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Nutritional condition of two coastal rocky fishes and the potential role of a marine protected area

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Abstract

Knowledge of the nutritional conditions of coastal commercial fish populations is key to understanding stock health status, and is essential when making reasonable exploitation and management plans. Here, we present the first results on the condition and feeding preferences of two coastal fish species, *Diplodus sargus* (Linnaeus, 1758) and *Pagellus erythrinus* (Linnaeus, 1758). Using stable isotope and biochemical analyses, we tested the potential effects of a marine protected area (MPA) and the occurrence of a dramatic coastal storm on the condition and quality of nutrition. The results suggest that both condition (lipids) and nutrition quality (fatty acids, FAs) in *P. erythrinus* and *D. sargus* depend upon on food availability in the area in which they were captured. *Pagellus erythrinus* individuals inside the MPA stored higher quantities of lipids [46.73 ± 19.00 μg lipid·mg organic matter $(OM)^{-1}$] than those outside the MPA (15.63 ± 5.30 μg lipid·mg OM^{-1}) only before the storm. *Diplodus sargus* showed different FA signatures inside and outside the MPA before and after the storm. These results suggest that *D. sargus* increased their quality of nutrition inside (16.62 ± 3.17 μg FA·mg OM^{-1}) *versus* outside (7.88 ± 2.36 μg FA·mg OM^{-1}) the MPA, owing to increased food diversity and availability. Conversely, *P. erythrinus* did not show differences in nutritional quality inside (18.12 ± 1.13 μg FA·mg OM^{-1}) or outside (18.81 ± 1.42 μg FA·mg OM^{-1}) the MPA, possibly because of the increase in ingestion not affecting the studied parameters. In *P. erythrinus*, the FA concentration decreased after the storm, but in *D. sargus*, a change in lipid composition was observed. These results suggest that *P. erythrinus* appears to be more impacted by food quality (different saturated and unsaturated FAs) than *D. sargus*, owing to a more restrictive diet. We hypothesize that the observed differences between inside and outside the MPA are not only related to the degree of protection, but also to the feeding preferences and behaviour of both fishes.

Introduction

Marine protected areas (MPAs) are created for many reasons, with one of the more important being to aid in the reduction of human pressure on fish stocks (mainly caused by overfishing, Castilla 2000). One of the principal objectives of MPAs is fish protection, including highly restrictive areas (no-take areas or integral reserves), adjacent to a buffer area in which some artisanal fisheries may be permitted. In marine reserves, fish density, biomass and size/age relationship are, in general, higher than in non-protected areas (Stelzenmüller *et al.* 2007; Vandeperre *et al.* 2011). An added benefit in some MPAs is the improved fitness of some fish species, which is partially attributable to the higher complexity and better conservation status of the benthic community (*e.g.* algal canopy, sea grasses or coral reefs), allowing for increased food availability (Lloret & Planes 2003; Lloret *et al.* 2005; Baillon *et al.* 2012). These effects, together with increased abundance and size/age class of the organisms inside the MPA, increase the fecundity of marine populations and enhance the reproductive output (Plan Development Team 1990; Planes *et al.* 2000; Berkeley *et al.* 2004; Gerber & Heppell 2004; Birkeland & Dayton 2005). Conservation of larger predators and/or endangered species is also important for management purposes (Boudouresque *et al.* 2005) because their presence increases food web complexity (Sala & Zabala 1996; Sala *et al.* 1998; Libralato *et al.* 2010), functional diversity (Villamor & Becerro 2012) and possibly the biomass exported to the surrounding areas (Roberts & Polunin 1991; Rowley 1994; Gell & Roberts 2003; Alcala *et al.* 2005; Halpern *et al.* 2010). However, the aforementioned benefits are dependent on the biology of each species and the management/characteristics of the MPA (Gell & Roberts 2003).

One of the main indicators of nutritional condition is the capability to store energy (lipid, carbohydrate and protein), which depends upon the quantity and quality of available food (Davis & Olla 1992; Saito & Murata 1998; Lloret & Planes 2003; Lloret *et al.* 2005). Low energy reserves can reduce the reproductive potential of fish, decreasing fecundity and/or the quality of eggs and larvae released, causing a reduction in fitness (Kjesbu *et al.* 1992; Adams 1999; Marshall & Frank 1999; Lambert & Dutil 2000). Poor nutrition can also reduce survival probabilities of fish (Krivobok & Tokareva 1972; Love 1980; Adams 1999; Shulman & Love 1999).

Total lipids and fatty acids (FAs) have been documented as useful tools to determine the availability and the quality of food, respectively (Parrish 1988; Grémare *et al.* 1997; Rossi *et al.* 2003, 2013a). On the other hand, analyses of lipid, carbohydrate and protein concentration may be useful tools to quantify the nutritional condition of populations depending on food availability (Lloret & Planes 2003; Rossi *et al.* 2006a; Elias-Piera *et al.* 2013). Food and nutritional quality is reflected in the relative amounts of different FAs present, such as saturated fatty acids (SFAs), mono-unsaturated fatty acids (MUFAs) and especially poly-unsaturated fatty acids (PUFAs). PUFAs are especially important for the fitness and life-stage development of the individual, allowing for organismal synthesis and accumulation of the necessary trophic markers for their development (selective accumulation), which are obtained through nutrition (Müller-Navarra 1995; Jónasdóttir & Kiørboe 1996; Müller-Navarra *et al.* 2000; Wacker & von Elert 2001; Rossi *et al.* 2006b). Periodic FA determination and quantification, combined with analysis of stable isotope (SI) trophic signature and biochemical balance (protein–carbohydrate–lipid levels in the tissue), are powerful tools to determine the potential impact of food fluctuations over mid- or long-term periods, as well as the food preferences amongst different species living in the same area (Sargent *et al.* 1989; Mayzaud *et al.* 1999; Hudson *et al.* 2004; Rossi *et al.* 2006b; Kelly & Scheibling 2011; Gori *et al.* 2012).

Sparids, especially the genera *Diplodus* and *Pagellus*, are representative rocky fish groups for sublittoral areas of the Northwestern Mediterranean (Sala & Ballesteros 1997). *Diplodus sargus* (Linnaeus, 1758), also known as the white sea bream, is considered an omnivore (Joubert & Hanekom 1980; Coetzee 1986; Mann & Buxton 1992; Sala & Ballesteros 1997) and keystone coastal species (Sala & Ballesteros 1997), being one of the most important predators of sea urchins in the Mediterranean (Guidetti 2006). *Diplodus sargus* inhabits shallow rocky bottoms and *Posidonia oceanica* meadows, competing for space and resources with other *Diplodus* species (Sala & Ballesteros 1997). *Pagellus erythrinus* (Linnaeus, 1758), or the common pandora, is considered a carnivorous species. Its diet is composed of decapods, bivalves, polychaetes, Teleostei and Euphausides (Santic *et al.* 2011). *Pagellus erythrinus* dwells in rocky and sandy bottoms from 10 to 100 m (Spedicato *et al.* 2002). Both *D. sargus* and *P. erythrinus* are locally important fishing target species in coastal areas of the Mediterranean Sea (Pajuelo & Lorenzo 1998; Husseina *et al.* 2011; Martin *et al.* 2012). They are both also considered to display what is commonly called the 'reserve effect', whereby they have higher densities (García-Rubies & Zabala 1990) and larger size classes (Martin *et al.* 2012) inside the reserve, as well as in buffer areas. Greatly, the information on the nutritional condition of rocky fishes is lacking, especially when considering the 'reserve effect'. To the best of our knowledge, there has been only one study linking the nutritional condition (in the form of higher lipid reserves) inside and outside an MPA for *D. sargus* (Lloret & Planes 2003).

The present study aimed to assess nutrition type (from SIs), nutritional condition (from lipids) and quality (from FA), inside and outside a fully protected marine reserve. The effect of an unexpected stochastic event, a heavy easterly storm that took place in the research area during the sampling period, on these nutritional values was also analysed. The differential effects of heavy storms on benthic communities are well known. Much of the storm damage dependent on the state of the community before the disturbance (Ebeling *et al.* 1985), intensity or wave impact (Yoshioka & Yoshioka 1991) and/or the magnitude of storm energy (Mendoza *et al.* 2011). Heavy storms are one of the major causes of disturbance in coastal ecosystems, mainly resulting from (1) sediment re-suspension (Grémare *et al.* 2003; Sánchez-Vidal *et al.* 2012) and (2) detachment of benthic species (Dayton & Tegner 1984; Ebeling *et al.* 1985; Navarro *et al.* 2011; Hereu *et al.* 2012). Sediment re-suspension can alter nutrient levels, light and organic matter availability, and result in the burial of living three-dimensional structures such as seagrass meadows (Grémare *et al.* 2003; Sánchez-Vidal *et al.* 2012). The detachment of benthic species causes a decrease in the number of ecosystem engineers, causing simplification of the benthic ecosystem (Dayton & Tegner 1984; Ebeling *et al.* 1985; Navarro *et al.* 2011; Hereu *et al.* 2012). The above-mentioned impacts may cause changes in the lower levels of the trophic network (*e.g.* algae and sea grass; Navarro *et al.* 2011), with a 'domino' effect on other ecosystem components, including the main prey of coastal fishes (crabs, fish, molluscs, *etc.*).

The current study analysed the reserve effects on the nutritional condition of *D. sargus* and *P. erythrinus* before (April 2008) and after (April 2009) an exceptional storm that occurred along the Catalan coast on 26 December 2008 (Mendoza *et al.* 2011; Sánchez-Vidal *et al.* 2012). To test these effects, we used the different but complementary parameters of: the biochemical balance (protein–lipid–carbohydrate levels), FA concentration and composition, and the C and N SIs. Using these biochemical parameters, we attempted to understand how the effects of a significant local eradication of seaweeds, seagrasses, gorgonians and sea urchins (García-Rubies *et al.* 2009; Navarro *et al.* 2011; Hereu *et al.* 2012; Sánchez-Vidal *et al.* 2012; Teixidó *et al.* 2013) affected the condition of the two rocky fish populations four months after the extreme event. This information is essential to (i) elucidate the effect of MPAs on the nutritional condition of the species studied, and (ii) better understand the influence of processes that act at different spatial and temporal scales on the structure of trophic assemblages, which is of great importance for the conservation of the species studied and for the management of MPAs.

Material and Methods

Study area and sampling methodology

This study was conducted in the NW Mediterranean Sea, inside and outside the MPA of the Medes Islands Marine Reserve (MIMR), which is located one nautical mile offshore of L'Estartit (3°13′ E, 42°16′ N). The MPA consists of seven small, uninhabited islands and islets and a few rocky reefs (Fig. 1). The MPA has a total area of 511 ha (emerged zone not included) formed by a buffer area (418 ha), and an integral reserve (no-take area; 93 ha). Inside the MPA, diving, anchoring/mooring and navigation are regulated. In the no-take area all fishing practices are forbidden (excluding investigative), and in the buffer area recreational and artisanal fishing are allowed in boats based in L'Estartit. As a result of regulations on fishing frequency (allowed 5 days a week) and meteorological conditions, the fishing association of L'Estartit fishes for an average of 120 days per year.

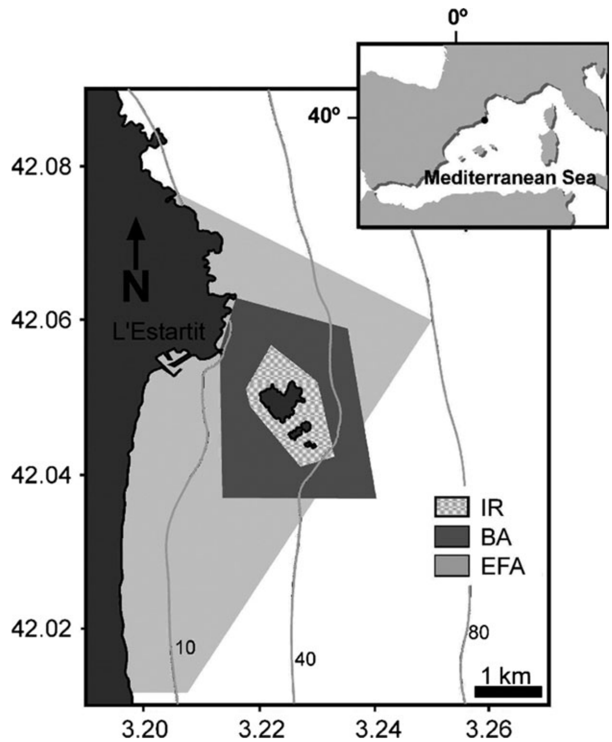


Fig. 1. Study area showing the MPA limits of the Medes Islands Marine Reserve (IR, integral reserve; BA, buffer area; EFA, estimated fishing area). The location of the village of L'Estartit is also displayed (from Martin *et al.* 2012).

Sampling of *Diplodus sargus* and *Pagellus erythrinus* occurred in April 2008 and April 2009 with trammel nets and long lines, which were deployed between 10 and 20 m depth inside (no-take area; 3°23′ E, 42°05′ N) and outside (3°22′ E, 42°02′ N) the MPA at the same type of rocky locations (boulders surrounded by sand and soft-bottom gravel substrates). In total, 20 *D. sargus* (19–27 cm length) and 20 *P. erythrinus* (19–35 cm length) were fished and frozen (−20 °C) prior to analysis. Pieces of white muscle were dissected, without defrosting, and then stored at −80 °C prior to analysis.

Environmental conditions of the 2008 storm

In the Medes Islands, storms with waves of 3 m in height have an average frequency of three times per year (PRUG 2008). On 26 December 2008, the Medes Islands were struck by a severe easterly storm, with wind speeds higher than 85 km·h^{-1} and wave heights mean of 8 m, with peaks of 14.4 m (Mendoza *et al.* 2011). This storm has been described as the most intense (and with a higher impact) to occur in the last 50 years (García-Rubies *et al.* 2009; Mendoza *et al.* 2011; Sánchez-Vidal *et al.* 2012). The effects of the storm were similar outside and inside the MPA, impacting the benthic communities up to 20 m depth (Navarro *et al.* 2011; Hereu *et al.* 2012). At depths of up to 10 m, boulders on the rocky bottoms of around 0.3 m in diameter were found displaced or turned upside down. Movement of these boulders resulted in remarkable benthic organismal loss as a result of abrasion, smashing and erosion (Sánchez-Vidal *et al.* 2012). Algal- and gorgonian-dominated communities, as well as boring species such as *Litophaga litophaga*, were also decimated (García-Rubies *et al.* 2009; Navarro *et al.* 2011; Teixidó *et al.* 2013).

Stable isotope analysis

The composition of the SIs $\delta^{13}C$ and $\delta^{15}N$ in fish muscle were analysed in three fish (adequate number of samples to make statistical comparisons; see Estrada *et al.* 2005; Jacob *et al.* 2005) from each area (inside and outside the MPA) for both study species (*Diplodus sargus* and *Pagellus erythrinus*) in April 2008 and 2009. In total, 24 fish were analysed. Approximately 0.4 mg (±0.001 mg) of muscle dry weight (DW, dried at 80 °C for 24 h and weighed using a Mettler Toledo model XS3DU balance) was taken from each sample and analysed for carbon (C) and nitrogen (N) content. SI compositions of $\delta^{13}C$ and $\delta^{15}N$ were determined by using a Thermo Flash EA 1112 analyser and a Thermo Delta V Advantage spectrometer, following the same methodology as Gori *et al.* (2012) and Elias-Piera *et al.* (2013). Isotope ratios are expressed as parts per thousand (‰; difference from a standard reference material) according to the following equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 103 \qquad 1$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio, C^{13}/C^{12} or N^{15}/N^{14}. R standards for C^{13} and N^{15} are from PeeDee Belemnite (PDB) and atmospheric N^2, respectively (Jacob *et al.* 2005; Carlier *et al.* 2007a).

Fatty acid analysis

To determine the composition of FAs, five fish samples from both areas (inside and outside the MPA) and both species (*Diplodus sargus* and *Pagellus erythrinus*) in April 2008 and 2009 were collected (total 40 fishes). Previous studies have shown that three samples are sufficient for statistical comparisons in determinations of FA composition (Wheeler & Morrissey 2003; Rossi *et al.* 2006b). FA samples were analysed following the methodology of Rossi & Fiorillo (2010), Soler-Membrives *et al.* (2011), Gori *et al.* (2012) and Rossi *et al.* (2012, 2013a). Approximately 14 to 16 mg (±0.1 mg) of DW muscle from each sample was dissolved in 3:1 dichlorolmethane : methanol and FAs were quantified with gas chromatography (GC) analysis performed with an Agilent Technologies 7820A GC system instrument equipped with a DB–5ms Agilent column (60 m length, 0.25 mm internal diameter and 0.25 μm phase thickness). Oven temperature was programmed to increase from 50 to 180 °C at 10 $°C \cdot min^{-1}$ and from 180 to 320 °C at 4 $°C \cdot min^{-1}$. Injector and detector temperatures were 300 and 320 °C, respectively. Methyl esters of FAs (FAMEs) were identified by comparing retention times with those of standard FAs (37 FAME compounds, Supelco™ Mix C^4-C^{24}; trophic markers). FAs were quantified by integrating areas under peaks in the GC traces (CHROMQUEST 4.1 software), with calibrations derived from standard FAs. The results are presented as μg FA·mg organic matter $(OM)^{-1}$, % of SFAs, MUFAs, PUFAs and % of each trophic markers.

Organic matter analysis

The OM in the white muscle of *Diplodus sargus* and *Pagellus erythrinus* was measured using samples of five fish per species from both areas (inside and outside the MPA) in April 2008 and 2009 (a total of 40 fish). Approximately 100 mg (±0.1 mg) of DW muscle from each sample was reduced to ash for 4 h at 500 °C in a muffle furnace (Relp 2H-M9). The percentage of OM was calculated as the difference between dry and ash weight (Slattery & McClintock 1995).

Biochemical analyses

Carbohydrate, protein and lipid analyses were carried out using five fish samples (adequate number of samples to make statistical comparisons; see Sabatés *et al.* 2003) from both areas (inside and outside the MPA) and both species (*Diplodus sargus* and *Pagellus erythrinus*) in April 2008 and 2009, giving a total of 40 fish. The methodology was that previously applied by Sabatés *et al.* (2003) and Rossi *et al.* (2006a). Approximately 7–10 mg (±0.1 mg) of DW muscle from each sample was homogenized in 3 ml double-distilled water, and carbohydrates were quantified colorimetrically according to the method of Dubois *et al.* (1956), with glucose as a standard. Approximately 7–10 mg (±0.1 mg) of DW muscle from each sample were homogenized in 2 ml hydroxide sodium one normality, and proteins were quantified colorimetrically according to the method of Lowry *et al.* (1951), with albumin as a standard. Finally, approximately 10 mg (±0.1 mg) of DW muscle from each sample was homogenized in 3 ml chloroform : methanol (2:1), and total lipids were quantified colorimetrically according to the method of Barnes & Blackstock (1973), with cholesterol as a standard. Results are presented in μg carbohydrate/protein/lipid·mg OM^{-1}.

Statistical analyses

Statistical analyses were conducted to test for the potential differences in SI composition, total FA concentration and quality (SFA, MUFA, PUFA), and biochemical content (carbohydrate, protein, lipid) between *Diplodus sargus* and *Pagellus erythrinus* inside and outside the MPA, as well as before and after the storm event.

The independent variables used in the statistical analysis to assess the condition were: (i) year (to determine storm effect), (ii) area (inside and outside the MPA) and (iii) species (*D. sargus* and *P. erythrinus*). A two-way analysis of variance (ANOVA; year and area as fixed effects) and a Tukey's *post hoc* test were used to compare the response of each species. We also applied a three-way ANOVA and a Tukey's *post hoc* test to compare all of the independent variables. Prior to performing the ANOVA analyses, normality (Shapiro test) and variance homogeneity (Bartlett test) tests were conducted. When variances were not homogeneous, the transformations necessary to achieve normality were applied. All of these tests were performed with the R language function aov (Chambers *et al.* 1992) by the R software platform.

In order to document the individual FA signature (in %) within each species, a similarity percentage (SIMPER) analysis was conducted. SIMPER results show the percentage contribution of each trophic marker (FAs). SIMPER analysis was performed with the R-language function simper (Clarke 1993) from vegan library (Oksanen *et al.* 2005) and permute library (Simpson, 2012).

Finally, to test if there exists a relationship between fish size and total lipid contents in the muscle of the fishes, a Pearson product-moment correlation coefficient was applied (Zar 1996).

Results

Stable isotopes

In *Diplodus sargus* and in *Pagellus erythrinus*, no significant differences was found between the areas and years (two-way ANOVA; $P > 0.05$; Table 1). However, in both species, variability was higher inside than outside the MPA, being larger for *D. sargus* than for *P. erythrinus* (Fig. 2). Significant differences were detected between the species (three-way ANOVA; $P < 0.01$; Table 2), mostly caused by variation in $\delta^{15}N$ (Fig. 2).

Fatty acids

The total concentration of FAs showed significant differences inside and outside the MPA for *Diplodus sargus* (two-way ANOVA; $P < 0.001$; Tables 1 and 3; Fig. 3), but not for *Pagellus erythrinus* (two-way ANOVA; $P = 0.929$). The total concentration of FAs for *D. sargus* was higher inside than outside the MPA. This difference agrees with the results obtained for PUFA concentration (two-way ANOVA; $P < 0.001$; Table 1; Fig. 4). Significant differences in the concentration of FAs were detected between the two years, but only for *P. erythrinus* (two-way ANOVA; $P < 0.001$; Tables 1 and 3; Fig. 3), showing lower values in April 2009 compared with April 2008. PUFA concentration displayed a similar pattern (two-way ANOVA; $P < 0.01$; Table 1; Fig. 4). Comparing the total concentration of FAs for both species, *D. sargus* showed a lower concentration outside the MPA compared with *P. erythrinus* (three-way ANOVA; $P < 0.001$; Tables 3 and 4; Fig. 3); however, inside the MPA both species had a similar pattern (three-way ANOVA; $P > 0.01$; Tables 3 and 4; Fig. 3). The same pattern was displayed for SFAs, MUFAs and PUFAs (three-way ANOVA; $P < 0.001$; Table 4; Fig. 4).

A total of 27 FAs (trophic markers) was identified in the muscle of both fish species (Table 5). SIMPER analysis showed that the trophic markers 22:6(n–3) and 16:0 for *D. sargus* contributes mainly to differences between inside and outside the MPA, being more abundant inside (Tables 5 and 6). These trophic markers [22:6(n–3) and 16:0] showed a drastic decrease inside the MPA in April 2009, after the storm. No significant differences were found in the SIMPER analyses for *P. erythrinus* (Table 7).

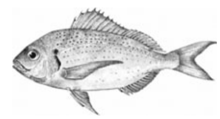
Organic matter

The abundance of OM did not show any significant differences within either species for the two areas and sampling periods (two-way ANOVA; $P > 0.05$; Tables 1 and 3). However, the percentage of OM was significantly higher in *Diplodus sargus* with respect to *Pagellus erythrinus* inside the MPA (three-way ANOVA; $P < 0.01$; Tables 2 and 3).

Biochemical analyses

Carbohydrate and protein content did not display any significant differences between areas, years (two-way ANOVA; $P > 0.05$; Tables 1 and 3) or species (three-way ANOVA; $P > 0.05$; Tables 2 and 3). Lipids did not show any significant differences for *Diplodus sargus* between areas and years (two-way ANOVA; $P > 0.05$; Tables 1 and

Table 1. Results of the two-way analysis of variance (ANOVA) comparing the different parameters analysed in *Diplodus sargus* and *Pagellus erythrinus* for the two years studied (April 2008 before and April 2009 after a heavy storm) and areas (inside and outside the marine protected area).



	Diplodus sargus				*Pagellus erythrinus*			
Parameters analysed	df	MS	F	P	df	MS	F	P
stable isotopes								
year	1	111.00	0.395	0.547	1	22.74	0.293	0.603
area	1	137.70	0.490	0.504	1	0.27	0.004	0.954
year × area	1	524.60	1.868	0.209	1	4.39	0.057	0.818
residuals	8	280.90			8	77.57		
organic matter								
year	1	0.0038	1.418	0.252	1	0.0018	0.657	0.430
area	1	0.0027	1.001	0.333	1	0.0105	3.812	0.070
year × area	1	0.0044	1.632	0.221	1	0.0000	0.012	0.915
residuals	15	0.0027			15	0.0028		
carbohydrates								
year	1	13.339	0.768	0.396	1	1.972	1.072	0.316
area	1	0.110	0.006	0.938	1	5.579	3.034	0.101
year × area	1	0.477	0.027	0.871	1	1.972	1.072	0.316
residuals	14	17.371			16	1.839		
proteins								
year	1	11,691	2.116	0.168	1	4	0.001	0.970
area	1	10,271	1.859	0.194	1	5096	2.000	0.181
year × area	1	10,583	1.915	0.188	1	2920	1.146	0.304
residuals	14	5525			13	2548		
lipids								
year	1	0.01	0.000	0.988	1	42.10	0.273	0.610
area	1	10.04	0.176	0.681	1	309.50	2.009	0.180
year × area	1	34.47	0.604	0.449	1	2384.10	15.481	0.00171**
residuals	15	57.06			13	154.00		
total fatty acids								
year	1	104,067,04	1.298	0.270	1	83916647	30.328	1.35E-4***
area	1	335,101,744	41.807	1.48E-05***	1	22779	0.008	0.929
year × area	1	112,223,62	1.400	0.256	1	1370570	0.495	0.495
residuals	14	801,5457			12	2766932		
SFAs								
year	1	0.000	0.001	0.975	1	0.024	0.009	0.926
area	1	39.970	44.867	1.01E-05***	1	1.685	0.639	0.440
year × area	1	0.300	0.338	0.570	1	1.921	0.728	0.410
residuals	14	0.890			12	2.638		
MUFAs								
year	1	0.0010	0.003	0.957	1	2.3532	2.138	0.169
area	1	6.7580	30.614	7.37E-05***	1	0.9127	0.829	0.380
year × area	1	0.0000	0.000	0.987	1	0.0459	0.042	0.842
residuals	14	0.2210			12	1.1007		
PUFAs								
year	1	10.38	3.180	0.096	1	55.83	16.182	1.69E-3**
area	1	88.04	26.984	1.36E-05***	1	4.42	1.281	0.280
year × area	1	7.80	2.392	0.144	1	0.00	0.000	1.000
residuals	14	3.26			12	3.45		

F = F ratio; MS = mean square; MUFAs = mono-unsaturated fatty acids; PUFAs = poly-unsaturated fatty acids; SFAs = saturated fatty acids. Probability values (P) considered significant: **<0.01; ***<0.001.

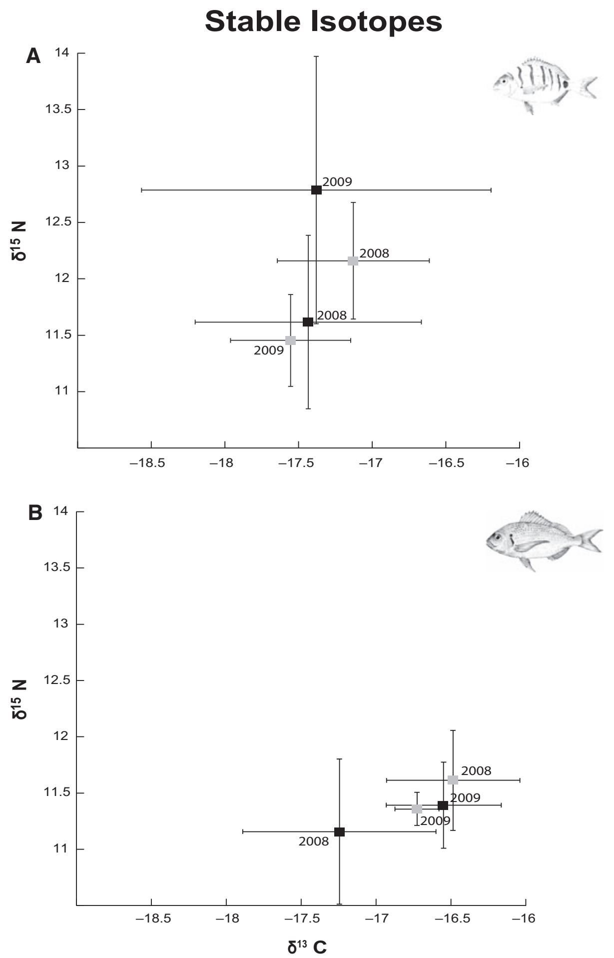


Fig. 2. Stable isotope ($\delta^{15}N$ and $\delta^{13}C$) composition inside (black squares) and outside the marine protected area (grey squares) for the two years analysed (April 2008 before and April 2009 after a heavy storm) in (A) *Diplodus sargus* and (B) *Pagellus erythrinus.* n = 3 for each point, mean ± SD.

3; Fig. 3). However, lipid content displayed significant differences for *Pagellus erythrinus* when considering the interaction of the two factors 'year' and 'area'. For this species, the lipid content in 2008 was higher inside the MPA, but was higher in April 2009 outside the MPA (two-way ANOVA; $P < 0.01$; Tables 1 and 3; Fig. 3). This same difference was also detected when considering both species, sampling areas and periods (three-way ANOVA; $P < 0.001$; Table 2).

No significant relationship was found between size (S; cm) and total lipid (TL; mg $Li \cdot g^{-1}$) content in the muscle for either species, validating the results of our study. In the case of *D. sargus*, the linear relationship was TL = 46.41–0.559 S, R = 0.227, $P > 0.05$. For *P. erythrinus*, it was TL = 56.19–0.835 S, R = 0.268, $P > 0.05$.

Discussion

Trophic ecology of *Diplodus sargus* and *Pagellus erythrinus* indicated by SI and FA analyses

There are numerous studies on the biology and ecology of *Diplodus sargus* and *Pagellus erythrinus*, owing principally to their commercial importance (*e.g.* Cejas *et al.* 2004; Özyurt *et al.* 2005; Kousoulaki *et al.* 2007; Pérez *et al.* 2007; Özbilgin *et al.* 2012; amongst others). One study analysed the potential relationship between nutritional condition and MPAs in *D. sargus* (Lloret & Planes 2003). However, to our knowledge, the present study is the first to test the condition and quality of nutrition in these two species (by means of trophic markers and biochemical analysis) inside and outside an MPA and under the effect of an extreme storm event.

For both species the SI analysis revealed a diet based on benthic organisms ($\delta^{13}C$ between −16‰ and −18‰; Dunton & Schell 1987; Thomas & Cahoon 1993; Carlier *et al.* 2007a) consuming mainly invertebrate prey ($\delta^{15}N$ between 11‰ and 13.5‰ in both species; Thomas & Cahoon 1993; Carlier *et al.* 2007a,b). The variability observed in the SI results was much higher (especially the $\delta^{15}N$) for *D. sargus* than for *P. erythrinus* (Fig. 2), possibly because of the wider variability of prey typical of omnivorous behaviour, as documented by several authors for *D. sargus* (Sala & Ballesteros 1997; Rodríguez-Ruiz *et al.* 2011), compared with the carnivorous behaviour of *P. erythrinus* (Santic *et al.* 2011).

The FA analyses for both species showed signals from invertebrate organisms [18:1(n–9) and 20:4(n–6)], as well as from macroalgae [18:2(n–6) and 18:1(n–7)], corresponding to Chlorophyta, Phaeophyceae and Rhodophyta (Dalsgaard *et al.* 2003; Kelly & Scheibling 2011). These signals presented a high concentration for both species, inside and outside the MPA (Table 5). This may indicate that *D. sargus* and *P. erythrinus* graze on the apical part of the algae in order to feed on the associated fauna, as observed in a previous study for another species (Soler-Membrives *et al.* 2011). The FAs of *D. sargus* showed higher variability than those of *P. erythrinus,* with a higher concentration of macroalgae markers, again supporting the omnivorous behaviour of *D. sargus*. These results also agree with the recent review by Kelly & Scheibling (2011), which suggested that all of the above-mentioned FAs combined with SI analysis can be useful in interpreting the trophic preferences of rocky fishes, as well as other organisms.

The MPA effect on fish condition and nutritional quality

In previous studies investigating the effects of European MPAs on *Diplodus sargus* and *Pagellus erythrinus*, both

Table 2. Results of the three-way analysis of variance comparing stable isotope and biochemical analyses amongst species (*Diplodus sargus* and *Pagellus erythrinus*), years (April 2008 before and April 2009 after a heavy storm) and area (inside and outside the marine protected area).

	df	MS	F	P
stable isotopes				
year	1	16.6	0.093	0.765
area	1	62.9	0.351	0.562
species	1	1895.5	10.576	0.005**
year × area	1	216.5	1.208	0.288
year × species	1	117.1	0.653	0.432
area × species	1	75.1	0.419	0.527
year × area × species	1	312.5	1.744	0.205
residuals	16	179.2		
organic matter				
year	1	0.000183	0.067	0.797
area	1	0.001284	0.472	0.498
species	1	0.021472	7.887	0.009**
year × area	1	0.002585	0.950	0.338
year × species	1	0.005440	1.998	0.168
area × species	1	0.011921	4.378	0.045*
year × area × species	1	0.001833	0.673	0.418
residuals	30	0.002772		
carbohydrates				
year	1	2.23	0.246	0.624
area	1	2.85	0.314	0.579
species	1	13.21	1.454	0.237
year × area	1	2.24	0.246	0.623
year × species	1	12.44	1.369	0.251
area × species	1	3.47	0.382	0.541
year × area × species	1	0.21	0.023	0.879
residuals	30	272.62		
proteins				
year	1	6081	1.486	0.233
area	1	800	0.196	0.662
species	1	3239	0.792	0.282
year × area	1	14,264	3.486	0.073
year × species	1	5112	1.249	0.274
area × species	1	13,653	3.337	0.079
year × area × species	1	1029	0.251	0.620
residuals	27	4092		
lipids				
year	1	19.0	0.186	0.669
area	1	100.5	0.984	0.330
species	1	16.4	0.160	0.692
year × area	1	836.9	0.200	0.008**
year × species	1	41.1	0.402	0.531
area × species	1	226.5	2.219	0.147
year × area × species	1	1560.8	15.291	5.34E-4***
residuals	28	102.1		

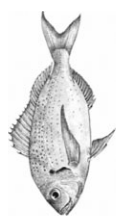
F = F ratio; MS = mean square.
Probability values (P) considered significant: *<0.05; **<0.01; ***<0.001.

species displayed clear signals of a 'reserve effect', showing much larger size/age classes for the individuals inside the no-take areas (see García-Rubies & Zabala 1990; González-Wangüemert *et al.* 2004; Martin *et al.* 2012; Horta e Costa *et al.* 2013). However, size is not necessarily a direct indicator of favourable conditions, due to the MPAs have different effects on different fish species, behaviour and developmental stage (larva, settler, adult; Lloret & Planes 2003; Lloret *et al.* 2005; Stelzenmüller *et al.* 2007).

No significant differences were found between the samples from the no-take area and from outside the MPA in

Table 3. Mean values and SD for the analysed parameters: organic matter (amount), carbohydrates, proteins, lipids and fatty acids (μg mg OM-1)for both species inside the marine protected area (MPA) and outside the MPA in the two analysed years (April 2008 before and April 2009 after a heavy storm). n = 5 in each case.

Diplodus sargus	April 2008				April 2009			
	inside MPA		outside MPA		inside MPA		outside MPA	
	mean	SD	mean	SD	mean	SD	mean	SD
organic matter	0.89	0.05	0.84	0.06	0.88	0.06	0.89	0.04
carbohydrates	14.47	2.76	14.30	2.81	12.41	3.22	17.17	2.87
proteins	455.10	86.68	489.46	79.98	349.99	79.51	446.86	82.72
lipids	36.61	5.30	43.61	10.33	42.02	10.68	40.26	10.68
total fatty acids	17.58	3.38	6.89	2.05	15.43	2.84	8.34	2.68



Pagellus erythrinus	April 2008				April 2009			
	inside MPA		outside MPA		inside MPA		outside MPA	
	mean	SD	mean	SD	mean	SD	mean	SD
organic matter	0.81	0.03	0.86	0.05	0.79	0.,06	0.84	0.06
carbohydrates	12.88	1.57	11.20	1.00	10.50	1.75	12.75	1.16
proteins	475.75	67.96	372.25	105.25	491.06	80.66	447.00	37.09
lipids	46.73	19.00	15.63	5.30	27.82	1.90	44.30	12.43
total fatty acids	18.12	1.13	18.81	1.42	14.22	1.42	13.72	2.34

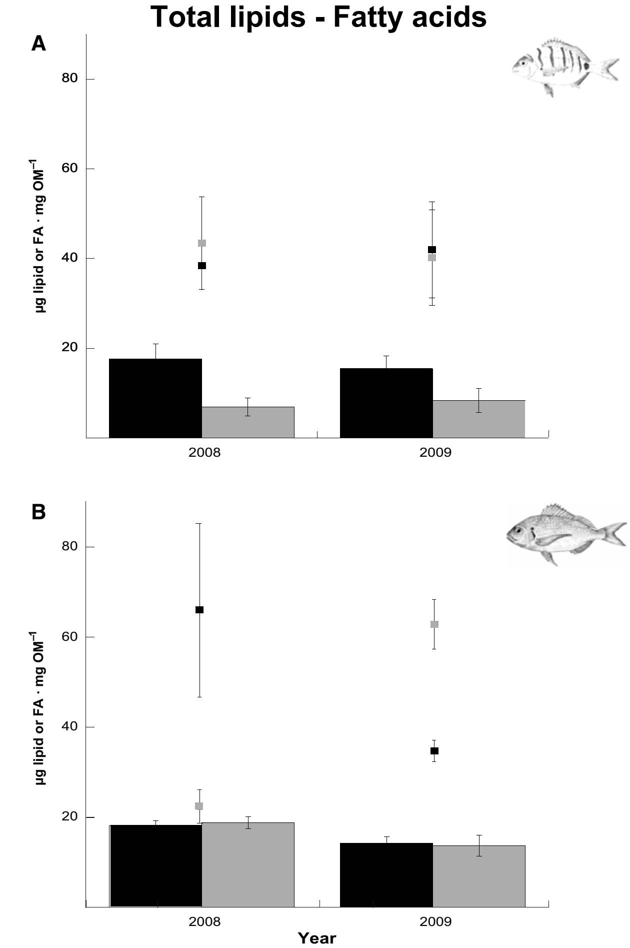


Fig. 3. Lipids (squares) and fatty acids (FAs; histogram bars) inside (black) and outside the marine protected area (grey) in the two years analysed (April 2008 before and April 2009 after a heavy storm) for (A) *Diplodus sargus* and (B) *Pagellus erythrinus*. n = 5 for each point, mean ± SD. OM, organic matter.

protein, carbohydrate or total OM concentrations, suggesting that the structural functions (protein) and use of quick energy (carbohydrate) did not differ between the study areas. However, the lipid and FA analyses suggested that nutritional condition (lipids) and nutrition quality (FA) in *P. erythrinus* and *D. sargus* depends on food availability within the different areas (inside/outside the MPA) that they inhabit (Fig. 3). *Diplodus sargus* did not show any 'reserve effect' with respect to lipid storage, similar to the results of Lloret & Planes (2003). Although our lipid results for *P. erythrinus* in 2008 suggest a clear 'reserve effect', currently we are unable to conclude that this is the only factor affecting this potential energy accumulation. However, it is important to highlight that a total lipid analysis alone may not be sufficient to understand fish conditions (Rainuzzo *et al.* 1997). Therefore,

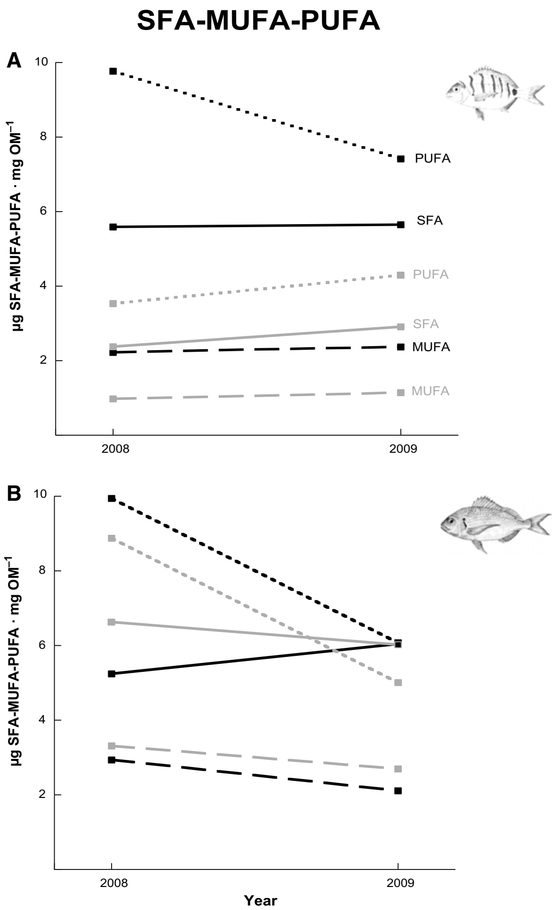


Fig. 4. Saturated (SFAs), mono-unsaturated (MUFAs) and poly-unsaturated fatty acids (PUFAs) inside (black lines) and outside the protected area (grey lines) in the two years analysed (April 2008 before and April 2009 after a heavy storm) for (A) *Diplodus sargus* and (B) *Pagellus erythrinus*. n = 5 for each point; mean ± SD. OM, organic matter.

we suggest that, considering the results for the total concentration and type of FAs, *D. sargus* has better nutrition in the no-take area of the study zone than *P. erythrinus*, which is reflected in the higher total concentration of FAs and, more specifically, PUFAs (Fig. 4). This could be the result of an increase in optimal food availability inside the Medes Islands Marine Reserve, owing to the higher complexity and diversity of habitat (Hereu *et al.* 2006; Forcada *et al.* 2009).

The high variability found within the SI results, combined with the higher concentration of 18:2(n-6) (trophic marker that may be partly of phanerogam origin; Kelly & Scheibling 2011 and references therein), inside the MPA for both species, may reflect a higher density and better conservation status of the seagrass meadows inside the MPA (Gili & Ros 1985; Romero-Romero & Yufera 2012). This result is in agreement with previous studies documenting that *D. sargus* prefer this habitat for living and feeding on the seagrass epibionts (García-Rubies & Zabala 1990; Gordoa & Moli 1997; Sala & Ballesteros 1997). Sánchez-Jerez & Ramos-Esplá (1996) demonstrated that a diet based on epibionts may also contain leaves and rhizome pieces of the seagrass meadows ingested with the prey.

Interestingly, the marker 22:6(n-3) (dinoflagellate origin, Dalsgaard *et al.* 2003; Kelly & Scheibling 2011 and references therein) showed the highest concentration of all of the specific microalgae markers analysed in both species and areas. This high concentration is not simply explained by a dinoflagellate bloom, it is more likely to be a result of selective accumulation, obtained from metabolic reactions and helped by high amount of PUFAs aforementioned. This selective accumulation of specific trophic markers can change according to the metabolic needs of the species and depends on the trophic markers accumulated within the diet, as has been previously observed for *D. sargus* (Özyurt *et al.* 2005) and other fish, even in larval stages (Sargent *et al.* 1987; St. John & Lund 1996; Rossi *et al.* 2006b). Previous studies have described that during the reproductive period and developing stages, the accumulation of certain PUFAs can be selective, and a key factor for the survivorship of adults and larvae (with a higher transfer of the reserves to the gonads, Cejas *et al.* 2004). Amongst the PUFAs examined here, the 22:6(n-3) marker is considered essential to understanding the population fitness and viability of fish offspring (Bell & Sargent 1996), and it has been demonstrated that deficiencies of the (n-3) highly unsaturated fatty acids (HUFAs) negatively affect the fecundity, fertilization and hatching rates in brood stocks of many fish species (Rainuzzo *et al.* 1997). Based on the concentration of HUFAs, our results suggest a higher rate of fertilization and/or survival for *D. sargus* individuals inside the MPA. The SIMPER analysis showed an average dissimilarity of 48% between the sites in 2008, to which 22:6(n-3) was the most important contributor, with 12.27% (Table 6). These results, together with the highest gonadosomatic index values inside the Medes Islands MPA (Micale et al. 1987; Mouine *et al.* 2007; Orejas & Fernández 2010), would demonstrated that reproductive conditions of *D. sargus* are better in no-take areas of the MPA. However, this assertion should be considered with caution, as no information on fertilization or survival rates is available for the species at this time.

Potential effects of heavy storms on fish conditions

One of the novel results of this study is the records of SI, biochemical balance and FAs for the same fish

Table 4. Results of the three-way analysis of variance comparing fatty acids and class of fatty acids amongst species (*Diplodus sargus* and *Pagellus erythrinus*), the two years analysed (April 2008 before and April 2009 after a heavy storm) and area (inside and outside the marine protected area).

	df	MS	F	P
total fatty acids				
year	1	745,000,23	13.320	0.002**
area	1	141,674,062	25.330	3.08E-5***
species	1	154,761,195	27.670	1.69E-5***
year × area	1	214,3399	0.383	0.541
year × species	1	371,887,88	6.649	0.016*
area × species	1	158,141,162	28.275	1.46E-5***
year × area × species	1	986,9278	1.765	0.196
residuals	27	559,3061		
SFAs				
year	1	0.02	0.010	0.922
area	1	11.15	6.566	0.017*
species	1	34.32	20.221	1.27E-4***
year × area	1	0.24	0.143	0.708
year × species	1	0.25	0.149	0.703
area × species	1	27.29	16.373	4.14E-4***
year × area × species	1	1.93	1.134	0.297
residuals	26	44.13		
MUFAs				
year	1	1.068	1.704	0.203
area	1	0.814	1.299	0.265
species	1	10.726	17.110	3.27E-4***
year × area	1	0.012	0.019	0.891
year × species	1	1.646	2.626	0.117
area × species	1	6.100	9.730	0.004**
year × area × species	1	0.023	0.037	0.850
residuals	26	0.627		
PUFAs				
year	1	55.79	16.659	3.78E-4***
area	1	58.70	17.525	2.87E-4***
species	1	10.93	3.263	0.082
year × area	1	3.41	1.019	0.322
year × species	1	18.60	5.553	0.026*
area × species	1	23.37	6.976	0.014*
year × area × species	1	3.63	1.084	0.307
residuals	26	3.35		

F = F ratio; MS = mean square; MUFAs = mono-unsaturated fatty acids; PUFAs = poly-unsaturated fatty acids; SFAs = saturated fatty acids.
Probability values (P) considered significant: *<0.05; **<0.01; ***<0.001.

populations before (April 2008) and after (April 2009) the storm of December 2008. These results must be considered with caution as this is the first time that these techniques have been used to contrast feeding behaviour, nutritional condition and quality of nutrition in fish populations before and after a catastrophic event. However, the results indicate tendencies that deserve discussion. The literature available for the effects of this storm on the benthic sessile species in the study area (algae, phanerogams, gorgonians, *etc*.; Navarro *et al.* 2011; Hereu *et al.* 2012; Teixidó *et al.* 2013), together with our results, can help to interpret the possible consequences for *D. sargus* and *P. erythrinus*.

Although *D. sargus* did not show any significant differences in lipid and FA concentration, the FA composition (trophic markers) showed a different spectra of biomolecules, suggesting changes in food availability from April 2008 to April 2009. An increase in the algal component [18:1(n-7)], was detected in 2009, which may have originated from organic detritus (Soler-Membrives *et al.* 2011). By contrast, other macroalgal trophic markers detected in this fish species decreased considerably from April 2008 to April 2009, possibly as a result of the reduction in algal coverage in the study area after the storm (>70% in some areas, Navarro *et al.* 2011; Hereu *et al.* 2012). Seasonal changes in the feeding behaviour of

Table 5. Mean values (% of total fatty acids) and SD of trophic markers and saturated (SFAs), mono-unsaturated (MUFAs) and poly-unsaturated fatty acids (PUFAs) for both species (*Diplodus sargus* and *Pagellus erythrinus*) inside and outside the marine protected area (MPA) for the two years analysed (April 2008 before and April 2009 after the storm). n = 3 in each case.

																
	Diplodus sargus								*Pagellus erythrinus*							
	April 2008				April 2009				April 2008				April 2009			
	inside MPA		outside MPA		inside MPA		outside MPA		inside MPA		outside MPA		inside MPA		outside MPA	
fatty acids	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
13:0	2.97	0.73	3.65	2.25	3.11	1.38	4.42	0.91	0.00	0.00	1.56	2.19	4.46	1.65	5.54	1.87
14:1(n-5)	0.13	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.46	0.63	0.31	0.03	0.17	0.17
14:0	0.69	0.32	0.70	0.30	0.81	0.04	0.52	0.31	0.66	0.32	1.19	1.10	1.15	0.51	1.55	1.16
15:0	0.32	0.09	0.40	0.24	0.40	0.07	0.34	0.21	0.41	0.08	0.47	0.23	0.58	0.18	0.77	0.32
16:1(n-7)	2.49	1.08	3.70	1.93	3.99	0.91	3.87	0.55	2.76	0.70	4.00	2.57	4.67	2.05	6.85	3.15
16:0	19.74	1.88	21.12	5.39	24.08	1.85	19.98	2.16	16.97	0.59	22.51	3.68	23.29	4.12	27.49	0.76
17:1(n-7)	0.34	0.08	0.45	0.09	0.36	0.04	0.41	0.24	0.00	0.00	0.34	0.48	0.49	0.10	0.76	0.31
17:0	0.59	0.17	0.76	0.25	0.61	0.13	0.75	0.13	0.69	0.14	0.67	0.21	0.85	0.26	1.16	0.48
18:3(n-6)	0.22	0.23	0.46	0.29	0.95	0.57	0.24	0.24	0.00	0.00	0.21	0.29	0.49	0.10	0.37	0.05
18:2(n-6)	7.52	4.16	2.69	0.58	6.11	3.54	2.97	1.70	1.61	0.89	0.97	0.19	5.14	7.23	1.32	0.18
18:1(n-9)	4.14	1.30	4.82	3.09	4.37	1.12	2.95	0.78	5.71	1.75	5.58	1.08	3.21	1.23	3.98	1.58
18:1(n-7)	4.98	0.94	3.60	2.18	5.90	1.09	5.29	1.17	4.00	1.06	4.71	1.45	5.39	0.69	6.20	1.47
18:0	6.70	1.41	6.21	1.71	7.07	0.88	7.33	1.42	5.06	0.67	5.66	1.12	6.01	0.39	6.35	0.86
20:5(n-3)	6.70	3.77	4.73	5.46	7.48	2.50	13.80	4.02	6.12	1.59	6.17	1.56	6.43	2.14	4.81	0.87
20:4(n-6)	7.89	1.84	10.01	3.37	10.03	3.15	11.73	2.98	16.41	2.91	12.35	4.99	10.60	1.56	7.72	2.30
20:3(n-6)	4.37	7.38	10.09	10.61	1.49	0.61	1.59	1.03	2.54	0.79	1.29	0.32	1.38	0.19	1.05	0.37
20:2(n-6)	0.32	0.19	0.20	0.08	0.23	0.02	0.23	0.14	0.95	0.21	0.41	0.22	0.26	0.03	0.23	0.07
20:1(n-9)	0.37	0.11	0.42	0.13	0.40	0.05	0.50	0.13	1.14	0.26	0.85	0.12	0.30	0.07	0.48	0.10
20:0	0.00	0.00	0.13	0.10	0.05	0.04	0.17	0.17	0.06	0.10	0.07	0.05	0.09	0.03	0.17	0.13
21:0	0.37	0.51	0.01	0.02	0.02	0.03	0.21	0.29	0.97	0.23	1.00	0.67	5.21	10.32	0.07	0.03
22:6(n-3)	27.90	4.50	24.84	4.32	21.82	9.62	21.77	5.22	27.40	6.62	25.09	8.94	18.87	11.45	21.82	6.18
22:2(n-6)	0.16	0.11	0.13	0.14	0.14	0.01	0.15	0.09	0.00	0.00	0.75	1.06	0.08	0.03	0.12	0.05
22:1(n-9)	0.23	0.28	0.09	0.06	0.08	0.06	0.23	0.13	0.23	0.41	0.35	0.71	0.08	0.03	0.14	0.05
22:0	0.23	0.14	0.69	1.37	0.25	0.15	0.31	0.28	1.52	0.24	0.73	0.62	0.23	0.09	0.30	0.06
23:0	0.11	0.24	0.01	0.02	0.00	0.00	0.00	0.00	0.20	0.35	0.64	0.88	0.11	0.02	0.11	0.05
24:1(n-9)	0.16	0.11	0.02	0.03	0.19	0.04	0.06	0.06	2.19	0.44	1.15	0.17	0.19	0.10	0.22	0.18
24:0	0.36	0.30	0.04	0.06	0.07	0.05	0.19	0.18	2.39	0.66	0.78	0.67	0.05	0.02	0.19	0.14
SFAs	31.79		34.51		36.59		34.87		28.93		35.23		42.48		43.87	
MUFAs	12.65		14.15		15.35		13.67		16.19		17.58		14.80		19.65	
PUFAs	55.56		51.33		48.06		51.46		54.88		47.18		42.71		36.49	

Table 6. Results of similarity percentage (SIMPER) analysis of fatty acids for *Diplodus sargus* between area [inside and outside the marine protected area (MPA)] and year (April 2008 before and April 2009 after a heavy storm.

process					
group 1	group 2	average dissimilarity (%)	trophic markers	average abundance per group	average dissimilarity per group (%)
inside MPA	outside MPA	48	22:6(n-3)	4968.48	12.27
April 2008	April 2008		16:0	3451.43	7.89
			20:3(n-6)	931.78	4.70
inside MPA	inside MPA	22	22:6(n-3)	4968.48	6.74
April 2008	April 2009		16:0	3451.43	2.35
			20:3(n-6)	931.78	2.23
inside MPA	outside MPA	40	22:6(n-3)	4968.48	12.28
April 2008	April 2009		16:0	3451.43	6.96
			18:2(n-6)	1295.01	4.18
inside MPA	outside MPA	44	16:0	1477.34	9.77
April 2009	April 2008		22:6(n-3)	1882.26	7.59
			20:5(n-3)	2819.78	3.74
inside MPA	outside MPA	29	20:5(n-3)	2819.13	5.33
April 2009	April 2008		20:3(n-6)	8570.66	4.90
			22:6(n-3)	1882.58	4.16
inside MPA	outside MPA	35	16:0	3722.64	8.66
April 2009	April 2009		22:6(n-3)	3355.62	6.85
			20:4(n-6)	1577.34	3.12

Table 7. Results of similarity percentage (SIMPER) analysis of fatty acids for *Pagellus erythrinus* between area [inside and outside the marine protected area (MPA)] and year (April 2008 before and April 2009 after a heavy storm).

process					
group 1	group 2	average dissimilarity (%)	trophic markers	average abundance per group	average dissimilarity per group (%)
inside MPA	outside MPA	20	22:6(n-3)	4915.97	3.94
April 2008	April 2008		16:0	3070.37	3.12
			20:4(n-6)	2993.32	2.16
inside MPA	inside MPA	31	22:6(n-3)	4915.97	7.17
April 2008	April 2009		20:4(n-6)	2993.32	4.59
			21:0	174.73	2.66
inside MPA	outside MPA	31	22:6(n-3)	4915.97	6.40
April 2008	April 2009		20:4(n-6)	2993.32	6.11
			13:0	0	2.36
inside MPA	outside MPA	29	22:6(n-3)	4730.9	7.15
April 2009	April 2008		16:0	4222.58	3.07
			20:4(n-6)	2308.53	2.85
inside MPA	outside MPA	26	22:6(n-3)	4730.9	6.07
April 2009	April 2008		20:4(n-6)	2308.53	3.98
			16:0	4222.58	2.29
inside MPA	outside MPA	21	22:6(n-3)	2590.86	3.61
April 2009	April 2009		21:0	810.47	2.76
			16:0	3300.3	2.53

D. sargus have been previously reported (Rodríguez-Ruiz *et al.* 2011), showing that changes in diet may be related to food availability. Owing to their omnivorous and opportunistic feeding behaviour, an increase in variability of markers is expected as a result of type prey available.

In *P. erythrinus*, the total concentration of FAs and the percentage of PUFAs (Fig. 4) decreased in both areas from April 2008 to April 2009, but not significantly. Our results suggest that *P. erythrinus* has a more restricted diet than *D. sargus* (as mentioned above), and that food availability was lower after the storm, probably because of the mass mortality of the first levels of the food web and loss of biomass and complexity all trophic network (García-Rubies *et al.* 2009; Hereu *et al.* 2012; Sánchez-Vidal *et al.* 2012; Teixidó *et al.* 2013). Previous studies have shown that in the study area, the pluriannual algae *Cystoseira zosteroides* experienced a decrease in density of ~80% in some patches (Navarro *et al.* 2011), and densities of gorgonians, seagrasses and other sessile components of the benthic community also showed significant reductions after the storms (Sánchez-Vidal *et al.* 2012; Teixidó *et al.* 2013). These reductions may potentially have affected the nutritional condition of organisms belonging to higher trophic levels, especially in organisms with more restricted diets.

The present study suggests that the large storm that occurred in December 2008 could have influenced the condition of both studied rocky fish species. Differences observed between the two species could be related to their feeding preferences and behaviour. Favourable conservation statuses of 'sea benthic forests' and other habitat-forming species are essential to maintain and increase the nutritional condition of fishing stocks (Rossi 2013b), which may affect populations' persistence. Consequently, the future viability of fish populations in MPAs will also depend on the complexity and diversity of these structures, which may be affected not only by the design and conservation plans of the MPA, but also by stochastic events.

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