Comparison of ecosystem modelling and isotopic approach as ecological tools to investigate food webs in the NW Mediterranean Sea

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Abstract

Understanding how marine ecosystems are structured and how they function is a scientific challenge. Nowadays, to learn about ecosystem trophic dynamics ecologists use two complementary methodological approximations, mass-balance ecosystem models and isotopic tools. However, despite the wide applications of these methodologies, the comparison and combination of both are still scarce. The aim of this study was to examine the trophic dynamics of representative species of the South Catalan marine ecosystem (North-western Mediterranean) using the Ecopath ecosystem modelling tool and the stable isotope approach. By using the two methodologies we depicted the trophic position (trophic level and $\delta^{15}\text{N}$ values) and trophic width (omnivory index and total isotopic area) of several species of fish, cephalopods, cetaceans, seabirds and one sea turtle. Our results show a clear correlation between the trophic levels estimated by the Ecopath model and the $\delta^{15}\text{N}$ values, which validate the trophic position of several species in the study area and indicate that both methodologies are useful to determine the trophic position of marine species in Mediterranean marine food webs. In contrast, the two estimators of trophic width (the omnivore index and the total isotopic area) were not strongly related, since the relationship between modelling and stable isotope results was evident only for some species. Further comparisons of trophic width calculated in other marine food webs may provide a better understanding of our results and validate the accuracy of both methodological approaches to calculate trophic width.

Keywords: Ecopath model, ecosystem model, marine food-webs, marine predators, trophic position, stable isotopes.

1. Introduction

Marine food webs are subjected to anthropogenic and environmental disturbances that can impact ecosystem structure and functioning, and thus affect natural resource availability (e.g. Jackson et al., 2001; Lotze et al., 2006). To progress towards an understanding on how marine ecosystems are structured is a fundamental but also challenging scientific task. In this context, ecologists are now focused on novel ecosystem models or biogeochemical approximations that complement classical approaches representing marine food webs and are very useful not only to study the structure of ecosystems, but also to detect, prevent and mitigate environmental and human changes (e.g. Christensen et al. 2004; Peterson and Fry, 1987).

Ecosystem models are particularly useful because they allow the study of marine ecosystems as a whole (Plagányi, 2007; Fulton, 2010). For example, the Ecopath modelling approach, a mass-balance model integrated in the Ecopath with Ecosim software (Christensen and Walters 2004) that provides a representation of the food web, can be used to depict the structure and functioning of marine ecosystem and calculate different ecological indicators (Pauly et al., 2000; Christensen and Walters, 2004). Based on this methodology, we can estimate the trophic levels of species or functional groups included in the model. We can also measure how specialist or generalist these species or groups are through the calculation of the omnivory index (Christensen and Walters, 2004; Libralato, 2008).

The use of intrinsic markers such the stable isotopes (SIA) of nitrogen ($^{15}\text{N}/^{14}\text{N}$, denoted as $\delta^{15}\text{N}$) and carbon ($^{13}\text{C}/^{12}\text{C}$, denoted as $\delta^{13}\text{C}$) are widely used as dietary tracers and can depict trophic position and trophic habits of species (Peterson and Fry, 1987; Kelly, 2000; Layman et al., 2007; Newsome et al., 2007). This approach is based on the fact that $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values are transformed from dietary sources to consumers in a predictable manner (Peterson and Fry, 1987; Kelly, 2000). Nitrogen isotopic values show a predictable

77 increase in the isotopic ratio throughout the trophic levels, i.e., typically from 2.5‰ to 5‰
78 (Post, 2002; Kelly, 2004). Carbon isotopic values show little change with trophic transfers,
79 but are useful indicator of the dietary source of carbon (De Niro and Epstein, 1981).

80 Despite the extensive use of both mass-balance models and SIA approaches, the
81 comparison and combination of both approaches in ecological studies are still scarce (but
82 see Kline and Pauly, 1998 and Dame and Christian, 2008). The application of both
83 methodologies simultaneously could provide more accurate information of the structure and
84 functioning of marine food webs and be a useful tool to validate results. One of the most
85 common comparative studies is the calculation of trophic levels from both methodologies;
86 however, to our knowledge, comparison of trophic width or omnivory index calculated from
87 these two methodologies has not yet been pursued. Moreover, although comparative studies
88 have been done including different functional groups from marine systems, top predators
89 such as cetaceans or seabirds have not been included (e.g. Nilsen et al., 2008).

90 The Mediterranean Sea has been subjected to several quantitative studies on trophic
91 ecology and ecosystem modelling (e.g. Polunin et al., 2001; Coll and Libralato, 2009;
92 Forero and Hobson, 2003). A highly studied Mediterranean marine area is the South
93 Catalan Sea, in the NW Mediterranean (Fig. 1), where various Ecopath models have recently
94 been developed (Coll et al., 2006; Coll et al., 2008). These models revealed that the
95 Southern Catalan Sea supports a complex food web with a strong link between the pelagic
96 and the benthic compartments (Fig. 2; Coll et al., 2006; Coll et al., 2008). In addition,
97 several isotopic studies have investigated the trophic ecology of different organisms in this
98 area including fishes, seabirds, cetaceans or marine turtles (e.g. Cardona et al., 2007; Ramos
99 et al., 2009; Navarro et al., 2009; Navarro et al., 2010). Due to the large amount of
100 information available about the structure and functioning of the food web in this marine
101 ecosystem, the South Catalan Sea provides an excellent case study to analyse similarities
102 and discrepancies between ecosystem modelling and stable isotope methodologies.

Therefore, the aim of the present study was to analyse the trophic structure of several representative species present in the food web of the South Catalan marine ecosystem by using the two complementary approaches: Ecopath modelling and stable isotope analysis. Specifically, we compared the results for trophic position (trophic level and $\delta^{15}\text{N}$ values, from Ecopath and SIA, respectively) and of the trophic width (omnivore index and total isotopic area, from Ecopath and SIA, respectively). We focussed on predatory species of this marine ecosystem including several fishes, cephalopods, cetaceans, marine turtles and seabirds (Table 1).

2. Material and methods

2.1. Study area and species

The South Catalan Sea comprises the continental shelf and upper slope associated with the Ebro River Delta (Fig. 1). The area used for the modelling exercises has a depth range between 50 m and 400 m and covers a total area of soft bottom sediments of 4500 km². Generally this region is oligotrophic, where nutrient enrichment occurs due to regional environmental events related to wind episodes, the conditions of a temporal thermocline, and a shelf slope current and river discharges (Salat, 1996).

We focused our study in 24 representative species on this ecosystem, including 16 fishes (anchovy *Engraulis encrasicolus*, blackbelly rosefish *Helicolenus dactylopterus*, blotched picarel *Spicara flexuosa*, blue whiting *Micromesistius poutassou*, bluefin tuna *Thunnus thynnus*, boarfish *Capros aper*, European hake *Merluccius merluccius*, glasshead grenadier *Hymenocephalus italicus*, greater forkbeard *Phycis blennoides*, jewel lanternfish *Lampanyctus crocodilus*, pilchard *Sardina pilchardus*, poor cod *Trisopterus minutus*, red bandfish *Cepola macrophthalma*, shortnose greeneye *Chlorophthalmus agassizi*, silvery cod *Gadiculus argenteus*, and tonguesole *Symphurus nigrescens*) and 2 cephalopods (common

bobtail *Sepieta oweniana* and common squid *Allotheuthis subulata*), 2 cetaceans (bottlenose dolphin *Tursiops truncatus* and striped dolphin *Stenella coeruleoalba*), 3 seabird species (Audouin's gull *Larus audouinii*, Balearic shearwater *Puffinus mauretanicus*, and yellow-legged gull *Larus michahellis*), and 1 marine turtle (loggerhead sea-turtle *Caretta caretta*) (Table 1).

2.2. Mass-balance ecosystem model

Ecopath is the mass-balance routine of Ecopath with Ecosim software that allows building averaged trophic models of food webs (e.g. Polovina, 1984; Pauly et al., 2000; Christensen and Walters, 2004; Christensen et al., 2005). This modelling tool describes the ecosystem considering functional groups, each representing a species, a sub-group of a species or a group of several species that have functional and ecological similarities. The basic routine of Ecopath describes the balance between production of the functional groups and consumption flows, including fishing activities. A detail description of the main equations of the Ecopath model is described in Annex 1.

In the present study, we modified a recently developed mass-balance model that originally represented the Southern Catalan ecosystem during the mid-late 1990s (details are fully described in Coll et al. 2006). This model had 40 functional groups and was calibrated with time series data using independent fishing effort and fishing mortality, biomass and catch data from 1978 to 2003 to assess the capability of the model to hindcast ecosystem dynamics (Coll et al. 2008). Detailed information on the model development and data limitations is provided in Coll et al. (2006, 2008).

Here we modified the 1990s model to explicitly separate those species with detailed information from stable isotopes (see following section) that were included as individual functional groups in the model. To do so we used the information available regarding diet composition, biomass, catch, production/biomass, and consumption/biomass ratios for those

species (see full references in Coll et al., 2006, and specifically Macpherson, 1981; Würtz and Marrale, 1993; Bosch et al., 1994; Orsi Relini et al., 1995; Oro et al., 1997; Granadeiro et al., 1998; Arcos, 2001; Blanco et al., 2001). The modified version of the model was initially balanced so no further modifications were performed.

Once the South Catalan Sea model was modified, we analysed the trophic levels (TL_i) by functional group. The trophic level (TL_i) was first defined as an integer identifying the position of organisms within food webs (Lindeman, 1942) and it was later modified to be fractional (Odum & Heald 1975). Following an established convention, a TL of 1 is given to primary producers and detritus and the TL can be formulated as follows:

$$TL_j = 1 + \sum_{i=1}^n DC_{ji} \cdot TL_i \quad (1)$$

where j is the predator of prey i , DC_{ji} is the fraction of prey i in the diet of predator j and TL_i is the trophic level of prey i .

Ecopath models also allow calculating the Omnivory Index (OI_i), a measure of the distribution of feeding interactions among trophic levels by a species or functional group (Christensen and Walters, 2004; Libralato, 2008). The OI_i is calculated as the variance of TL of the preys j :

$$OI_i = \sum_{j=1}^n \left[TL_j - (TL_j - 1) \right]^2 \cdot DC_{ij} \quad (2)$$

where the contribution of each prey j to the variance of the consumer i is a proportion of the fraction of prey j in the diet of consumer i (DC_{ij}). When the OI_i is zero, the consumer is specialized, i.e. it feeds on a single trophic level. A high value of OI_i indicates that the consumer feeds on many trophic levels and thus shows a more generalist strategy. The OI is dimensionless and the square root of the OI represents the standard error of the TL (i.e. a

measure of the *TL*'s uncertainty about the *TL* due to omnivory and sampling variability, Christensen and Walters, 2004; Libralato, 2008).

2.3. Stable isotope data

We used published isotopic data ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values) from 15 species (Table 1; Borell et al., 2006; Cardona et al., 2007; Revelles et al., 2007; Ramos et al., 2009; Navarro et al., 2009; Navarro et al., 2010). In addition to these data, we performed stable isotopic analysis on 7 fish species and 2 species of cephalopods (Table 1). Samples for these species were collected during May 2003 from an experimental oceanographic trawling cruise along the continental shelf of the Ebro Delta (MEDITS- Mediterranean International Trawl Survey; Bertrand et al., 2002). From each individual, we sampled a small portion of muscle that was stored frozen at -21°C until stable isotope analyses. Each sample was lyophilized and one aliquot was immediately prepared for $\delta^{15}\text{N}$ analysis. The other underwent lipid extraction prior to $\delta^{13}\text{C}$ analysis (Sholto-Douglas et al., 1991; Schlechtriem et al., 2003). Lipids were extracted using the methods described by Smedes (1999). All samples (about 0.5-1 mg) were combusted at $1,020^{\circ}\text{C}$ using continuous-flow isotope-ratio mass spectrometry (CFIRMS) system by means of a Carlo Erba 1500NC elemental analyser interfaced with a Delta Plus CL mass spectrometer. All isotope abundances were expressed in δ - notation as parts per thousands (‰) deviation from IAEA standards AIR (^{15}N) and VPDB (^{13}C). Based on within-run replicate measurements of laboratory standards, we estimated measurement error to be ± 0.2 and ± 0.1 ‰ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively. The average of the $\delta^{15}\text{N}$ values was used as a proxy of the trophic level of each species (Peterson and Fry, 1987; Kelly, 2000).

To explore the functional relationship between the predators and their potential prey, we predicted the range of expected $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopic values to be found in a potential

predator that consume the species located at lower trophic levels (fish and cephalopods). For this prediction, the range was calculated by two times the standard deviation of the mean isotopic values ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) of all fish (excluding the bluefin tuna and the tonguesole) and cephalopods species after consumer-prey correction by an isotopic fractionation of +3 and +1 for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, respectively (Kelly, 2000).

As trophic width isotopic estimators, we used the total isotopic area (*TA*) represented by the convex hull polygon for all individuals of each species (Layman et al., 2007). Convex hull polygon is the total area encompassed by the smallest convex polygon containing these individuals in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ niche dimensions (Layman et al., 2007). To calculate *TA* we needed the isotopic data of all individuals from each species. We had these information for those species of fish and cephalopods sampled in the present study (Table 1) and also for some fish, cephalopods and seabirds, which individual stable isotope values were provided from authors of these studies (Navarro et al., 2009; Navarro et al., 2010). As the number of values for individuals of the two seabirds' species was higher than for the fish species, the convex hull area was calculated for 100 subsets of 10 individuals randomly selected from the total seabird individuals. The convex hull areas were calculated using the Animal Movement Extension in the ArcView GIS 3.3 (ESRI, Redlands, CA, USA). Additional information on application of convex hulls in ecological studies can be found in Cornwell et al. (2006) and Layman et al. (2007).

2.4. Comparison between Ecopath and isotopic results

Comparisons between trophic level and trophic width measurements calculated from ecosystem modelling and SIA were made at the level of species. The trophic level *TL* estimated by the Ecopath model was plotted against the corresponding average $\delta^{15}\text{N}$ values and their correlation was calculated by means of the Spearman-rank correlation coefficient

(Zar, 1984). Similarly, we analyzed the correlation between the *OI* estimated from the Ecopath model and the *TA* estimated with the isotopic values.

3. Results

3.1. Ecopath model: trophic level and omnivory index

Trophic levels calculated with the Ecopath model revealed that the studied species (see Table 1) were located between a trophic level of 2.97 (European sardine) and a trophic level of 4.51 (bottlenose dolphin). Seabirds, cetaceans and two species of fish (bluefin tuna and blackbelly rosefish) showed the higher trophic levels (Fig. 2). In contrast, the remaining fishes and cephalopods, and the loggerhead sea-turtle showed lower trophic levels (Fig. 2).

The *OI* estimated with the Ecopath model indicated that species ranged from highly specialized consumers (thus with an $OI = 0$ for anchovy and shortnose greeneye) to generalist predators ($OI = 1.05$ for Balearic shearwater). Higher *OI* values were found for three species of seabirds and for the European hake and poor cod (Table 1) indicating that these organisms feed in a diverse range of trophic levels. Conversely, lower *OI* values were found for some fishes, including anchovy and bluefin tuna, and striped dolphin (Table 1).

3.2. Isotopic results

$\delta^{15}\text{N}$ values varied between 6.81‰ (Silvery cod) and 12.68‰ (bottlenose dolphin) (Fig. 3A). Higher $\delta^{15}\text{N}$ values (indicating higher trophic positions in the food web) were found for the two cetaceans, seabirds and two fishes (bluefin tuna and tonguesole). In contrast, the loggerhead sea-turtle, the remaining fishes, and cephalopods showed the lower $\delta^{15}\text{N}$ values (Fig. 3A). In the case of $\delta^{13}\text{C}$, the values varied between -19.93‰ (anchovy) and -17.37‰ (tonguesole) (Fig. 3A).

The predicted range of expected isotopic values to be found in a potential predator that consume the different species located at lower trophic level (Fig. 3B, see material and methods) matched with the trophic position of the dolphins, bluefin tuna, two seabirds (Balearic shearwater and yellow-legged gull) and the fish tonguesole (Fig. 3B). In contrast, the Audouin's gull, although showed $\delta^{15}\text{N}$ values similar to the predicted range, showed $\delta^{13}\text{C}$ values lower than the $\delta^{13}\text{C}$ values of the predicted range (Fig. 3B). Similarly, the loggerhead sea-turtle mismatched with the predicted range (Fig. 3B), indicating that these species fed different preys than the ones studied here.

3.3. Comparison between Ecopath and isotopic results

The trophic levels estimated by the Ecopath model were highly and positively correlated with the $\delta^{15}\text{N}$ values for the different species (Fig. 4A. Spearman-rank correlation coefficient, $R_s=0.69$, $p<0.0001$). These results show that higher trophic levels from the food-web model also showed higher $\delta^{15}\text{N}$ values, thus evidencing a good agreement between both methodologies. In addition, the *OI* estimated by the Ecopath model was marginally correlated with the *TA* estimated by the isotopic values (Fig. 4B. $R_s=0.44$, $p=0.06$). However, these results showed higher variability between both methods and only some species such as the Audouin's gull showed a good correspondence between both measures of trophic width (Fig. 4B).

4. Discussion

By using two different methodologies we were able to depict and confirm the trophic position and trophic width of several species of fish, cephalopods, cetaceans, seabirds and one marine turtle presents in the food web of the South Catalan Sea, in the North-Western Mediterranean. Our results revealed a clear correlation between the trophic levels estimated

by the food-web model and the $\delta^{15}\text{N}$ values. This indicates that both methodologies are useful to determine the trophic position of marine organisms in the Mediterranean marine food webs. In addition, although the two estimators of trophic width (the omnivore index and the total isotopic area) were also marginally correlated, this relationship was only clear for some species.

The good relationship between the trophic models estimated by the Ecopath model and the $\delta^{15}\text{N}$ values highlights that the diet information that was used for the species in the Ecopath models was accurate (Coll et al., 2006). This result is similar to other comparative studies between the two methodologies (Kline and Pauly, 1998; Dame and Christian, 2008) and extends previous results to the top end of the food web.

As expected, the high trophic level and high $\delta^{15}\text{N}$ values of cetaceans, seabirds and the bluefin tuna confirm their position as the main top predators in the NW Mediterranean food web, and indicate that the major part of the fishes and cephalopods species present in the lower and intermediate trophic levels can be part of their diet (i.e. Würtz and Marrale, 1993; Estrada et al., 2005; Ramos et al., 2009; Navarro et al., 2009; Navarro et al., 2010, Orsi Relini et al., 1995). Reinforcing this result, the isotopic position regarding $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of dolphins, bluefin tuna and two seabirds (Balearic shearwater and yellow-legged gull) matched with the predicted range of expected isotopic values to be found in a potential predator that consume the different species located at lower trophic level, suggesting that these predators consume these preys. Among apical predators, only the Audouin's gull did not match with this predicted range. This is probably a consequence of the consumption by Audouin's gull of freshwater resources such the American crayfish *Procambarus clarkii* that shows low $\delta^{13}\text{C}$ values (Fig. 3B, $\delta^{13}\text{C} = -27.73 \pm 1.56\text{‰}$, Navarro et al. 2010). This would impact the C isotopic results of Audouin's gull towards depleted values (Navarro et al. 2010).

In contrast, the lower trophic levels and lower $\delta^{15}\text{N}$ values of fish species and cephalopods confirmed their intermediate positions in this marine food web and the fact that

they are mainly exploiting the benthos, phytoplankton and zooplankton resources (Tudela and Palomera, 1997; Stergiou and Karpouzi, 2002; Coll et al., 2006). In the particular case of the loggerhead sea-turtle, both methodologies indicated its low trophic position in the food web, close to the fish and cephalopods. Moreover, this marine turtle was completely segregated from the isotopic range predicted for a potential predator consuming fishes and cephalopods. All these results confirmed that the loggerhead sea-turtle exploits resources located at a lower trophic level of the food- web such as planktonic prey or benthic invertebrates (Revelles et al., 2007).

The largest discrepancy in the estimation of trophic level was found for the demersal tonguesole. Although the Ecopath model estimated a low trophic level, the isotopic analysis showed high $\delta^{15}\text{N}$ values, close to the main top predators. Moreover, the isotopic values of the tonguesole matched with the isotopic range of a predator consuming fishes and cephalopods. This discrepancy was probably a consequence of assigning trophic level 1 to detritus in the Ecopath model (Burns, 1989; Nilsen et al., 2008). The scavenger benthic community consumes the death fish and invertebrates provided by the fisheries discards (Bozzano and Sardà, 2002; Kaiser and Hiddink, 2007), and thus they integrate the isotopic values of these prey that in turn enrich the $\delta^{15}\text{N}$ values of their predators (in our case the tonguesole; Stergiou and Karpouzi, 2002). Due to this reason, $\delta^{15}\text{N}$ is a better estimator than Ecopath model of the high trophic position of scavenger species in the studied food web if this is not directly taken into account in the trophic model and highlights the importance of assigning in specific occasions a higher trophic level to detritus in the food-web calculations.

Regarding trophic width estimations, the omnivore index and the total isotopic area were not strongly correlated. Based on our previous knowledge on the ecosystem, we expected to find different dietary preferences among the studied species, from highly specialist large predatory fishes such as the bluefin tuna to generalist seabirds (i.e. Macpherson, 1981; Stergiou and Karpouzi, 2002; Estrada et al., 2005; Ramos et al., 2009).

However, the omnivory index was the only indicators that captured the different degree of trophic width among the different species. The omnivory index was very low (close to 0) for specialist top predators, such the striped dolphin and the bluefin tuna or fish specialist species such shortnose greeneye, and was higher for opportunistic species such the seabirds, bottlenose dolphin or few fish such as the European hake or poor cod. In contrast, despite the isotopic methodology indicated a high isotopic area for the seabird species (Audouin's gull and Balearic shearwater), the other species had similar and small trophic width, thus showing a lower correlation between both trophic width indicators. These results may highlight that the trophic width measured using the total isotopic area is most useful for generalist predators (Hoeinghaus and Zeug, 2008). In fact, for the Audouin's gull, a well-known opportunist species that exploit resources with very different isotopic values, from demersal fish or pelagic fish to freshwater invertebrates (Navarro et al., 2010), the relationship between both food-web and isotopic methodologies was similar. Moreover, we have to take into account that we could not estimate the total trophic area for all the species included in this study (see Material and Methods), therefore we could not compare trophic width estimations of other species with contrasting trophic ecology, such as bluefin tuna (high specialist, Stergiou & Karpouzi, 2002) or yellow-legged gulls (high generalist, Ramos et al., 2009).

In conclusion, this study highlights that the trophic position of Mediterranean marine organisms can be correctly estimated by using the Ecopath model (trophic level) and the isotopic approach ($\delta^{15}\text{N}$ values). Since both methodologies are being widely applied in the Mediterranean Sea, these results highlight that they could be used together to depict the structure of Mediterranean marine food webs. In contrast, the trophic width estimated with the Ecopath model (omnivory index) and with the isotopic approach (total isotopic area) did not show such a clear correlation. Further studies should include additional data on the trophic width calculated with the isotopic approach to contrast our results from Mediterranean marine food webs.

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388 **References**

- 389 Allen, K.R., 1971. Relation between production and biomass. Journal of Fisheries Research Board of
390 Canada 28, 1573-1581.
- 391 Arcos, J., 2001. Foraging ecology of seabirds at sea: significance of commercial fisheries in the
392 Northwest Mediterranean. PhD Thesis. Departament de Biologia Animal. Universitat de
393 Barcelona, Barcelona, Spain.
- 394 Bertrand, J.A., Gil de Sola, L., Papaconstantinou, C., et al., 2002. The general specifications of the
395 MEDIS surveys. Scientia Marina 66, 9-17.

396 Blanco, C., Salomón, O., Raga, J., 2001. Diet of the bottlenose dolphin (*Tursiops truncatus*) in the
 397 Western Mediterranean Sea. Journal of Marine Biological Association of the UK 81, 1053-
 398 1058.

399 Borell, A., Aguilar, A., Tornero, V., et al., 2006. Organochlorine compounds and stable isotopes
 400 indicate bottlenose dolphin subpopulation structure around the Iberian Peninsula.
 401 Environmental Pollution 32, 516-523.

402 Bosch, M., Oro, D., Ruiz, X., 1994. Dependence of Yellow-legged gulls (*Larus cachinnans*) on food
 403 from human activity in two Western Mediterranean colonies. Avocetta 18, 135-139.

404 Bozzano, A., Sardà, F., 2002. Fishery discard consumption rate and scavenging activity in the
 405 northwestern Mediterranean Sea. ICES Journal of Marine Science 59, 15-28.

406 Burns, T., 1989. Lindeman's contradiction and the trophic structure of ecosystems. Ecology 70,
 407 1355-1362.

408 Cardona, L., Revelles, M., Sales, M., et al., 2007. Meadows of the seagrass *Posidonia oceanica* are a
 409 significant source of organic matter for adjoining ecosystems. Marine Ecology Progress Series
 410 335, 123-131.

411 Christensen, V., Walters, C., 2004. Ecopath with Ecosim: methods, capabilities and limitations.
 412 Ecological Modelling 172, 109-139.

413 Christensen, V., Walters, C., Pauly, D., 2005. Ecopath with Ecosim: a User's Guide. Fisheries
 414 Centre, University of British Columbia, Vancouver, Canada.

415 Coll, M., Libralato, S., 2009. Modelling marine food webs in the Mediterranean Sea. Fisheries
 416 Centre Research Report 17, 124-125.

417 Coll, M., Palomera, I., Tudela, S., et al., 2008. Food-web dynamics in the South Catalan Sea
 418 ecosystem (NW Mediterranean) for 1978–2003. Ecological Modelling 217, 95-116.

419 Coll, M., Palomera, I., Tudela, S., et al., 2006. Trophic flows, ecosystem structure and fishing
 420 impacts in the South Catalan Sea, Northwestern Mediterranean. Journal of Marine Systems 59,
 421 63-96.

422 Cornwell, W., Schwik, D., Ackerly, D., 2006. A trait-based test for habitat filtering: convex hull
 423 volume. Ecology 87, 1465-1471.

424 Dame, J., Christian, R., 2008. Evaluation of ecological network analysis: validation of output.
 425 Ecological Modelling 210, 327-338.

426 De Niro, M., Epstein, S., 1981. Influence of diet on the distribution of nitrogen isotopes in animals.
 427 Geochimica et Cosmochimica Acta 45, 341-351.

428 Estrada, J.A., Lutcavage, M., Thorrold, S.R., 2005. Diet and trophic position of Atlantic bluefin tuna
 429 (*Thunnus thynnus*) inferred from stable carbon and nitrogen isotope analysis. Marine Biology
 430 147, 37-45.

431 Forero, M.G., Hobson, K.A., 2003. Using stable isotopes of nitrogen and carbon to study seabird
 432 ecology: applications in the Mediterranean seabird community. Scientia Marina 67, 23-32.

433 Fulton, E., 2010. Approaches to end-to-end ecosystem models. Journal of Marine Systems 81, 171-
 434 183.

435 Granadeiro, J., Monteiro, L., Furness, R., 1998. Diet and feeding ecology of Cory's shearwater
 436 *Calonectris diomedea* in the Azores, north-east Atlantic. Marine Ecology Progress Series 166,
 437 267-276.

438 Hoeinghaus, D.J., Zeug, S.C., 2008. Can stable isotope ratios provide for community-wide measures
 439 of trophic structure? Comment. Ecology 89, 2353-2357.

440 Jackson, J., Kirby, M., Berger, W., et al., 2001. Historical overfishing and the recent collapse of
 441 coastal ecosystems. Science 293, 629-638.

442 Kaiser, M., Hiddink, J., 2007. Food subsidies from fisheries to continental shelf benthic scavengers.
 443 Marine Ecology Progress Series 350, 267-276.

444 Kelly, J.F., 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian
 445 trophic ecology. Canadian Journal of Zoology 78, 1-27.

446 Kline, T., Pauly, D., 1998. Cross-validation of trophic level estimates from a mass-balance model of
 447 Prince William Sound using $^{15}\text{N}/^{14}\text{N}$ data. In: Funk, T.J., Quinn, J. Heifetz, J.N., et al. (eds.),
 448 Fishery stock assessment models, pp. 693-702. Alaska Sea Grant College Program Report No
 449 AK-SG-98-01, University of Alaska, Fairbanks, USA.

450 Layman, C.A., Arrington, D., Montaña, C., et al., 2007. Can stable isotope ratios provide quantitative
 451 measures of trophic diversity within food webs? Ecology 88, 42-48.

452 Libralato, S., 2008. System Omnivory Index. In: Jorgensen, S.E., Fath, B.D. (eds), Ecological
 453 Indicators, Vol. 4 of Encyclopedia of Ecology, pp. 3472-3477. Elsevier. Oxford, UK.
 454 Lindeman, R., 1942. The trophic-dynamic aspect of the Ecology. Ecology 23, 399-418.
 455 Lotze, H.K., Lenihan, H.S., Bourque, B.J., et al., 2006. Depletion, degradation, and recovery
 456 potential of estuaries and coastal seas. Science 312, 1806-9.
 457 Macpherson, E., 1981. Resource Partitioning in a Mediterranean Demersal Fish Community. Marine
 458 Ecology Progress Series 4, 183-193.
 459 Navarro, J., Louzao, M., Igual, J.M., et al., 2009. Seasonal changes in the diet of a critically
 460 endangered seabird and the importance of trawling discards. Marine Biology 159, 2571-2578.
 461 Navarro, J., Oro, D., Bertolero, A., et al., 2010. Age and sexual differences in the exploitation of two
 462 anthropogenic food resources for an opportunistic seabird. Marine Biology *in press*.
 463 Newsome, S.D., Martinez del Rio, C., Bearhop, S., et al. 2007. A niche for isotopic ecology.
 464 Frontiers in Ecology and the Environment 5, 429-436.
 465 Nilsen, M., Pedersen, T., Nilssen, E.M., et al., 2008. Trophic studies in a high-latitude fjord
 466 ecosystem - a comparison of stable isotope analyses ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and trophic-level estimates
 467 from a mass-balance model. Canadian Journal of Fisheries and Aquatic Sciences 65, 2791-2806.
 468 Odum, W., Heald, E., 1975. The detritus-based food web of an estuarine mangrove community. In:
 469 Cronin, L.E. (ed). Estuarine Research, Vol. 1, pp. 265-286. Academic Press, New York, USA.
 470 Oro, D., Ruíz, X., Jover, L., et al., 1997. Diet and adult time budget of Audouin's Gull *Larus*
 471 *audouinii* in response to changes in commercial fisheries. Ibis 139, 631-637.
 472 Orsi Relini, L., Garibaldi, F., Cima, C., et al., 1995. Feeding of the Swordfish, the bluefin and other
 473 pelagic nekton in the Western Ligurian Sea. ICCATT Collection Volume Science Papers 44,
 474 283-286.
 475 Pauly, D., Christensen, V., Walters, C., 2000. Ecopath, Ecosim, and Ecospace as tools for evaluating
 476 ecosystem impact of fisheries. ICES Journal of Marine Science 57, 697-706.
 477 Peterson, B., Fry, B., 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and
 478 Systematics 18.
 479 Plagányi, É., 2007. Models for an Ecosystem Approach to Fisheries. FAO Fisheries Technical Paper.
 480 Food and Agriculture Organization, Rome, Italy.

481 Polunin, N., Morales-Nin, B., Pawsey, W., et al., 2001. Feeding relationships in Mediterranean
 482 bathyal assemblages elucidated by stable nitrogen and carbon isotope data. *Marine Ecology*
 483 *Progress Series* 220, 13-23.

484 Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and
 485 assumptions. *Ecology* 83, 703-718.

486 Ramos, R., Ramírez, F., Sanpera, C., et al., 2009. Feeding ecology of yellow-legged gulls *Larus*
 487 *michahellis* in the western Mediterranean: a comparative assessment using conventional and
 488 isotopic methods. *Marine Ecology Progress Series* 377, 289-297.

489 Revelles, M., Cardona, L., Aguilar, A., et al., 2007. The diet of pelagic loggerhead sea turtles off the
 490 Balearic archipelago: relevance of long-line baits. *Journal of the Marine Biological Association*
 491 *of the UK* 87, 805-813.

492 Salat, J., 1996. Review of hydrographic environmental factors that may influence anchovy habitats in
 493 northwestern Mediterranean. *Scientia Marina*, 60 (Suppl. 2), 21-32.

494 Schlechtriem, C., Focken, U., Becker, K., 2003. Effect of different lipid extraction methods on $\delta^{13}\text{C}$
 495 of lipid and lipid-free fractions of fish and different fish feeds. *Isotopes in Environmental and*
 496 *Health Studies* 39, 135-140.

497 Sholto-Douglas, A., Field, J., James, A., et al., 1991. $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ isotope ratios in the
 498 Southern Benguela Ecosystem: indicators of food web relationships among different size-
 499 classes of plankton and pelagic fish; differences between fish muscle and bone collagen tissues.
 500 *Marine Ecology Progress Series* 78, 23-31.

501 Smedes, F., 1999. Determination of total lipid using non-chlorinated solvents. *Analyst* 124, 1711-
 502 1718.

503 Stergiou, K.I., Karpouzi, V.S., 2002. Feeding habits and trophic levels of Mediterranean fish.
 504 *Reviews in Fish Biology and Fisheries* 11, 271-254.

505 Tudela, S., Palomera, I., 1997. Trophic ecology of European anchovy *Engraulis encrasicolus* in the
 506 Catalan Sea (Northwestern Mediterranean). *Marine Progress Marine Series* 160, 121-134.

507 Würtz, M., Marrale, D., 1993. Food of striped dolphin, *Stenella coeruleoalba*, in the Ligurian Sea.
 508 *Journal of Marine Biological Association of the United Kingdom* 73, 571-578.

509 Zar, J., 1984. *Biostatistical analysis*. Prentice-Hall, London, UK.

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Fig. 1. Map of the South Catalan Sea study area (modified from Catalano-Balearic Sea-Bathymetric chart 2005; www.icm.csic.es/geo/gma/MCB).

Fig. 2. Overview of the flow diagram of the modified mass-balance model of the South Catalan Sea (NW Mediterranean, Coll et al., 2006; Coll et al., 2008) to specifically visualize the species with stable isotopes data. The components of the ecosystem are structured along the vertical axis according to their trophic level (estimated with Ecopath). All species used in the isotopic approach are indicated in larger letters and are within a box). The area of each red circle is proportional to the biomass of each functional group during the mid-late 1990s.

Fig. 3. Mean $\pm$ standard error of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for: (A) fishes (black circles), cephalopods (white circles), seabirds (white triangles), cetaceans (black triangles) and marine turtles (white square) from the NW Mediterranean Sea, and (B) apical predators (seabirds, cetaceans and a marine turtle, the Tonguesole fish and a pool of fishes and cephalopods (black and white circle)). The grey box represents the range of expected isotopic values from the predators, considering two times the standard deviation of the mean for the pool of fishes and cephalopods, accounting for prey-predators isotopic fractionation of 3‰ and 1‰ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, respectively. Abbreviations of the different species and the source of the isotopic values are given in Table 1. Values of freshwater American crayfish *Procambarus clarkii* (CRAY) are also reported in panel (B) (from Navarro et al. 2010).

Fig. 4. Relationships between: (A) the trophic level (mean) calculated with the Ecopath model and the $\delta^{15}\text{N}$ values (mean), and (B) the omnivory index (*OI*) calculated with Ecopath with the total area (*TA*) calculated with the isotopic values for fishes (black circles), cephalopods (white circles), seabirds (white triangles), cetaceans (black triangles), and

marine turtles (white squares) from the NW Mediterranean Sea. Abbreviations of the different species and the source of the isotopic values are given in Table 1.

Fig. 3

Table 1

Trophic level (mean $\pm$ sd) and trophic width estimators, total area (TA) and omnivory index (OI), for fish, cephalopods, seabirds, cetaceans and marine turtles from the South Catalan Sea (NW Mediterranean). The source of the stable isotope and the sample sizes for each species (n) are also indicated.

Species	TL	OI	TA	n	source
Fish					
Anchovy <i>Engraulis encrasicolus</i> (ENG)	3.05 $\pm$ 0.01	0	0.18	9	(Navarro et al., 2009)
Blackbelly rosefish <i>Helicolenus dactylopterus</i> (HEL)	4.14 $\pm$ 0.26	0.19	0.12	10	Present study
Blotched picarel <i>Spicara flexuosa</i> (SPI)	3.49 $\pm$ 0.35	0.35	0.08	9	(Navarro et al., 2009)
Blue whiting <i>Micromesistius poutassou</i> (MIC)	3.40 $\pm$ 0.41	0.46	0.14	9	(Navarro et al., 2009)
Bluefin tuna <i>Thunnus thynnus</i> (THU)	4.42 $\pm$ 0.32	0.11	ND	2	(Cardona et al., 2007)
Boarfish <i>Capros aper</i> (CAP)	3.65 $\pm$ 0.28	0.22	0.12	10	Present study
European hake <i>Merluccius merluccius</i> (MER)	3.39 $\pm$ 0.61	1	0.07	9	Present study
Glasshead grenadier <i>Hymenocephalus italicus</i> (HYM)	3.61 $\pm$ 0.24	0.32	0.05	6	Present study
Greater forkbeard <i>Phycis blennoides</i> (PHY)	3.89 $\pm$ 0.05	0.01	0.03	9	(Navarro et al., 2009)
Jewel lanternfish <i>Lampanyctus crocodilus</i> (LAM)	3.49 $\pm$ 0.35	0.35	0.18	8	Present study
Pilchard <i>Sardina pilchardus</i> (SAR)	2.97 $\pm$ 0.27	0.21	1.01	9	(Navarro et al., 2009)
Poor cod <i>Trisopterus minutus</i> (TRI)	3.25 $\pm$ 0.51	0.71	0.21	10	Present study
Red bandfish <i>Cepola macrophthalma</i> (CEP)	3.25 $\pm$ 0.30	0.25	0.53	9	Present study
Shortnose greeneye <i>Chlorophthalmus agassizi</i> (CHL)	3.05 $\pm$ 0.01	0	0.01	5	Present study
Silvery cod <i>Gadiculus argenteus</i> (GAD)	3.18 $\pm$ 0.37	0.38	1.01	9	(Navarro et al., 2009)
Tonguesole <i>Symphurus nigrescens</i> (SYM)	3.31 $\pm$ 0.45	0.56	0.08	8	(Navarro et al., 2009)
Cephalopods					
Common bobtail <i>Sepieta oweniana</i> (SEP)	3.08 $\pm$ 0.29	0.24	0.06	9	Present study
Common squid <i>Allotheuthis subulata</i> (ALL)	3.66 $\pm$ 0.53	0.77	0.45	6	(Navarro et al., 2009)
Cetaceans					
Bottlenose dolphin <i>Tursiops truncatus</i> (TUR)	4.51 $\pm$ 0.50	0.25	ND	7	(Borell et al., 2006)
Striped dolphin <i>Stenella coeruleoalba</i> (STE)	4.22 $\pm$ 0.26	0.07	ND	5	(Cardona et al., 2007)
Seabirds					
Audouin's gull <i>Larus audouinii</i> (AUD)	4.01 $\pm$ 0.95	0.91	2.44	107	(Navarro et al., 2010)
Balearic shearwater <i>Puffinus mauretanicus</i> (PUF)	3.96 $\pm$ 1.02	1.05	1.35	145	(Navarro et al., 2009)
Yellow-legged gull <i>Larus michahellis</i> (MIC)	4.27 $\pm$ 0.93	0.87	ND	23	(Ramos et al., 2009)
Marine turtle					
Loggerhead sea-turtle <i>Caretta caretta</i> (CAR)	3.06 $\pm$ 0.79	0.63	ND	27	(Revelles et al., 2007)