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Meta-analysis review of fish trophic levels in marine protected areas based on stable isotope data

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Abstract

Stable isotopes ($\delta^{15}N$) have been used to determine trophic levels in marine food webs. This study assessed if Marine Protected Areas (MPAs) affect the trophic levels of fishes based on stable isotopes in the western Mediterranean. A total of 22 studies including 600 observations were found and the final dataset consisted of 11 fish species and 146 observations comparing trophic levels inside and outside MPAs. The database was analysed by meta-analysis and the covariate selected was the level of protection (inside *vs*. outside MPAs). The results indicate significant differences between trophic levels inside and outside the MPAs; however, results differ from expectations since the trophic level was lower inside than outside MPAs. Three habitats were analysed (coastal lagoons, demersal, and littoral) and significant statistical differences were found among them: trophic level was higher in demersal habitats than in coastal lagoons and littoral areas. No significant differences were found in species classified by trophic functional groups. Several hypotheses are considered as possible explanations linked to protection level, time since protection, and MPA size. We debate the suitability of using the stable isotope $\delta^{15}N$ as a direct indicator of trophic level in evaluating MPA effects on food webs.

Keywords: Diet, fisheries effects, marine reserves, Mediterranean Sea.

Introduction

Marine Protected Areas (MPAs) have been established to prevent or reduce impacts exerted by humans in marine ecosystems. The benefits of the establishment of MPAs have been described both inside (García-Rubies & Zabala, 1990; Francour, 1994; Guidetti, 2002) and outside no-take areas (Russ & Alcala, 1996; Ojeda-Martinez *et al.*, 2007).

The trophic level (TL) of a species is an ecological trait that might provide valuable information on the ecological processes deriving from spatial regulations on fish catches in marine protected areas. TL could be determined by fish stomach contents analyses or by quantifying stable isotopes ratios of nitrogen (N). Stomach contents analysis provides information on prey consumption and is based on the transfer of organic matter through food chains. Species position within the food chain is determined by their diet and weight (Polis & Strong, 1996). There are several limitations to this methodology: it only provides a snapshot of the ingestion at a particular time, there exists the difficulty of distinguishing between assimilate and non- assimilated food sources, the amount that has been assimilated, and that which has been the contribution of primary producers.

Stable isotope signatures rely on the assumption of ‘we are what we eat’ and are based on the transformation of the carbon isotope (^{12}C) and nitrogen isotope (^{14}N) within an organism to its most stable isotopes, ^{13}C and ^{15}N, respectively. This process takes place because when an organism feeds, the food tends to stabilize. The ratio of stable isotopes of proteins in consumer species reflects those proteins in the diet in a predictable way (Hobson & Clark, 1992; Hobson, 1999).

This study focuses on $\delta^{15}N$ since that isotope is used to estimate the trophic level of marine organisms, especially when the base level is the primary producers (Cabana & Rasmussen, 1996). The abundance of $\delta^{15}N$ in the tissues of the consumer species is enhanced, more or less, by 3.4 ‰ relative to their prey (Vander Zanden *et al.*, 1997; Post, 2002). This type of analysis overcomes the limitations of analysis by stomach content. The results reflect the actual assimilation of nutrients and can be used for studies over a longer period of time because assimilation is reflected in the tissue analysis.

Meta-analyses have been widely applied as a tool to assess processes in MPAs so as to demonstrate the variations in ecological traits and fish life histories (Claudet *et al.*, 2008), effects of MPAs on fish populations (Cotê *et al.*, 2001) as well as the interactions between cod and shrimp in oceanic food webs (Worm & Myers, 2003).

Although some meta-analyses on fisheries research have been performed since the 1990s, to our knowledge our study is the first to assess trophic level changes linked to Marine Protected Areas through the evaluation of stable isotopes in fishes.

In this context, the main aim of the present study was to investigate changes in trophic levels based on stable isotopes for fish species both inside Marine Protected Areas and outside MPAs. We have considered only data derived from stable isotopes in our analysis, not taking into consideration shifts in biomass, abundance or density related to fisheries effects. The initial hypothesis is that the fishing pressure exerted outside protected areas determines lower trophic levels of fishes with respect to those measured inside the protected areas. For that reason, we aimed to analyse how fish trophic level is influenced by protection. Additionally, we wanted to investigate differences in trophic level (TL) by habitat types, and assess differences in TL regarding trophic functional groups.

Material and Methods

This study has focused on the western Mediterranean, considering large number of observations of fish species that cover a vast array of ecosystems, coastal lagoons, demersal, and littoral areas and several functional trophic groups, carnivorous, omnivorous and piscivorous (according to FISHBASE[1]).

TL for a given species can be modelled as a function of the , according to the equation:

$$TL_i = \frac{\delta^{15}N_i - \delta^{15}N_{ref}}{TE} + TL_{ref}$$

where TE represents the trophic enrichment and TL_{ref} refers to base trophic level in the studied ecosystem. For the Mediterranean, the final equation is:

$$TL_i = \frac{\delta^{15}N_i - \delta^{15}N_{ref}}{3.4} + 2$$

where 3.4 is the enrichment in each trophic level increase (Post, 2002) and 2 is the trophic level base at the Mediterranean Sea.

Database

Data have been obtained from a search in the ISI Web of Science using “trophic”, “stable isotopes”, and “Mediterranean” as keywords. A total of 22 studies were found until 2008, which corresponds to 600 observations. Subsequently, the information was summarised in a database in which an identity code was provided for each observation including different fields: study author, fish species and family, fish size, depth average, stable isotope ratios (carbon and nitrogen data), standard deviation, location, geographical coordinates, country, type of habitat, diet, protection effect (inside *vs*. outside), protection status, trophic level obtained from our calculations from stable isotope data, trophic level recorded from bibliographical cites at www.fishbase.org, and trophic level based on stomach contents reported in Stergiou & Karpouzi (2002).

From the original pool of studies, the observations lacking sample size and therefore without standard deviation estimates were rejected. The observations retained were those concerning species with information from both inside and outside the Marine Protected Area. Thus, the final dataset consists of 11 species and 146 observations. The covariate selected is the level of protection (inside *vs*. outside the marine protected area) (Table 1).

Analysis

To test the differences between trophic levels inside and outside the marine protected areas, the size effect (e_i) was utilised, with log-response ratio (Hedges *et al.*, 1999):

$$e_i = \ln\left(\frac{TL_{iin}}{TL_{iout}}\right)$$

where TL_{iin} and TL_{iout} are the trophic levels inside and outside the marine protected area, respectively. Subsequently, variance of the size effect (V_i) was calculated. V_i is used to derive weights in the meta-analysis to increase both the precision and the power of the test (Gurevitch & Hedges, 1999; Osenberg *et al.*, 1999). Thus, studies with more observations provide a greater weight. Variance of the size effect was calculated according to the equation:

$$V_i = \frac{(S_{iin})^2}{N_{iin}(\overline{TL}_{iin})^2} + \frac{(S_{iout})^2}{N_{iout}(\overline{TL}_{iout})^2}$$

where V_i is the variance associated to e_i, N_{iin} and N_{iout} corresponds to the number of observations inside and outside the protected area respectively, *TLiin* and *TLiout* are the mean trophic levels inside and outside the protected area respectively, and S_{iin} and S_{iout} are the standard deviations associated with the trophic levels. The variance is applied to calculate the weight for meta-analysis:

$$w_i = \frac{1}{V_i}$$

where w_i is the weight associated to the size effect *ei*; V_i is the variance defined above. Weighted cumulative effect was then calculated as:

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[1] Diet based on fishbase.org:
Piscivorous: Feed mainly on fishes and invertebrates.
Carnivorous: Feeds on crustaceans, decapods, mysids, worms, and molluscs
Omnivorous: Feed on seaweeds and small invertebrates, algae, crustaceans, etc

Table 1. Author, species, stable isotope ratios trophic level (TL), standard deviation, number of samples, habitat and diet (fishbase).

		No-take areas			Take areas				
Author	**Species**	**TL δ^{15}N**	**SD**	**n samples**	**TL δ^{15}N**	**SD**	**n samples**	**Habitat**	**Diet (fishbase)**
Vizzini & Mazzola, 2008	*Boops boops*	2.90	0.10	52.00	-	-	-	Littoral	Omnivorous
Carlier *et al*., 2007	*Boops boops*	-	-	-	3.63	0.70	3.00	Littoral	Omnivorous
Vizzini & Mazzola, 2008	*Boops boops*	2.96	0.10	27.00	-	-	-	Littoral	Omnivorous
Pinnegar *et al*., 2000	*Boops boops*	-	-	-	2.63	0.36	3.00	Littoral	Omnivorous
Vizzini & Mazzola, 2008	*Boops boops*	2.96	0.10	27.00	-	-	-	Littoral	Omnivorous
-	*Boops boops*	-	-	-	3.13	0.53	3.00	-	-
Vizzini & Mazzola, 2008	*Boops boops*	2.96	0.10	27.00	-	-	-	Littoral	Omnivorous
-	*Boops boops*	-	-	-	3.13	0.53	3.00	-	-
Vizzini & Mazzola, 2008	*Coris julis*	3.01	0.20	62.00	-	-	-	Littoral	Carnivorous
Jennings *et al*., 1997	*Coris julis*	-	-	-	3.81	0.11	4.00	Littoral	Carnivorous
Vizzini & Mazzola, 2008	*Coris julis*	3.10	0.20	60.00	-	-	-	Littoral	Carnivorous
Cardona *et al*., 2007	*Coris julis*	-	-	-	4.10	0.70	10.00	Littoral	Carnivorous
Pinnegar *et al*., 2000	*Coris julis*	3.19	0.50	58.00	-	-	-	Littoral	Carnivorous
Jennings *et al*., 1997	*Coris julis*	-	-	-	4.22	0.27	6.00	Littoral	Carnivorous
Vizzini & Mazzola, 2008	*Coris julis*	3.22	0.20	70.00	-	-	-	Littoral	Carnivorous
Jennings *et al*., 1997	*Coris julis*	-	-	-	4.25	0.11	5.00	Littoral	Carnivorous
-	*Coris julis*	3.04	0.19	47.88	-	-	-	-	-
Vizzini & Mazzola, 2008	*Coris julis*	-	-	-	3.19	0.50	58.00	Littoral	Carnivorous
-	*Coris julis*	3.04	0.19	47.88	-	-	-	-	-
Lepoint *et al*., 2000	*Coris julis*	-	-	-	3.34	0.40	2.00	Littoral	Carnivorous
Vizzini & Mazzola, 2008	*Diplodus annularis*	2.99	0.60	10.00	-	-	-	Littoral	Carnivorous
Jennings *et al*., 1997	*Diplodus annularis*	-	-	-	3.93	0.13	5.00	Littoral	Carnivorous
Vizzini & Mazzola, 2008	*Diplodus annularis*	3.13	0.20	13.00	-	-	-	Littoral	Carnivorous
Cardona *et al*., 2007	*Diplodus annularis*	-	-	-	4.25	0.50	10.00	Littoral	Carnivorous
Vizzini & Mazzola, 2008	*Diplodus annularis*	3.16	0.30	18.00	-	-	-	Littoral	Carnivorous
Jennings *et al*., 1997	*Diplodus annularis*	-	-	-	4.40	0.20	5.00	Littoral	Carnivorous
-	*Diplodus annularis*	3.09	0.37	13.67	-	-	-	-	-
Jennings *et al*., 1997	*Diplodus annularis*	-	-	-	4.40	0.20	5.00	Littoral	Carnivorous
-	*Diplodus annularis*	3.09	0.37	13.67	-	-	-	-	-
Lepoint *et al*., 2000	*Diplodus annularis*	-	-	-	3.07	0.40	2.00	Littoral	Carnivorous
-	*Diplodus annularis*	3.09	0.37	13.67	-	-	-	-	-
Pinnegar *et al*., 2000	*Diplodus annularis*	-	-	-	3.13	0.27	3.00	Littoral	Carnivorous
-	*Diplodus annularis*	3.09	0.37	13.67	-	-	-	-	-
Deudero *et al*., 2004	*Diplodus annularis*	-	-	-	3.44	0.18	10.00	Littoral	Carnivorous
Badalamenti *et al*., 2008	*Lophius budegassa*	3.90	0.15	6.00	-	-	-	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Lophius budegassa*	-	-	-	3.86	0.16	6.00	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Merluccius merluccius*	3.60	0.07	6.00	-	-	-	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Merluccius merluccius*	-	-	-	3.34	0.11	6.00	Demersal	Piscivorous
Badalamenti *et al*., 2002	*Merluccius merluccius*	3.43	0.10	80.00	-	-	-	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Merluccius merluccius*	-	-	-	3.36	0.08	6.00	Demersal	Piscivorous
Badalamenti *et al*., 2002	*Merluccius merluccius*	3.46	0.10	80.00	-	-	-	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Merluccius merluccius*	-	-	-	3.41	0.12	6.00	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Merluccius merluccius*	3.48	0.11	6.00	-	-	-	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Merluccius merluccius*	-	-	-	3.42	0.17	6.00	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Merluccius merluccius*	3.49	0.19	6.00	-	-	-	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Merluccius merluccius*	-	-	-	3.45	0.10	6.00	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Merluccius merluccius*	3.49	0.19	6.00	-	-	-	Demersal	Piscivorous
Badalamenti *et al*., 2002	*Merluccius merluccius*	-	-	-	3.46	0.10	80.00	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Merluccius merluccius*	3.49	0.09	6.00	-	-	-	Demersal	Piscivorous

(continued)

Table 1 *(continued)*

		No-take areas			Take areas				
Author	**Species**	**TL δ^{15}N**	**SD**	**n samples**	**TL δ^{15}N**	**SD**	**n samples**	**Habitat**	**Diet (fishbase)**
Badalamenti *et al*., 2008	*Merluccius merluccius*	-	-	-	3.48	0.12	6.00	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Merluccius merluccius*	3.50	0.12	6.00	-	-	-	Demersal	Piscivorous
Badalamenti *et al*., 2002	*Merluccius merluccius*	-	-	-	3.49	0.10	80.00	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Merluccius merluccius*	3.51	0.12	6.00	-	-	-	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Merluccius merluccius*	-	-	-	3.50	0.18	6.00	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Merluccius merluccius*	3.53	0.14	6.00	-	-	-	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Merluccius merluccius*	-	-	-	3.55	0.09	6.00	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Merluccius merluccius*	3.56	0.10	6.00	-	-	-	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Merluccius merluccius*	-	-	-	3.55	0.17	6.00	Demersal	Piscivorous
-	*Merluccius merluccius*	3.50	0.12	19.45	-	-	-	-	-
Polunin *et al*., 2001	*Merluccius merluccius*	-	-	-	3.59	0.21	3.00	Demersal	Piscivorous
Badalamenti *et al*., 2008	*Mullus barbatus*	3.60	0.39	6.00	-	-	-	Demersal	Carnivorous
Badalamenti *et al*., 2002	*Mullus barbatus*	-	-	-	3.61	0.41	6.00	Demersal	Carnivorous
Badalamenti *et al*., 2008	*Mullus barbatus*	3.82	0.16	6.00	-	-	-	Demersal	Carnivorous
Badalamenti *et al*., 2008	*Mullus barbatus*	-	-	-	3.62	0.19	6.00	Demersal	Carnivorous
Badalamenti *et al*., 2008	*Mullus barbatus*	3.84	0.51	6.00	-	-	-	Demersal	Carnivorous
Badalamenti *et al*., 2008	*Mullus barbatus*	-	-	-	3.67	0.19	6.00	Demersal	Carnivorous
Badalamenti *et al*., 2008	*Mullus barbatus*	3.88	0.08	6.00	-	-	-	Demersal	Carnivorous
Badalamenti *et al*., 2008	*Mullus barbatus*	-	-	-	3.70	0.57	6.00	Demersal	Carnivorous
Badalamenti *et al*., 2008	*Mullus barbatus*	3.49	0.38	6.00	-	-	-	Demersal	Carnivorous
Badalamenti *et al*., 2002	*Mullus barbatus*	-	-	-	3.72	0.10	74.00	Demersal	Carnivorous
Badalamenti *et al*., 2008	*Mullus barbatus*	3.58	0.26	6.00	-	-	-	Demersal	Carnivorous
Badalamenti *et al*., 2002	*Mullus barbatus*	-	-	-	3.72	0.10	74.00	Demersal	Carnivorous
-	*Mullus barbatus*	3.70	0.30	6.00	-	-	-	-	-
Badalamenti *et al*., 2008	*Mullus barbatus*	-	-	-	3.76	0.35	6.00	Demersal	Carnivorous
-	*Mullus barbatus*	3.70	0.30	6.00	-	-	-	-	-
Bautista-Vega *et al*., 2008	*Mullus barbatus*	-	-	-	3.82	0.62	62.00	Littoral	Carnivorous
-	*Mullus barbatus*	3.70	0.30	6.00	-	-	-	-	-
Bautista-Vega *et al*., 2008	*Mullus barbatus*	-	-	-	3.83	0.81	78.00	Littoral	Carnivorous
-	*Mullus barbatus*	3.70	0.30	6.00	-	-	-	-	-
Bautista-Vega *et al*., 2008	*Mullus barbatus*	-	-	-	3.87	1.53	46.00	Littoral	Carnivorous
-	*Mullus barbatus*	3.70	0.30	6.00	-	-	-	-	-
Bautista-Vega *et al*., 2008	*Mullus barbatus*	-	-	-	3.88	1.01	47.00	Littoral	Carnivorous
-	*Mullus barbatus*	3.70	0.30	6.00	-	-	-	-	-
Carlier *et al*., 2007	*Mullus barbatus*	-	-	-	3.96	0.50	3.00	Littoral	Carnivorous
-	*Mullus barbatus*	3.70	0.30	6.00	-	-	-	-	-
Badalamenti *et al*., 2008	*Mullus barbatus*	-	-	-	3.16	0.07	6.00	Coastal Lagoon	Carnivorous
Vizzini & Mazzola, 2008	*Mullus surmuletus*	2.93	0.40	19.00	-	-	-	Littoral	Carnivorous
Bautista-Vega *et al*., 2008	*Mullus surmuletus*	-	-	-	3.77	0.39	44.00	Littoral	Carnivorous
Vizzini & Mazzola, 2008	*Mullus surmuletus*	2.96	0.20	38.00	-	-	-	Littoral	Carnivorous
Bautista-Vega *et al*., 2008	*Mullus surmuletus*	-	-	-	3.83	0.96	35.00	Littoral	Carnivorous
Vizzini & Mazzola, 2008	*Mullus surmuletus*	3.10	0.30	33.00	-	-	-	Littoral	Carnivorous
Bautista-Vega *et al*., 2008	*Mullus surmuletus*	-	-	-	3.88	0.70	37.00	Littoral	Carnivorous
-	*Mullus surmuletus*	3.00	0.30	30.00	-	-	-	-	-
Cardona *et al*., 2007	*Mullus surmuletus*	-	-	-	3.90	1.20	10.00	Littoral	Carnivorous
-	*Mullus surmuletus*	3.00	0.30	30.00	-	-	-	-	-
Bautista-Vega *et al*., 2008	*Mullus surmuletus*	-	-	-	3.91	1.06	35.00	Littoral	Carnivorous
-	*Mullus surmuletus*	3.00	0.30	30.00	-	-	-	-	-
Magni *et al*., 2008	*Mullus surmuletus*	-	-	-	4.46	0.20	4.00	Coastal Lagoon	Carnivorous
-	*Mullus surmuletus*	3.00	0.30	30.00	-	-	-	-	-
Pinnegar *et al*., 2000	*Mullus surmuletus*	-	-	-	2.73	0.17	3.00	Littoral	Carnivorous

(continued)

Table 1 *(continued)*

		No-take areas			Take areas				
Author	**Species**	**TL δ^{15}N**	**SD**	**n samples**	**TL δ^{15}N**	**SD**	**n samples**	**Habitat**	**Diet (fishbase)**
-	*Mullus surmuletus*	3.00	0.30	30.00	-	-	-	-	-
Deudero *et al.*, 2004	*Mullus surmuletus*	-	-	-	3.38	0.03	1.00	Littoral	Carnivorous
-	*Mullus surmuletus*	3.00	0.30	30.00	-	-	-	-	-
Deudero *et al.*, 2004	*Mullus surmuletus*	-	-	-	3.59	0.14	1.00	Littoral	Carnivorous
Vizzini & Mazzola, 2008	*Serranus cabrilla*	2.99	0.30	43.00	-	-	-	Littoral	Piscivorous
Vizzini & Mazzola, 2006	*Serranus cabrilla*	-	-	-	3.60	0.30	1.00	Littoral	Piscivorous
Vizzini & Mazzola, 2008	*Serranus cabrilla*	3.13	0.20	41.00	-	-	-	Littoral	Piscivorous
Vizzini & Mazzola, 2006	*Serranus cabrilla*	-	-	-	3.13	0.20	41.00	Littoral	Piscivorous
Vizzini & Mazzola, 2008	*Serranus cabrilla*	3.16	0.30	4.00	-	-	-	Littoral	Piscivorous
Vizzini & Mazzola, 2006	*Serranus cabrilla*	-	-	-	3.51	0.40	1.00	Littoral	Piscivorous
Vizzini & Mazzola, 2008	*Serranus cabrilla*	3.25	0.30	35.00	-	-	-	Littoral	Piscivorous
-	*Serranus cabrilla*	-	-	-	3.42	0.30	14.33	-	-
Vizzini & Mazzola, 2008	*Serranus scriba*	3.10	0.20	8.00	-	-	-	Littoral	Piscivorous
Jennings *et al.*, 1997	*Serranus scriba*	-	-	-	4.04	0.29	6.00	Littoral	Piscivorous
Vizzini & Mazzola, 2008	*Serranus scriba*	3.25	0.20	9.00	-	-	-	Littoral	Piscivorous
Cardona *et al.*, 2007	*Serranus scriba*	-	-	-	4.37	0.40	10.00	Littoral	Piscivorous
Vizzini & Mazzola, 2008	*Serranus scriba*	3.28	0.10	35.00	-	-	-	Littoral	Piscivorous
Jennings *et al.*, 1997	*Serranus scriba*	-	-	-	4.51	0.23	6.00	Littoral	Piscivorous
Pinnegar *et al.*, 2000	*Serranus scriba*	3.31	0.20	13.00	-	-	-	Littoral	Piscivorous
Jennings *et al.*, 1997	*Serranus scriba*	-	-	-	4.57	0.17	6.00	Littoral	Piscivorous
-	*Serranus scriba*	3.24	0.18	16.25	-	-	-	-	-
Vizzini & Mazzola, 2008	*Serranus scriba*	-	-	-	3.31	0.20	3.00	Littoral	Piscivorous
-	*Serranus scriba*	3.24	0.18	16.25	-	-	-	-	-
Deudero *et al.*, 2004	*Serranus scriba*	-	-	-	3.44	0.17	10.00	Littoral	Piscivorous
-	*Serranus scriba*	3.24	0.18	16.25	-	-	-	-	-
Deudero *et al.*, 2004	*Serranus scriba*	-	-	-	3.46	0.33	1.00	Littoral	Piscivorous
Vizzini & Mazzola, 2008	*Spicara maena*	2.78	0.10	39.00	-	-	-	Littoral	Carnivorous
Pinnegar *et al.*, 2000	*Spicara maena*	-	-	-	2.73	0.32	3.00	Littoral	Carnivorous
Vizzini & Mazzola, 2008	*Spicara maena*	2.78	0.10	39.00	-	-	-	Littoral	Carnivorous
-	*Spicara maena*	-	-	-	2.73	0.32	3.00	-	-
Vizzini & Mazzola, 2008	*Spicara maena*	2.84	0.10	18.00	-	-	-	Littoral	Carnivorous
-	*Spicara maena*	-	-	-	2.73	0.32	3.00	-	-
Vizzini & Mazzola, 2008	*Symphodus ocellatus*	3.22	0.60	6.00	-	-	-	Littoral	Omnivorous
Vizzini *et al.*, 2002	*Symphodus ocellatus*	-	-	-	2.54	0.10	8.00	Coastal Lagoon	Omnivorous
-	*Symphodus ocellatus*	3.22	0.60	6.00	-	-	-	-	-
Pinnegar *et al.*, 2000	*Symphodus ocellatus*	-	-	-	3.29	0.23	3.00	Littoral	Omnivorous
Vizzini & Mazzola, 2008	*Symphodus tinca*	2.96	0.40	68.00	-	-	-	Littoral	Carnivorous
Jennings *et al.*, 1997	*Symphodus tinca*	-	-	-	3.84	0.86	6.00	Littoral	Carnivorous
Vizzini & Mazzola, 2008	*Symphodus tinca*	2.99	0.50	38.00	-	-	-	Littoral	Carnivorous
Cardona *et al.*, 2007	*Symphodus tinca*	-	-	-	3.99	0.40	10.00	Littoral	Carnivorous
Vizzini & Mazzola, 2008	*Symphodus tinca*	3.01	0.40	10.00	-	-	-	Littoral	Carnivorous
Jennings *et al.*, 1997	*Symphodus tinca*	-	-	-	4.04	0.14	6.00	Littoral	Carnivorous
Vizzini & Mazzola, 2008	*Symphodus tinca*	3.40	0.30	88.00	-	-	-	Littoral	Carnivorous
Jennings *et al.*, 1997	*Symphodus tinca*	-	-	-	4.10	1.42	6.00	Littoral	Carnivorous
-	*Symphodus tinca*	3.09	0.40	51.00	-	-	-	-	-
Pinnegar *et al.*, 2000	*Symphodus tinca*	-	-	-	3.13	0.40	3.00	Littoral	Carnivorous

$$\bar{e}_k = \frac{\sum_{i=1}^{n_k} w_i e_i}{\sum_{i=1}^{n_k} w_i}$$

where n_k is the number of studies for consideration and e_i and w_i are defined above. The fixed effect model was used since, among variance studies, it is lower than 0 and therefore, relative weights for the fixed effect model are more balanced than those for the random effect model. Total heterogeneity was obtained according to Hedges & Olkin (1985):

$$Q_{T_k} = \sum_{i=1}^{n_k} w_i \left(e_i - \bar{e}_k\right)^2$$

and the corresponding significance is tested against X^2 distributions with n_k-*1* degrees of freedom. The meta-analysis was performed using R and Excel software packages.

The equation was proposed as a method to quantify heterogeneity and it is expressed in percentage of the total variability in a set of size effects due to true heterogeneity, that is, between-studies variability (Higgins & Thompson, 2002).

$$I^2 = \left(\frac{(Q - df)}{Q}\right) \times 100$$

where *Q* stands for "heterogeneity" and stands for "degrees of freedom".

Results

The results indicate significant differences between fish trophic levels inside and outside MPAs. However, those differences differ from initial expectations, with higher TL outside MPAs despite the protection level (Fig. 1).

Total heterogeneity was obtained according to Hedges & Olkin (1985) by applying Q test measures heterogeneity among studies (Qt = 1638.93, p < 0.001, df = 145). The result points out the corresponding size effects were heterogeneous among the studies.

The heterogeneity (I^2) calculated in this meta-analysis was 98.71%. This result suggests that mean differences of δ^{15}N-fish varied more between protected areas and non-protected areas than could be expected by random error alone.

The final dataset used consists of eleven species and the covariate selected was the level of protection (inside *vs*. outside MPAs). The meta-analysis provided the following results on the carnivorous species: *Coris julis* (ei = -0.055 ± 0.019, 95% CI, n = 145), *Diplodus annularis* (ei = -0.195 ± 0.023, 95% CI, n = 135), *Mullus barbatus* (ei = -0.002 ± 0.004, 95% CI, n = 498), *Mullus surmuletus* (ei = -0.207 ± 0.018, 95% CI, n = 440), *Spicara maena* (ei= 0.026±0.000, 95% CI n=105), *Symphodus tinca* (ei= -0.209±0.013, 95% CI, n=286); omnivorous species: *Boops boops* (ei= -0.055±0.019, 95% CI, n=145), *Symphodus ocellatus* (ei= 0.107±0.032, 95% CI, n=23); piscivorous species: *Merluccius merluccius* (ei= 0.010±0.000, 95% CI, n=50), *Serranus cabrilla* (ei=-0.085±0.006, 95% CI, n=180) and *Serranus scriba* (ei= -0.193±0.018, 95% CI, n=155). Significant size effect was found in all the fish species. In order to test habitat effects on the trophic level, the species were classified by habitat according to literature (Table 1). The meta-analysis showed significant size effect at all the habitats considered: coastal lagoons (ei = 0.037 ± 0.004, 95% CI, n = 5), demersal (ei= 0.006±0.001, 95% CI, n=41) and littoral (ei= -0.144±0.004, 95% CI, n= 100). The results exhibit higher trophic level in demersal areas than in littoral and coastal lagoons (Fig. 2).

Similarly, to evaluate trophic changes at MPAs, the species were classified by trophic functional groups (Table 1) (Fig.3): carnivorous (ei= -0.126±0.003, 95% CI, n= 86), omnivorous (ei= -0.001±0.008, 95% CI, n= 12) and piscivorous (ei= -0.065±0.001, 95% CI, n= 48). No significant effect was found in omnivorous species.

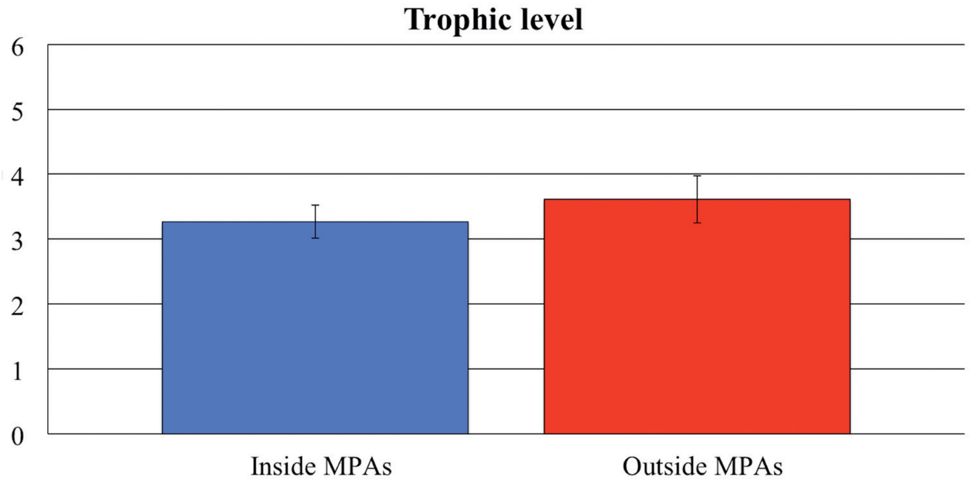

Fig. 1: Mean fish trophic level based on stable isotopes (±SD) inside and outside Marine Protected Areas.

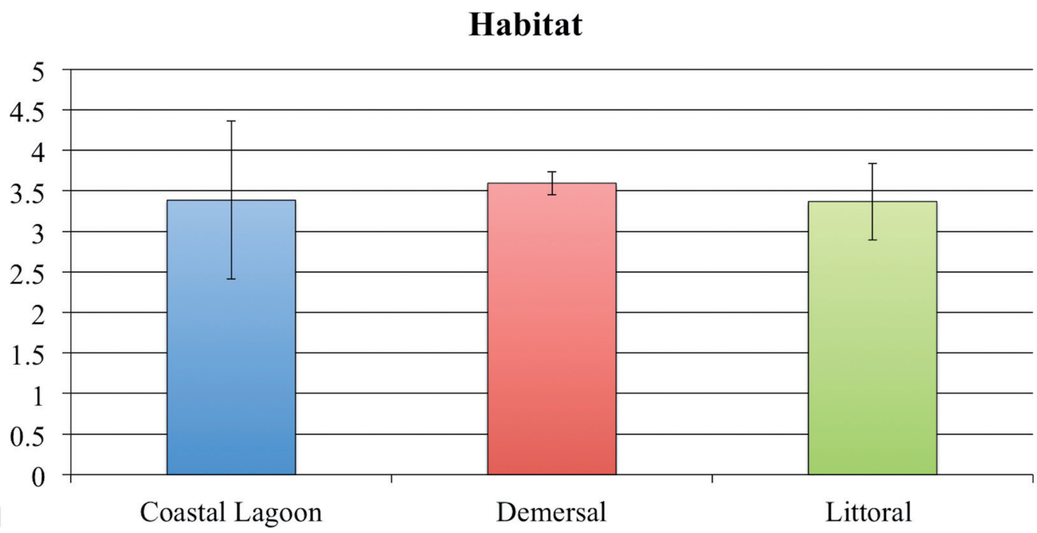

Fig. 2: Mean fish trophic level (± SD) calculated by habitat derived from fish stable isotope data.

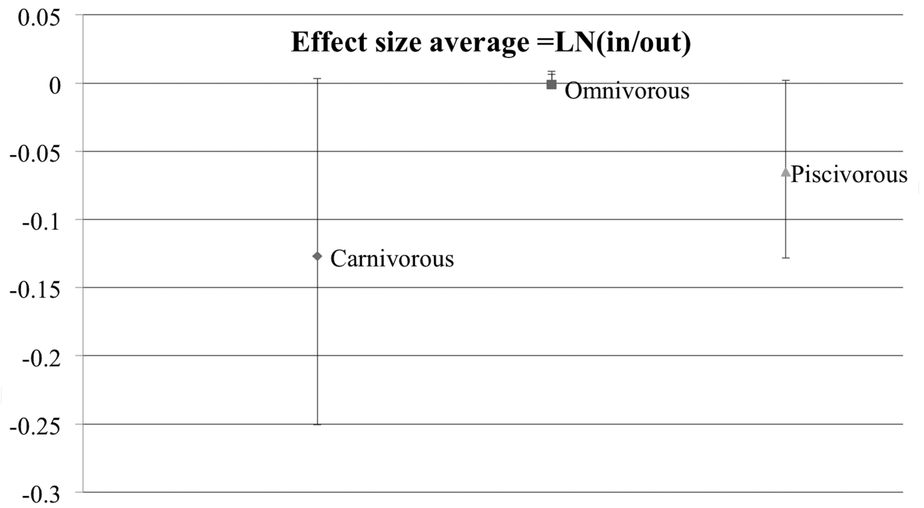

Fig. 3: Size effect average (± SD) by trophic functional groups of the fish species. No significant size effect has been found.

Discussion

The size effect for protection effects exhibits higher trophic levels outside Marine Protection Areas in spite of protection (Fig. 1). The initial hypothesis holds that inside MPAs with full protection, species have food resources available to achieve the maximum trophic level within the food web for each of the fish species considered. However, outside MPAs, species do not have plentiful food resources available because of fishing and overfishing effects so their trophic level is lower. The increased mortality caused by fishing changes both diet and trophic level (Pope & Knights, 1982). Also, a reduction in the average fish size associated with fishing could increase the apparent decline in the mean trophic level (Pauly *et al.*, 2000) and even change the trophic structure globally (Christensen *et al.*, 2014).

There is extensive literature about effects inside MPAs documenting increased density, biomass, individual size, and diversity in all functional groups (Dufour *et al.*, 1995; Halpern & Warner, 2002; Halpern, 2003), increased abundance and richness of fishes (Rowley, 1994; Bohnsack, 1998; Barrett *et al.*, 2007), improvement in the conservation and restoration of population and marine habitats (Fox *et al.*, 2012), avoidance of depletion of high trophic level populations due to fishing, and recovery of natural ecological structures (Jones, 2014) and management, education, and research issues (Angulo-Valdés & Hatcher, 2010). However, there is not much literature about how the establishment of MPAs modifies the species trophic level based on stable isotopes. Badalamenti *et al*. (2002) reported an increase of trophic level (based on $\delta^{15}N$) of some fish species (*M. merluccius*, *M. barbatus* and *Lophius budegassa*) after nine years of no-trawling at the Gulf of Castellammare.

Our overall results indicate that the trophic level does not increase with the presence of the protection itself. This conclusion is in line with previous studies indicating that trophic level does not necessarily increase with protection and in some cases it can even decrease (Deudero *et al.*, 2004; Vizzini & Mazzola, 2009; Sinopoli *et al.*, 2012).

The establishment of MPAs provides benefits within MPAs borders but also to adjacent no-take areas. For example, there are increases of fish abundance (Ojeda-Martinez *et al.,* 2007), density and species richness (Russ & Alcala, 1996) linked to the "spill-over effect" (Harmelin-Vivien *et al.*, 2008; Stobart *et al.*, 2009; Chateau & Wantiez, 2009), and/or a surplus of fish available in adjacent areas (Roberts & Polunin, 1991; DeMartini, 1993; McClanahan & Magni, 2000). However, no studies have been found to support the fact that trophic levels could be higher outside MPAs than inside in spite of protection, as this study concludes.

Protection Level

In this study, there is a wide array of protection status at the studied MPAs: fishing is prohibited in Columbretes and some parts of "Egadi Islands", trawling is prohibited in some parts of "Egadi Island" and Gulf of Castellammare, and professional fishing and recreational marine fishing are under regulation in Cala Ratjada. The different fishing types developed inside the MPAs could have indirect effects on trophic structures (Pauly *et al.*, 1998) and alter the sources of primary production (Kaiser & Spencer, 1994) with implications for prey (Pinnegar *et al.*, 2000). Each type of fishing is aimed at capturing a specific type of organism, which occupies a different position within the food chain (Stergiou *et al.*, 2004). For example, angling aims at fishing for larger species of a higher trophic level, while trawling, purse or dredging are directed towards catching smaller species placed at lower trophic levels, such as shellfish and crustaceans. These types of fishing have increased in the last 50 years, with a consequent reduction in both sizes and trophic level of the catches (Watson *et al.*, 2006).

In areas where fishing is restricted the target species increase both their size and biomass (Roberts & Polunin, 1991). For instance, piscivorous species (predators) prey on small fishes, decreasing both their numbers and sizes and, this in turn, causes an increase in invertebrate species at the base of the food chain. Therefore, one would expect a higher trophic level where fishing is restricted than in fishing areas (Pauly *et al.*, 2001; Pinnegar *et al.*, 2002). In the case of Mallorca Island, sport fishing impacts coastal fish communities: Morales-Nin *et al.* (2005) estimated that recreational fisheries exerted an impact on the majority of species within trophic level 4 (31%), representing at least 1209.25 tons/year catches. This might be a feasible explanation of the results obtained in this study, related to a low protection level in the MPAs analysed.

Establishment of MPAs

Other factors regarding processes at MPAs affecting life history and ecology of marine species are dependent on time since establishment of the MPAs and the size of the no-take zone (Claudet *et al.*, 2008). There are several analyses where the "reserve effect" emerges during different periods of time. For example, after the first year an increase in biomass was observed (Russ & Alcala, 1996); during the first three years of protection, increases in density, biomass, and diversity occur (Halpern & Warner, 2002) with a significant increase in density, species richness, and size of the target (Seytre & Francour, 2008). Six years after MPA establishment, significant differences were found in total abundance, species richness, and diversity on species such as *S. cabrilla* and *C. julis* (Claudet *et al.*, 2006); 5-10 years after protection, the "reserve effect" emerges (Gell & Roberts, 2003) and MPAs are more effective when maintained for at least 15 years following establishment (Molloy *et al.*, 2009).

In our study, several temporal scenarios in MPA designation are included. Thus, "Egadi Islands" were established in 1991, Gulf of Castellammare was established in 1990, Columbretes were established in 1990, and Cala Ratjada was established in 2007. Some studies reported increase abundance, biomass, mean body size, and trophic level (based on stomach content) after 8 - 16 years of no fishing at Columbretes (Stobart *et al.*, 2009). Therefore, contrasting responses at temporal scales seems to be relevant from studies including 15 years of effective protection.

The trophic level responses of fish at MPAs are in agreement with previous research from Claudet *et al.* (2008) that exhibited no effect on economic value, body size, habitat, depth range and schooling behaviour of several species at MPAs in 12 locations of the Mediterranean Sea. Similarly, Tunesi *et al.* (2006) did not record a clear "reserve effect" after five years of protection in spite of significant differences in the size of the target species. That lack of results could be due to the critical lack of empirical knowledge about MPAs and the unsuccessful conservation measures made (Planes *et al.*, 2000; Fraschetti *et al.*, 2002; Claudet & Fraschetti, 2010), or a lack of knowledge in important scientific facts such as knowledge about dispersal schemes, the geographical range of the population, an appropriate experimental design, and environmental evaluation (Claudet & Pelletier, 2004). Establishment of appropriate MPA designation within the proper context such as a large-scale marine spatial planning has to be made (Agardy *et al.*, 2011).

Food sources

Habitat effects on the trophic levels have been analysed. Our results showed significant size effect in all the habitats considered (Fig.2). However, in our analyses, demersal habitats have a trophic level higher than coastal lagoons and littoral areas, which have similar trophic levels.

The species found in demersal areas are *M. merluccius* and *M. barbatus*. *Mullus barbatus* is carnivorous, feeding mainly on benthic invertebrates (Labropoulou &

Eleftheriou, 1997) such as the shrimp *Leptochela pugnax* (Golani & Galil, 1991). During its life cycle an increase in consumption of polychaetes and shrimps with predator size occurs along with a decrease in the smaller crustaceans consumption (Bautista-Vega *et al.*, 2008). *Merluccius merluccius* is piscivorous but juveniles feed on small crustaceans such as euphausiids and mysids (Bozzano *et al.*, 1997), shifting to fish prey when older as *M. merluccius*, *Argentina sphyraena*, *Spicara flexuosa*, *Centracanthidae*, *M. barbatus* (Carpentieri et *al.*, 2005). Both fish species *Merluccius* and *Mullus* feed on organisms with high nitrogen content (Bautista-Vega *et al.*, 2008; Froese & Pauly, 2014), explaining the higher trophic level than species that live in littoral habitats and coastal lagoons.

Littoral habitats and coastal lagoons exhibit high fish trophic levels (Fig. 2) since both habitats are highly influenced by many human activities impacting the functioning of coastal marine ecosystems. Due to human influences the inputs of nutrients with $\delta^{15}N$ from sewage and aquaculture facilities are variable (Vizzini & Mazzola, 2004). Studies of the largest sources of nitrogen (air, fertilizer, soil, and waste water) show that waste water has the higher content $\delta^{15}N$ (the modal value of >15 ‰) compared to other sources with lower values of $\delta^{15}N$ (between -5 ‰ and 5 ‰). All these nitrogen loads combined with fraction occurring during the ammonification processes (Cabana & Rasmussen, 1996) increase nutrients and organic matter in the ecosystem, resulting in eutrophication. Eutrophication may cause a reduction in species richness and diversity and indirectly change the trophic level occupied by each species in the system. Enrichment of $\delta^{15}N$ is significantly higher in coastal areas than in marine ecosystems due to anthropogenic influences (McClelland & Valiela, 1998; McGhie *et al.*, 2000; Umezawa *et al.*, 2002). The rise in available nitrogen makes $\delta^{15}N$ values increase in all benthic primary producers occurring during the ammonification processes and in the main herbivorous species, such as *Paracentrotus lividus* and *Sarpa salpa* (Prado *et al.*, 2009).

The trophic level can be influenced both by species diet and by human-derived activities. The lack of knowledge about the appropriate determination of the food sources represents limitations of trophic level estimation by stable isotope analysis. The uncertainty about species diet could limit trophic level estimation due to the fact that diet is a direct reflection on the trophic level. For this reason, the combined use of stable isotopes and stomach analysis could decrease this uncertainty (Ramsvatn, 2013; Shannon *et al.*, 2014).

Among the aims of this study the size effect change of trophic functional groups was assessed (Fig.3). Omnivorous species did not show significant size effect (*B. boops* and *S. ocellatus*), linked to the generalist diet of omnivorous species. Accordingly, the diet of *B. boops* is composed of zooplanktonic crustaceans (copepods), fish eggs, gastropods, macroalgae, *Posidonia oceanica* (Cresson *et al.*, 2014), and *S. ocellatus* feeds on zoobenthos (gastropods, polychaetes, copepods), plants, and zooplankton (mainly fish eggs and larvae) (Froese & Pauly, 2014). Due to their diet, higher wide trophic levels could be expected in these species than in piscivorous species. Resource limitation outside the MPAs due to fishing and overfishing along with reserve effect within MPA boundaries could explain the lower trophic level recorded outside the MPA.

Life cycle

In this meta-analysis study, different trophic levels associated with fish species have been analysed, including different fish sizes. Size is a factor that might influence the species trophic level, linked to ontogenetic changes in fish diets and increasing TL with fish size (du Buit, 1995; Pinnegar *et al.*, 2003; Sarà & Sarà, 2007). The body mass of fish can increase by five times during their life cycle, and the trophic level increases with body size during ontogeny (Jennings *et al.*, 2002). Also, mean trophic level measured over time and space and its high variability may mask the potential of trophic level associated with the dynamics of the food chain (Greenstreet *et al.*, 1997) and ontogenetic change. Moreover, the trophic level of a species can be altered by the top-down and bottom-up dynamics within the trophic structure of an ecosystem (Devaraj, 2004; Shackell *et al.*, 2010).

$\delta^{15}N$ as direct indicator of trophic level

In this investigation, $\delta^{15}N$ has been considered as a direct indicator of trophic level; however, based on our results combined with published studies (Vanderklint & Ponsard, 2003; Schmidt *et al.*, 2004; Mancinelli *et al.*, 2013), other factors may bias trophic level interpretation. Thus, the isotopic fractionation should be considered. For example, with isotopic fractionation associated with herbivorous species, some studies show an increase higher than 3.4 ‰ relative to their prey, due to differences found in enzymatic and digestive systems of herbivorous species (Mill *et al.*, 2007; Wyatt *et al.*, 2010). Olive *et al.* (2003) indicated trophic level may be distorted by the feeding ratio, the nitrogen content of food, excretion rate, and assimilation efficiency.

The present study has showed differences in fish trophic level from stable isotope data obtained from the literature. Higher fish trophic levels are observed outside MPAs, indicating that many factors are involved in trophic responses and that protection by itself cannot guarantee the increase in food resources for fish species. The suitability of using the stable isotope ($\delta^{15}N$) as a direct indicator of trophic level in evaluating MPA effects on food webs should be considered carefully.

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