm)	Weight (g)	Colour	Position
CSM-AR	1	15	large	36.6 ± 0.3	750.7 ± 19.4	blue	left
	2	15	small	28.4 ± 0.3	359.6 ± 10.4	white	left
	3	10	large	36.8 ± 0.2	756.3 ± 39.0	yellow	left
	4	5	small	27.8 ± 0.5	347.2 ± 22.6	white	right
		5	large	36.9 ± 0.4	753.0 ± 39.6	blue	right
	5	10	small	28.2 ± 0.4	355.1 ± 23.3	green	left
	6	5	large	36.1 ± 0.5	717.6 ± 49.8	yellow	right
		5	small	28.2 ± 0.5	362.4 ± 19.0	green	right
TV-AR	1	5	large	37.0 ± 0.5	785.4 ± 44.7	blue	left
	2	5	small	29.0 ± 0.3	385.6 ± 24.1	green	left
	3	5	large	36.3 ± 0.4	758.0 ± 15.2	yellow	left
	4	5	small	29.4 ± 0.3	404.8 ± 14.8	white	left
	5	3	large	36.4 ± 0.5	728.3 ± 23.4	blue	right
		2	small	29.9 ± 0.1	402.5 ± 39.5	white	right

nique” (D’Anna *et al.*, 1999). All fishes inside and to a distance of 1.5 m from the pyramid were counted, so that a water volume of about 260 m^3 was surveyed at each pyramid. Benthic and epibenthic fishes were censused through two successive passages, and the position of individual fish with respect to pyramids and the sea bottom was recorded according to the method described by Charbonnel (1990). Data on dusky groupers included some fish characteristics, such as size (total length, TL), reaction to diver and position (i.e. depth, distance from substrate, level of pyramid), and some microhabitat features. During each survey, three pyramids per artificial reef system were censused.

Release protocol

The release protocol was designed to evaluate the influence of fish size and density at release on the settlement of dusky groupers on the artificial reefs. A total of 95 dusky grouper juveniles, which were 3 and 4 years old, were selected for release (Table 1). The fish sample was composed of 47 “small” and 48 “large” juveniles (25-32 cm and 33-40 cm TL respectively). Tags of different sizes (35 mm and 50 mm length), colours (white, green, blue, yellow) and points of insertion (right or left fish side) were utilized, according to fish size and release site. Groupers were released at the artificial reefs between the 4th and 11^{th} of October 2005. At TV-AR five small and/or large individuals were released per pyramid. At CSM-AR a higher density at release (ten and fifteen small and/or large individuals per pyramid) was used (Table 1).

Fish were transported from the farm to the release sites into plastic circular tanks (1 m^3) at approx. 20 Kg/m^3 density and then transferred into cylindrical cages, made with a structure of galvanized iron and plastic net, with a volume of 0.8 m^3. After a period of acclimatization at different depths, cages were transported to the seabed as close as possible to the pyramids and opened. Two divers recorded fish behaviour during release and over 10 min periods after release.

Monitoring dusky groupers after releases

Visual census surveys were periodically (1, 2, 6, 14 and 30 days after release, unless sea conditions were unfavourable) conducted at CSM-AR and TV-AR. All groupers encountered at each pyramid during the visual census were recorded. Tagged individuals were identified according to the colour and position of the tag. To estimate the dispersion pattern and fishing mortality of the tagged individuals, data on sightings and recapture by local fishermen and divers were also recorded. A questionnaire to describe sightings and recapture (e.g. date, location, fishing gear, fish size and weight) was distributed among the local diving and fishermen’s associations.

Data analysis

Fish assemblage composition and structure at CSM-AR and TV-AR were assessed by calculating species richness (S), diversity (Shannon-Wiener index, $H'=-\Sigma p_i \log_2 p_i$) and total density, species relative density (number of individuals per pyramid) and frequency of occurrence. A one-way ANOVA was used to test for differences between CSM-AR and TV-AR in the assemblage parameters (S, H’ and total density) and in the density of potential fish preys and competitors of *E. marginatus*. Homogeneity of variances was preliminarily tested with the Cochran's test.

Cluster analysis was used to check for similarities across localities and sampling periods in relation to the density of potential fish preys and competitors of the dusky grouper. This analysis was run using the average linkage algorithm (UPGMA method) of Euclidean distances among sampling sites.

The re-sighting rate of tagged juveniles at the artificial reefs within 30 days was used as a measure of grouper survival. The effect of fish density at release on grouper survival was evaluated by comparing a χ^2 test with the re-sighting rates obtained at CSM-AR and TV-AR. The effect of fish size on grouper survival was evaluated by comparing a χ^2 test with the re-sighting rates of small and large individuals at each locality.

Unless otherwise stated, $p<0.05$ was used as the criterion for significance in all tests. ANOVA tests were computed with the GMAV 5 software. Cluster analyses were carried out using the Statistica 5.0 (Statsoft) software package.

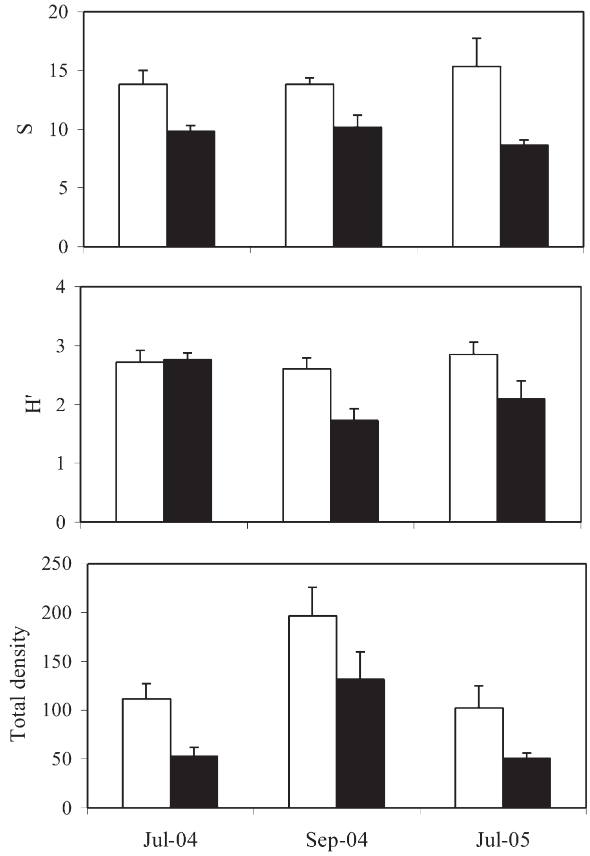

FIG. 2. – Mean (+SE) species richness (S), diversity (H') and total density (number of individuals per pyramid) of fishes recorded at Capo S. Marco (white bars) and Torre Verdura (black bars) according to sampling period.

RESULTS

Fish assemblages at the artificial reefs

The fish assemblages of CSM-AR and TV-AR consisted of 41 and 33 species respectively, mainly sparids and labrids. Species richness, diversity and total density were higher at CSM-AR than at TV-AR (Fig. 2) and these differences were significant (Table 2).

With few exceptions, the fish assemblage at CSM-AR and TV-AR were numerically dominated by the same species, namely C*hromis chromis*, *Apogon imberbis*, *Coris julis*, *Diplodus sargus* and *D. vulgaris.* Other abundant species were *Spicara maena*, *Boops boops* and *Spondyliosoma cantharus* at CSM-AR and *Parablennius rouxi* at TV-AR. In both localities, the potential fish preys of *E. marginatus* were mainly represented by *C. chromis* and *C. julis*, following by *P. rouxi*, *Serranus cabrilla*, *S. scriba* and *Diplodus annularis* (Fig. 3). Other potential preys, such as *Scorpaena porcus*, *S. Notata, Mullus surmuletus* and *M. barbatus* were also censused but at lower densities. *C. chromis*, *C. julis* and *P. rouxi* were usually more abundant in September than in July, whereas the opposite trend was observed in *D. annularis*. Among the potential competitors of the dusky grouper, *Conger conger* was more abundant than *Muraena helena*, regardless of the artificial reef

TABLE 2. – Results of ANOVA used to test for differences in the fish assemblage parameters and total density between the artificial reefs of Capo S. Marco (CSM-AR) and Torre Verdura (TV-AR). ** P<0.01; *** P<0.001.

		Richness (S)		Diversity (H')		Total density	
Effect	df	MS	F	MS	F	MS	F
Locality	1	1.17	27.42***	2,60	7.69**	30654.17	7.77**
Error	34	0.04		0.34		3944.55	
Transform			Ln (x+1)		-		-

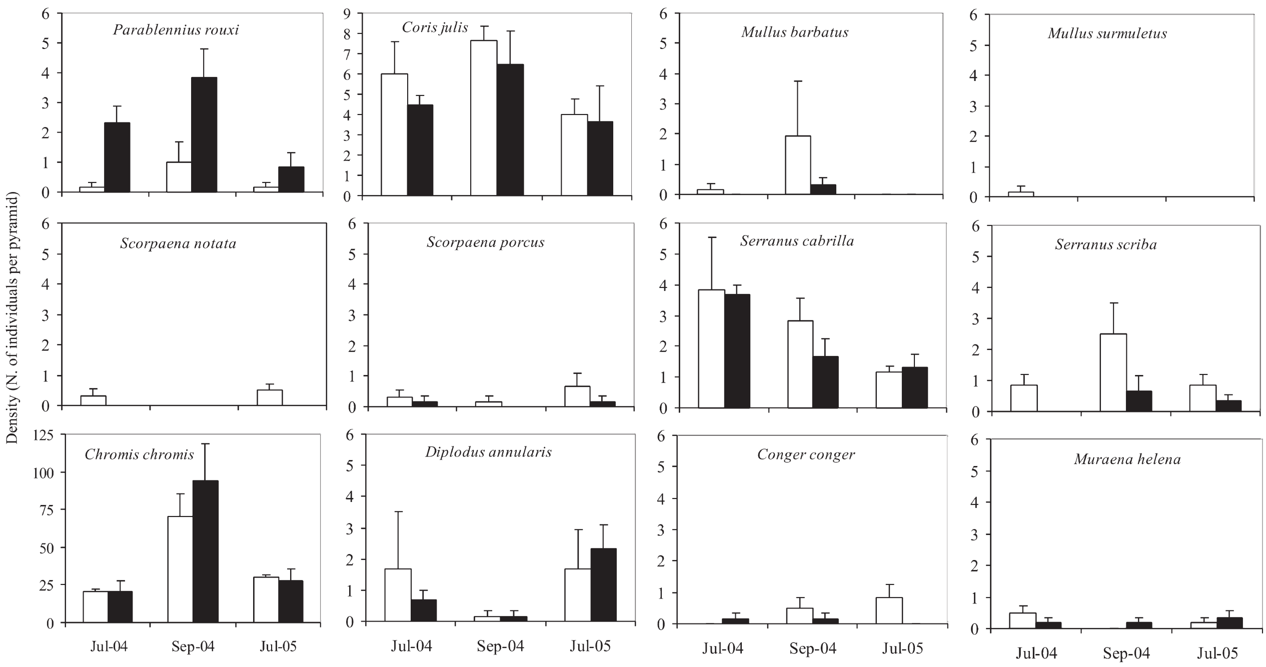

FIG. 3. – Mean (+SE) density (number of individuals per pyramid) of potential fish preys and competitors of *E. marginatus* recorded at Capo S. Marco (white bars) and Torre Verdura (black bars) according to sampling period.

TABLE 3. – Results of ANOVA used to test for differences in the density of potential fish preys and competitors (in bold) of *E. marginatus* between the artificial reefs of Capo S. Marco (CSM-AR) and Torre Verdura (TV-AR). **P<0.01; NS non-significant (p>0.05).

		Parablennius rouxi		*Coris julis*		*Mullus barbatus*	
Effect	df	MS	F	MS	F	MS	F
Locality	1	4.34	12.45**	9.00	0.88 NS	3.06	2.03 NS
Error	34	0.35		10.26		1.51	
Transform			Ln (x+1)		-		NT
		Mullus surmuletus		*Scorpaena notata*		*Scorpaena porcus*	
		MS	F	MS	F	MS	F
Locality	1	0.03	1.00 NS	0.69	6.54 NS[a]	0.69	2.35 NS
Error	34	0.03		0.11		0.29	
Transform			NT		NT		NT
		Serranus cabrilla		*Serranus scriba*		*Chromis chromis*	
		MS	F	MS	F	MS	F
Locality	1	0.00	0.01 NS	2.14	7.93**	410.06	0.22 NS
Error	34	4.33		0.27		1827.33	
Transform			Ln (x+1)		Ln (x+1)		-
		Diplodus annularis		*Conger conger*		*Muraena helena*	
		MS	F	MS	F	MS	F
Locality	1	0.11	0.03 NS	1.00	2.78 NS	0.00	0.00 NS
Error	34	3.85		0.36		0.18	
Transform			-		NT		-

[a] P<0.05 but not significant because higher than the setting value of α=0.01.
NT No transformation produced homogeneous variances.

(Fig. 3). All species except for *P. rouxi* and *S. scriba* showed no significant differences in density between localities (Table 3).

Figure 4 shows the cluster analysis dendrogram of the density of fish preys and competitors of *E. marginatus*. The survey period rather than locality seemed to be the main factor influencing the observed ordination pattern.

The larger prey species (e.g. *C. julis*, *S. porcus*, *S. cabrilla*, *S. scriba* and *D. vulgaris*) were usually rep-

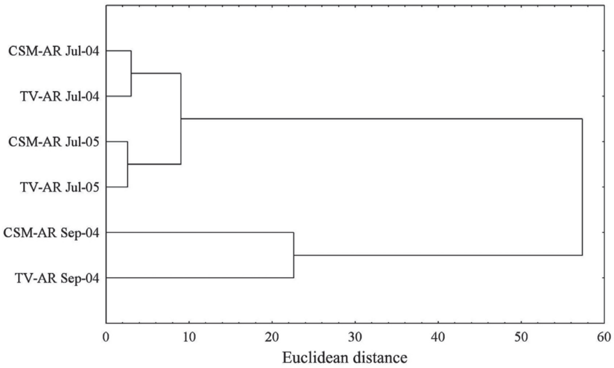

Fig. 4. – Results of cluster analysis of the sampling surveys carried out in the artificial reefs of Capo S. Marco (CSM-AR) and Torre Verdura (TV-AR) based on the density of potential fish preys and competitors of *E. marginatus*.

resented by small and medium specimens, i.e. those more likely to be preyed on by juvenile groupers. Most of the *C. conger* and *M. helena* were medium or large individuals in both localities.

The potential prey and competitors of dusky grouper can be assigned to four groups in relation to their location on the reefs. The first group included strictly benthic fishes, such as *P. rouxi*, *S. porcus* and *S. notata*, which were typically observed resting on the substrate. *C. conger* and *M. helena* both showed a close association with the reef, although they were mainly located within the cavities. Another group was composed by epibenthic species, namely *C. julis*, *Mullus barbatus*, *Mullus surmuletus* and *S. scriba* which inhabited both inside and close to the pyramids. The spatial distribution of *C. chromis*, *S. cabrilla* and *D. annularis* was quite similar, although they were sometimes observed far from the pyramids.

Only four wild dusky groupers were censused at CSM-AR and only one was censused at TV-AR during the pre-release surveys. These fish were larger than hatchery-released juveniles, and ranged in size from 45 to 70 cm TL. They were always observed inside the reefs, sometimes hidden in cavities, generally in the lower level of the pyramids. The observed groupers either escaped from or remained unconcerned by the diver. The substratum in the sighting sites was scarcely covered by small turf algae and other sessile organisms.

Monitoring dusky groupers after release

Abnormal swimming or behaviour was not observed in the dusky grouper juveniles during transportation to the sea-bed. Fish behaviour and position after the cages were opened are summarized in Table 4. At CSM-AR, all groupers came out of cages within two minutes, although some of them with re-

Table 4. – Behaviour and position of the dusky groupers released at the artificial reefs of Capo S. Marco (CSM-AR) (N=70) and Torre Verdura (TV-AR) (N=25) at different time intervals (0-2, 2-5 and 5-10 min.) after the cages were opened. Numbers of groupers are in parenthesis.

Reef	Time interval		Behaviour and position	
CSM-AR	0-2 min	Coming out of cage (70)	Among blocks (14)	Resting on substrate (2) Resting on substrate and schooling (6) Swimming (6)
			Out of diver's sight (56)	-
	2-5 min	"	Among blocks (10)	Resting on substrate (4) Resting on substrate and schooling (6)
			Out of diver's sight (60)	-
	5-10 min	"	Among blocks (10)	Resting on substrate (4) Resting on substrate and schooling (6)
			Out of diver's sight (60)	-
TV-AR	0-2 min	Coming out of cage (25)	Among blocks (17)	Resting on substrate (10) Swimming (4) Swimming and schooling (3)
			Around pyramid (1)	Swimming (1)
			Out of diver's sight (7)	-
	2-5 min	"	Among blocks (8)	Resting on substrate (2) Resting on substrate and schooling (4) Swimming (2)
			Out of diver's sight (17)	-
	5-10 min	"	Among blocks (7)	Resting on substrate (2) Resting on substrate and schooling (4) Swimming (1)
			Out of diver's sight (18)	-

Table 5. – Sightings of dusky groupers during the post-release monitoring surveys carried out in the artificial reefs of Capo S. Marco (CSM-AR) and Torre Verdura (TV-AR). Position: 1, on the pyramid of release; 2, among blocks of the pyramids; 3, around pyramids; 4, inside the holes of the blocks.

Reef	Days after release	Pyramid	Fish characteristics							Type of cover (%) in the sighting spot		
			Total length (cm)	Tag colour	Tag position	Reaction to diver	Position	Pyramid level	Depth (m)	Bare rock	Small turf algae	Long turf algae
CSM-AR	2	3	35	yellow	left	attraction	1,2,4	intermediate	17	0-50	>50	
	2	6	25-30			unconcern	2	intermediate	17.5	0-50	>50	
	6	1	45			escape	2	intermediate	17.3	>50	0-50	
	6	3	25			escape	2	upper	15.8	>50	0-50	
	6	6	45			unconcern	2	intermediate	17	0-50	>50	0-50
	6	5	25			escape	2	lower	19	0-50	>50	0-50
	12	6	26	white	left	escape	3	intermediate	16.8	0-50	>50	0-50
	12	3	45			unconcern	2	lower	20.7	>50	0-50	
	12	1	32			unconcern	2,4	intermediate	17.2	>50		
	12	5	65			unconcern	2,4	lower	19.1	>50	0-50	
	12	5	27	white	left	attraction	3	upper	15.4	0-50	>50	0-50
	30	1	35			escape	2	intermediate	17.5	>50	0-50	
	30	3	45			escape	3	intermediate	18	>50	0-50	
	30	3	38			escape	2,4	lower	19.4	>50	0-50	
	30	5	25	white	left	attraction	3	intermediate	16	>50	0-50	
	30	5	40	blue	left	attraction	2,4	intermediate	16.5	>50	0-50	
TV-AR	1	4	28	white	left	attraction	1,2,4	intermediate	17.3	>50	0-50	
	1	3	35	yellow	left	unconcern	1,2,4	upper	16.5	0-50	>50	0-50
	1	3	28	green	left	unconcern	2,4	upper	16.5	0-50	>50	0-50
	1	2	35	yellow	left	unconcern	2,4	upper	16.5	0-50	>50	0-50
	4	3	45			unconcern	2	intermediate	16.5	>50	0-50	
	4	2	30-35			unconcern	2	intermediate	16.8	>50	0-50	
	30	5	60			escape	2	lower	18.5	0-50	>50	0-50

luctance. The majority of fish disappeared soon after coming out. All the remaining groupers were located at the lower levels of pyramids, swimming around or resting on the blocks, and they remained close to each other. In the second time interval (2-5 min), four juveniles disappeared within the reefs, whereas the others were recognizable over the blocks or within the cavities and they did not move during the following time interval.

At TV-AR, all groupers came out of cages within the first time interval and most of them immediately after the cage was opened (Table 4). In this time interval, most of groupers were recorded inside the pyramids, swimming around or resting on the blocks. Some individuals displayed a clear schooling behaviour. Only a few juveniles remained within the diver's visual field in the following time intervals.

The sightings of released dusky groupers during the monitoring period are reported in Table 5. Sixteen groupers were recorded at CSM-AR and the number of groupers per survey did not change substantially throughout the monitoring period. Tags were only identified in five groupers, while in the remaining cases we only had a very transient and incomplete sight, due to the sheltered position of fish inside the pyramids or to their escape reaction. Two sightings of tagged juveniles (occurring 12 and 30 days after release respectively) could have concerned the same individual, as suggested by the similar fish size, location and tagging features. Six out of the eleven fish without a detectable tag were comparable in size to the released hatchery juveniles; the remaining fish were most likely the wild individuals observed during the pre-release surveys. As a result, the overall sighting rate (within the 30 day observation period) should increase from 5.7% (4 sightings / 70 released groupers, all the remaining sightings being considered as repeated counts of the same individuals) to 15.7% (11 sightings / 70 released groupers, if no repeated counts occurred).

Most of groupers inhabited inside the reef, usually inside cavities or close to the blocks of the mid and lower levels of the pyramids. Only one of the tagged juveniles was recorded in the pyramid of release. All but one of the tagged individuals were attracted to the diver, whereas all of the untagged groupers escaped from or were unconcerned by the diver. In the sighting sites, the substratum was partially covered by sessile organisms, especially small turf algae.

At TV-AR, 4 out of the total 7 groupers censused were tagged (Table 5). Nevertheless, the size of one individual without a detectable tag was comparable to that of the released juveniles. Hence, the overall sighting rate (within the 30 day observation period) was estimated at between 16% (4 sightings / 25 released groupers, one sighting was considered to be a repeated count of the same individual) and 20% (5 sightings / 25 released groupers, if no repeated counts occurred). Two tagged individuals were censused in the pyramid of release, whilst the other two were found further along the reef. Groupers were always located inside the reef, frequently inside cavities and close to the blocks of the mid and upper levels of the pyramids. The majority of groupers showed no reaction to the diver. As in CSM-AR, small turf algae dominated the biotic cover on the reef.

No significant difference in the re-sighting rate was observed either between localities (i.e. between different densities at release)(χ^2=0.21, p>0.05) or between small and large individuals (χ^2=0.07, p>0.05 and χ^2=0.48, p>0.05 at CSM-AR and TV-AR respectively).

Four tagged juveniles released at CSM-AR were recaptured by local fishermen. Two individuals were captured 14 days after release by surfcasters on a shallow sandy bottom facing CSM-AR. Another specimen was caught 45 days after release by a trammel net, on a rocky bank not very far (about 1 km) from CSM-AR. The last tagged juvenile was captured 98 days after release by a trammel net, at 25 m depth and more than 13 km from CSM-AR.

DISCUSSION

The fish assemblages of CSM-AR and TV-AR resembled those normally inhabiting infralittoral rocky shores, as they were dominated by labrids, sparids and serranids. Species richness, diversity and abundance of fishes at CSM-AR were higher than at TV-AR and comparable to those recorded in other artificial reef systems of the Mediterranean (D'Anna *et al*., 1995; Coll *et al*., 1998; Sánchez-Jerez and Ramos Esplá, 2000; Relini *et al.*, 2002). At TV-AR, the scarcity of other sessile organisms, which was probably related to higher seawater turbidity and abundance of silt, might be detrimental to fish diversity as it reduces the number of ecological niches.

A number of studies have pointed out that there are different fish species in the diet of *E. marginatus* (Derbal and Kara, 1996; Reñones *et al*., 2002; Linde *et al.*, 2004). The availability of these prey at CSM-AR and TV-AR confirmed the potential of these artificial environment to sustain the feeding requirements of juvenile dusky groupers. The suitability of the investigated reefs in terms of food resources was also supported by the presence of other piscivorous species, such as *C. conger* and *M. helena* (Bauchot, 1986; Bauchot and Saldanha, 1986). Nevertheless, we had no experimental evidence of these species, which are typically sedentary fish in the morning but wandering predators at night, using the artificial reefs as hunting territory.

The low number of wild dusky groupers recorded during the pre-release surveys was presumably an effect of overfishing, instead of due to the absence of proper habitats. *E. marginatus* has also occasionally been recorded in other studies on artificial reef fish assemblages (D'Anna *et al.*, 1995; Coll *et al.*, 1998; Sánchez-Jerez and Ramos-Esplá, 2000; Relini *et al.*, 2002). The suitability of artificial reefs appears unquestionable, at least in term of shelter opportunities, although most of them are spatially restricted environments. As a result, their carrying capacity is presumably limited, especially for territorial species like the dusky grouper. Taking into account these considerations, along with the cautionary approach frequently advocated by some authors (Leber *et al.*, 1998; Svåsand *et al.*, 2000; Fushimi, 2001), we planned to release a small number of juveniles, which should be reasonably detectable but also compatible with the habitat resources of the artificial reef.

We took care to collect information about the groupers' behaviour during transportation to the seabed and soon after release. Although the importance of acclimatization before being released into the natural environment has been largely demonstrated (e.g. Olla and Davis, 1989; Otterå *et al*., 1999; Kuwada *et al*., 2004), the operational details should be modulated according to the ecological features of the species. As they are a benthic fish, we believe it is better to release the dusky grouper juveniles close to the artificial reefs as they would probably have been unable to reach the pyramids quickly and autonomously if released on the sea-surface. As suggested by Olla *et al*. (1994), reared fish might be unable either to perceive environmental stimuli useful for their settlement or exploit available feeding resources.

During the transportation of fishes to the seabottom, no stress-related effects on fish swimming behaviour were observed. Conversely, most of groupers showed very low reactivity immediately after the cage was opened. The prevailing behaviour was to come slowly out of the cage and remain motionless at the bottom close to other individuals. This reaction, which has already been described in other fish by Henderson (1980) and Howell (1994), is most likely to be a "stand by" situation induced by a novel environment rather than an effect of unhealthy fish condition. According to Henderson (1980), the recovery of "normal" feeding behaviour in fish transplanted into a new, natural environment should occur through three steps: i) a relatively short period of recovery of movement, during which fish may exhibit some protective strategies such as freezing, hiding and schooling, ii) a period of familiarization with the new environment (particularly important for territorial fish) and iii) a period of adjustment of feeding habits.

The post-release monitoring of dusky grouper juveniles in the investigated artificial reefs provides encouraging results. Although apparently negligible in absolute terms, the number of censused groupers becomes conspicuous with respect to that of released fish. The sighting rates obtained at CSM-AR and TV-AR (from 5.7% to 15.7% and from 16% to 20% respectively) are higher than or comparable to those of other stock enhancement programmes. In *Diplodus sargus,* about 12% of the released juveniles were sighted over a 30 day period (which may be an overestimate, due to the possibility of re-sighting the same individuals) (D'Anna *et al.*, 2004). In repeated releases of hatchery reared *Sparus aurata*, the recapture rate by professional and recreational fishermen over several months ranged between 0% and 5% (Sánchez-Lamadrid, 2002). Similar results were attained with other species and in large-scale releases: 0-3.9% of recaptures in *Pagrus major* (Tsukamoto *et al.*, 1989), 0.6-3.3% in *Mugil cephalus* (Leber *et al.*, 1996), 0-2.3% and 2.9-10.5% in *Gadus morhua* (Otterå *et al.*, 1999).

Moreover, it can be supposed that some dusky groupers might not have been observed during the reef inspection due to the inaccessibility of the pyramid core (i.e. the central block of the lower level) and/or low underwater visibility. The actual number of released juveniles inhabiting the artificial reefs was thus presumably higher than that recorded by visual censuses.

The influence of size at release on survival of fish in the wild is still one of the most controversial topics of stock enhancement (Tsukamoto *et al.*, 1989; Yamashita *et al.*, 1994; Otterå *et al.*, 1999; Kristiansen *et al.*, 2000; Svåsand *et al.*, 2000). In several stocking experiments with reared fishes, both survival after release and recapture rate increased with increasing size at release (Tsukamoto *et al.*, 1989; Kristiansen *et al.*, 2000). The positive effect of fish size was frequently associated with a critical size as a consequence of selective predation on small fish (Tsukamoto *et al.*, 1989; Yamashita *et al.*, 1994). On the other hand, smaller individuals seemed more able to adapt to the natural feeding resources (Tsukamoto *et al.*, 1995). In the present study, the number of tagged juveniles recorded on the reefs was equally distributed between small and large individuals, apart from locality. However, only more extensive experimental trials with different sized fish and a cost benefit analysis can provide a decisive answer to the most appropriate size at release.

Fish density at release has frequently been recognized as a key factor for successful stock enhancement as it influences the survival of released fish (Svåsand *et al.*, 2000). In the stock enhancement of marine fish, the number of released individuals is usually very high (Leber *et al.*, 1998; Svåsand *et al.*, 2000; Fushimi, 2001). Without any knowledge on the extent of the dispersal of dusky grouper juveniles after release, we chose a density at release which would ensure a good chance of fish re-sighting. In the post release monitoring, no more than two groupers per pyramid were recorded. This number can be argued to be not very far from the maximum grouper density sustainable by the reefs. If this is true, the number of groupers released at CSM-AR and TV-AR abundantly exceeded the carrying capacity of the artificial reefs and consequently the comparison between the effect of different densities at release used in this experiment must be carefully interpreted.

Some hypotheses can be proposed to explain the disappearance of several groupers released on the reefs. The most reliable is that of dispersal, according to which some groupers would have left the reefs due to unsuccessful foraging and/or high competitiveness with their conspecific. The establishment of territories according to dominance hierarchies might have lead the weakest individuals to leave the pyramids. As reported elsewhere, competition between conspecifics can drastically reduce the post release survival of fish, especially in restricted environments or when feeding or habitat resources are limited (Svåsand *et al.*, 2000).

Some indications of the extent of dispersal of the released juveniles come from their movement in the studied area. Some groupers were not recorded on the pyramid of release but in other pyramids of the same artificial reef, which demonstrates their ability to move around the reef. The extent of dispersal of most individuals largely exceeded the area encompassed by the reefs, as suggested by the recapture of tagged groupers a few hundred meters to 13 km from the reef. The observed movement of groupers might be positively interpreted as a recovery of normal swimming activity or, conversely, a stress-induced condition induced by different causes, e.g. starvation.

Tag-release studies on juvenile dusky groupers have recently demonstrated high site fidelity, with daily displacements ranging from 9 to 93 m and a median home range area of 5312 m^2 (Lembo *et al.*, 2002). However, this experiment was performed on wild fish which were released in the same site of capture, that is, in a familiar environment. In hatchery reared groupers, which have absolutely no knowledge of the natural environment, site fidelity does not occur soon after their release into the sea, as they presumably need longer periods of adjustment. In tag-release studies on the red snapper *Lutjanus campechanus*, Schroepfer and Szedlmayer (2006) observed that when the species found a suitable habitat in the new artificial habitats, behavioural traits such as long-term residency and site fidelity were likely to reoccur.

The low number of records on groupers might also be determined by a high mortality of juveniles in the first days after release, a phenomenon frequently observed in other fish (Suboski and Templeton, 1989; Tsukamoto *et al.*, 1989; Howell, 1994; Svåsand *et al.*, 2000; D'Anna *et al.*, 2004). As in other large predators, the post release survival of dusky groupers is mainly related to the ability to exploit the available food. Starvation has indeed been demonstrated to be one of the main causes of mortality after release (Suboski and Templeton, 1989). Nevertheless, no dead or unhealthy groupers were observed over the monitoring period and around the study areas. Moreover, the trials of training dusky groupers with live prey, although performed in the laboratory, demonstrated the ability of the hatchery reared dusky grouper juveniles to capture and ingest live prey (Marino *et al.*, 2007).

The present study allows us to draw some preliminary conclusions about dusky grouper stock enhancement. We demonstrated the potential of hatchery reared juveniles of *E. marginatus* to survive after being released into the natural environment. Despite some constraints concerning a limited carrying capacity of the artificial reefs, these artificial environments can be considered valuable study areas for stock enhancement trials, and are important for integrated management of coastal resources. Nevertheless, the number of released fish should not exceed the carrying capacity of this restricted environment in relation to both feeding and habitat resources.

Some problems in evaluating stocking effectiveness arose from the difficulty of monitoring fish after release. Much experimental work remains to be done in order to obtain a better knowledge of habitat requirements and dispersal range of juvenile groupers after release.

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