Investigating feeding ecology of two anglerfish species, *Lophius piscatorius* and *Lophius budegassa* in the Celtic Sea using gut content and isotopic analyses

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Abstract :

We used stable isotope ratio and gut content analyses to determine and compare the feeding ecology of two commercially important predator species, *Lophius piscatorius* and *Lophius budegassa* in the Celtic sea, where data concerning their trophic ecology remain sparse. This study included two areas and two size-classes, showing that anglerfish in the Celtic sea are mainly piscivorous top predators as observed in other marine waters. However, a substantial part of the diet of the fish in the small size classes consists of benthic macro-invertebrates, mainly Crustaceans. Despite the common knowledge that they are opportunistic predators that display a low degree of prey selectivity, our results suggest that the two species have different trophic niches when they occur in the same area. In the shallow area, both small and large individuals of *L. budegassa* seemed to prefer Crustacean prey, whereas *L. piscatorius* showed a clear shift from Crustaceans to fish prey with increasing size-class in the two areas. Stable isotope data analysis support the common finding that trophic level increases with body size for *L. piscatorius* and that overlaps between the isotopic niche spaces are more pronounced in the deeper area. Ecosystem-based approaches to fisheries management requires the development of multi-species modelling based on food web structure and species interactions. Our results emphasize the importance of modelling the two species and size classes separately, as they indicate clear trophic segregation

Keywords : *Lophius*, gut content, stable isotopes, trophic niche, trophic segregation, top predator

The Ecosystem Approach to Fisheries Management is now recognized as a necessity, and numerous modelling studies are being conducted to support its implementation (Coll et al., 2015; Gascuel et al., 2011; Ramirez-Monsalve et al., 2016). The reliability of such models to provide diagnostics and scenarios depends on the availability and quality of the biological data on which they are based, especially those that describe biological interactions between species, including competition for food (Tremblay-Boyer et al., 2011). Feeding ecology is therefore a key component for understanding the position of marine organisms in the food chain and their relative roles in the ecosystem. Among the modelled species, it is essential to study predator's species as they exert important top-down control on the food web.

Anglerfish are known to be one of the top demersal predators in European waters. However, despite their high economic value, little is known about their biology and ecology (Farina et al., 2008; Landa et al., 2001). In the Celtic Sea, two species co-exist. Both have a wide mouth and use a modified first dorsal ray as a lure to ambush their prey. They are considered opportunistic predators, with whitefish, Crustacea and Cephalopoda identified as their main prey (Farina et al., 2008). Studies on the feeding ecology of anglerfish have been conducted in the Shetland Islands (Laurenson and Priede, 2005) and North Irish Sea (Crozier, 1989) for *L. piscatorius* and in the Cantabrian Sea (Preciado et al., 2006) and the Mediterranean Sea (Lopez et al., 2016; Negzaoui-Garali et al., 2008; Stagioni et al., 2013) for *L. budegassa*. However, to our knowledge no study investigated their feeding ecology in the Celtic sea.

The aim of this study was to investigate anglerfish feeding ecology in the Celtic sea using two complementary analyses. Gut content analysis was used to provide a snapshot of the composition of prey recently ingested by the two species. Stable isotope analysis of carbon and nitrogen was performed to reflect the composition of prey assimilated over longer time periods (Vander Zanden et al., 2015).

Fish were sampled during the EVHOE survey (part of the International Bottom Trawl Survey performed in November) using a GOV (Grande Ouverture Verticale) demersal trawl with a cod-end of 20-mm stretched mesh, towed for 30 min at a speed of approximately 3.5 knots by R/V "Thalassa" during day time. To account for potential ontogenetic diet shifts, size classes were determined according to the main modes observed in the size-distribution of each species, obtained in the same season during previous EVHOE campaigns (size split at 20 cm for L. budegassa and 25 cm for L. piscatorius). This is of the same order of magnitude than other size split reported in the literature (Laurenson and Priede, 2005; Lopez et al., 2016). To account for potential spatial variations of anglerfish prey, sampling was also performed in two contrasting areas: one shallow area between 30 and 120 m depth (hereafter referred to as area A) and a deeper one ranging from 121 to 259 m depth (hereafter referred to as area B), Figure 1. Numbers of studied individuals per species, size class and area are given in Table 1.

Only individuals that had at least one prey item in their gut contents were considered for the analyses. Prey were identified to the lowest taxonomic level possible and grouped to highlight the main contributors to predator diet (e.g., Crustacea, Cephalopoda, other invertebrates). As anglerfish are known to be piscivorous, fish prey were grouped in a more detailed manner than other prey (e.g., by order such as Gadiformes, by family such as Gobiidae, or by species such as *Trachurus trachurus*). Several metrics were calculated to characterize feeding strategies and to compare the diet between different groups of individuals: the relative abundance and occurrence of prey, the Shannon index (Shannon, 1949) and the Schoener overlap index (Schoener, 1970).

On a subset of individuals (Table 1), a sample of white dorsal muscle was dissected for determination of $\delta^{15}N$ $\delta^{13}C$ isotopic ratio. Trophic level (TL) of anglerfish were calculated following the Post equation (Post, 2002), with a trophic enrichment factor of 3.4 ‰ (Minagawa and Wada, 1984) and Pectinid bivalves collected as primary consumers in the two areas as trophic baseline (TL=2.5; Jennings and Warr, 2003). Effects of anglerfish groups (species/length class) and area were investigated through

two-way analysis of variance (ANOVA) on TL values after having checked their normal distribution by the mean of the Jarke-Bera test (1987). The ANOVA was followed by Tukey multiple-comparison tests. Isotopic niches were considered using Standard Ellipse Area (SEAc, (Jackson et al., 2011)) for the two species, size classes and areas. All the statistical analyses were performed using R 3.1.3 software (R Development Core Team, 2015).

Mean prey numbers per fish (Table 1) ranged between 1.1 and 2.5, with a maximum for large *L. piscatorius* in area B. Thirty-four taxa compose the diet of *L. piscatorius* and 32 that of *L. budegassa*. *L. piscatorius* had mainly fish prey (maximum: 100%, Table S1), as did *L. budegassa* (maximum: 84%), except for small individuals in area A, where Crustacean occurrence was the highest (62.5%). Figure 2 shows that for both areas, demersal and pelagic fishes such as Gadiformes, *Trachurus trachurus* and *Scomber scombrus* accounted for more than 60% of the diet of large *L. piscatorius*. Crustacean taxa were less observed than fish in the gut contents of the two species, and were totally absent from large *L. piscatorius* in area B. Cephalopod and other macro-invertebrates (Scaphopods, Gastropods, Bivalves, Polychaetes and Ophiurids) prey were less frequent (from 0 to 33%, Table S1). Diet composition of large *L. piscatorius* was quite similar between areas (Table S2 and Figure 2). For the small size class, the relative proportions of fish and invertebrates also remained consistent between the two areas, but fish species composition varied with an increase of Gobiidae and a lower proportion of Gadiformes in area B compared to area A. In contrast, *L. budegassa* had a more balanced diet than *L. piscatorius*, with 25 to 50% of benthic prey: Crustaceans (mainly shrimps and squat lobsters), flatfish and Gobiidae, depending on the area. Our results support the common finding that *Lophius* species are opportunistic predators that capture a wide range of prey types, since more than 50 taxa were identified in the gut contents. Fish were the primary food consumed by all size classes except for small *L. budegassa* in area A, for which 50% of the diet was composed of invertebrates. It is well known that anglerfish are sit-and-wait predators that preferentially feed on prey types that react to their lure, such as fish that themselves prey on mobile organisms and could confuse the lure

5

with their own prey (Gordoa and Macpherson, 1990). The most common fish prey type observed was Gadiformes for large individuals of both species and Gobiidae for small individuals. This is consistent with previous studies performed in European waters (Azevedo, 1996). Though *Lophius* is a benthic species, pelagic fish (e.g. *Trachurus trachurus* and *Scomber scombrus*) have been identified in the diet of large individuals, as in other areas (Negzaoui-Garali et al., 2008). One must assume that these pelagic fishes approach the bottom sufficiently for the anglerfish to catch them.

ANOVA analysis showed significant differences between anglerfish groups (p<0.0001) and areas (p<0.0001). In each area the estimated TL (Figure 3) of small *L. piscatorius* was significantly lower than that of larger ones (mean of 3.3 and 4.1 for small and large, respectively, Tukey p-value < 0.0001 for areas A and area B). For *L. budegassa*, the slight TL increase (from 3.8 to 4 between small and large fish) was not significant (Tukey p-value = 0.999 and 0. 963 for areas A and B, respectively). The TL obtained in this study are slightly lower than the value of other areas (Badalamenti et al., 2002). For both species, it is noteworthy that TLs in area B were significantly higher than in area A (Tukey p-value between areas > 0.0001). In shallow water (30–120 m), the trophic level of *L. piscatorius* was around 3.31 (no difference between size classes) while in the deepest area (>120 m) it was significantly higher for small (3.56) and large fish (4.49), nearing the 4.7 estimated in the North Sea for a 25.5–75.2 mm size range (Jennings et al., 2002). This suggests that prey are of lower trophic level in shallow water than in deeper water, especially for smaller fish.

Examination of Shoener's index (Table 2) suggests relatively weak diet overlaps between species and size classes in both areas. Overlaps were higher between the same size classes of the two species than between different size classes of the same species. The highest overlaps concerned large (0.49), and small fish (0.62) in area A and area B respectively. The isotopic niche was considered through SEAc estimates (Figure 4). For the same fish and length class, the SEA$_c$ were lower in area A than in area B (Table 2). SEA$_c$ estimates for *L. budegassa* of both size classes were lower than those of *L. piscatorius* in the two areas. For this fish, no niche overlap (Table 2) was observed between the two size classes

6

in area A and the niche overlap was quite small (0.03 $‰^2$) in area B. Niche overlap between small and large *L. budegassa* was also weak in area A (0.04 $‰^2$) and absent in area B. Maximum niche overlaps were observed in area A between large anglerfish of the two species (0.07 $‰^2$), and in area B, between small ones on the one hand (0.21 $‰^2$) and between large *L. budegassa* and small *L. piscatorius* on the other hand (0.18$‰^2$).

To summarize, trophic niche breadth was investigated through prey diversity and isotopic niche. Even if no clear pattern in term of number of prey could be detected between size classes, wider SEAc for smaller fish support a more diversified diet for small individuals. Prey diversity did not show any link to the area, while SEAc were systematically smaller in shallower waters, for each species and size class. Finally, the diet of *L. budegassa* was less diversified than that of *L. piscatorius*. The same pattern was observed for the isotopic niche with SEAc smaller for *L. budegassa* compared to *L. piscatorius*.

Ecosystem-based approaches to fisheries management requires the development of multi-species modelling based on food web structure and species interactions to investigate ecosystems functioning and responses to human disturbances. This type of data-oriented study is essential to inform and calibrate such trophic models. Considering the weak diet and SEA overlaps measured, we can conclude there is trophic segregation, limiting competition for food between the two species, especially in the shallow area. Our results suggest to model species and size classes separately to account for differences in feeding habits. We also highlighted that trophic preferences may vary between areas supporting the development of spatialized trophic models. Finally, our results suggest that the two species should be assess and manage separately instead of the currently in place common TAC (Total Allowable Catches).

Acknowledgements

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Tables and figures:

Table 1. Number of samples per species, size class and area for gut content and stable isotope analysis, with mean prey number per fish gut and total number of taxa.

Table 2. Shoener's and SEAc overlap index between species and size classes for the two areas studied.

Figure 1. Maps of sampled stations and area split. Triangles represent stations in area A and circles represent stations in area B.

Figure 2: Histogram of relative abundance of prey categories in the gut content of each species, size class and area combination.

Figure 3. Trophic level calculated for species, size classes and areas. LB indicates *L. budegassa* and LP indicates *L. piscatorius*. 1 refers to the small size class and 2 to the larger one. Grey boxplots represent area A and black boxplots area B.

Figure 4. Projection of SEA_C in the $\delta^{13}C$/ TL plane for the two species, size classes and areas. LB refers to *L. budegassa* and LP refers to *L. piscatorius*; 1 refers to small size class and 2 to the larger size class.
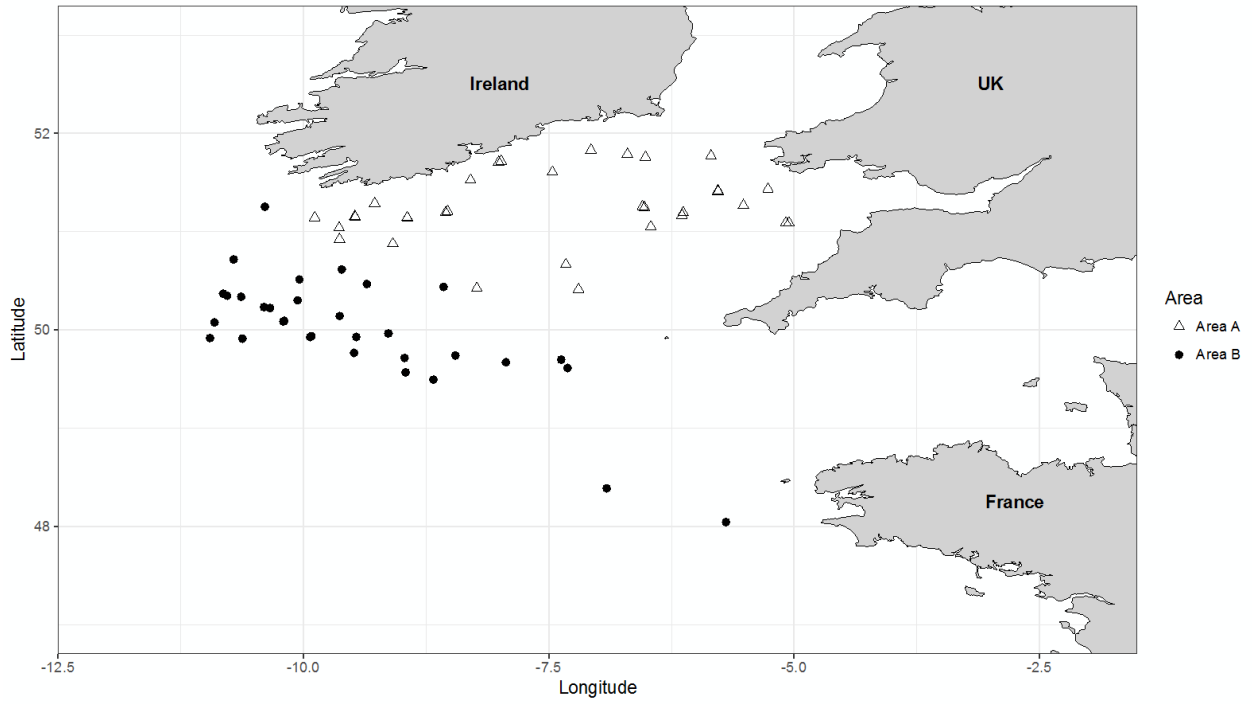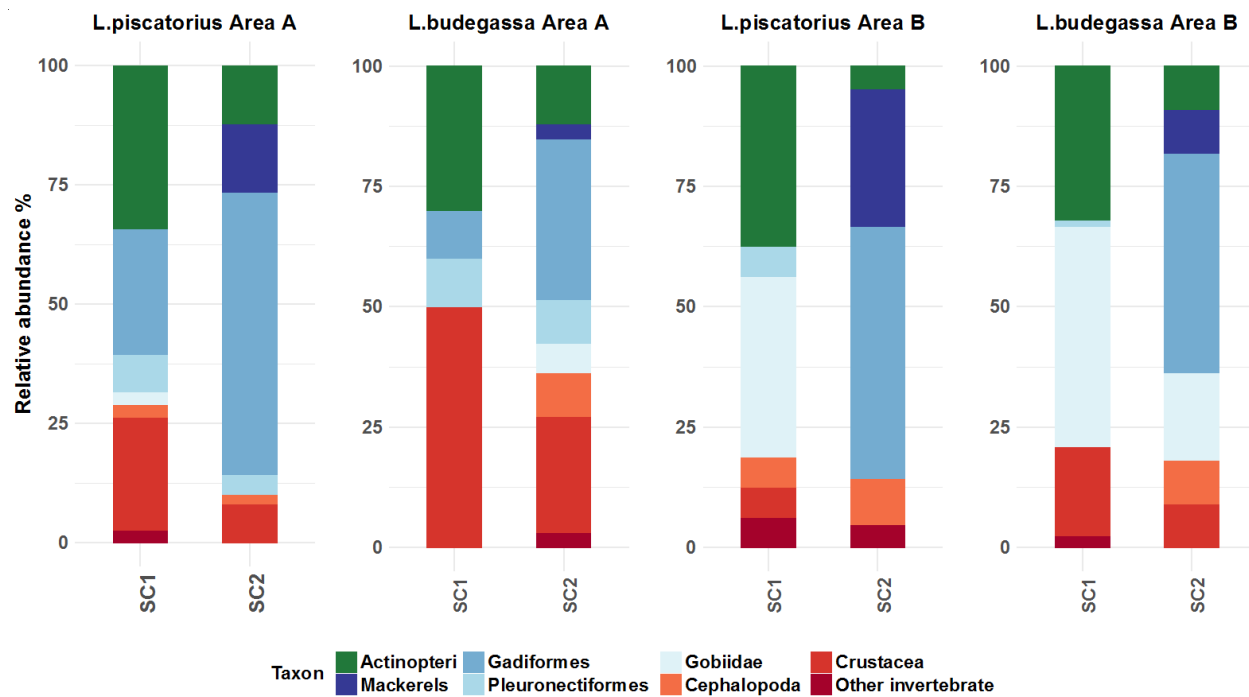
Figure 1

Figure 2

Figure 3
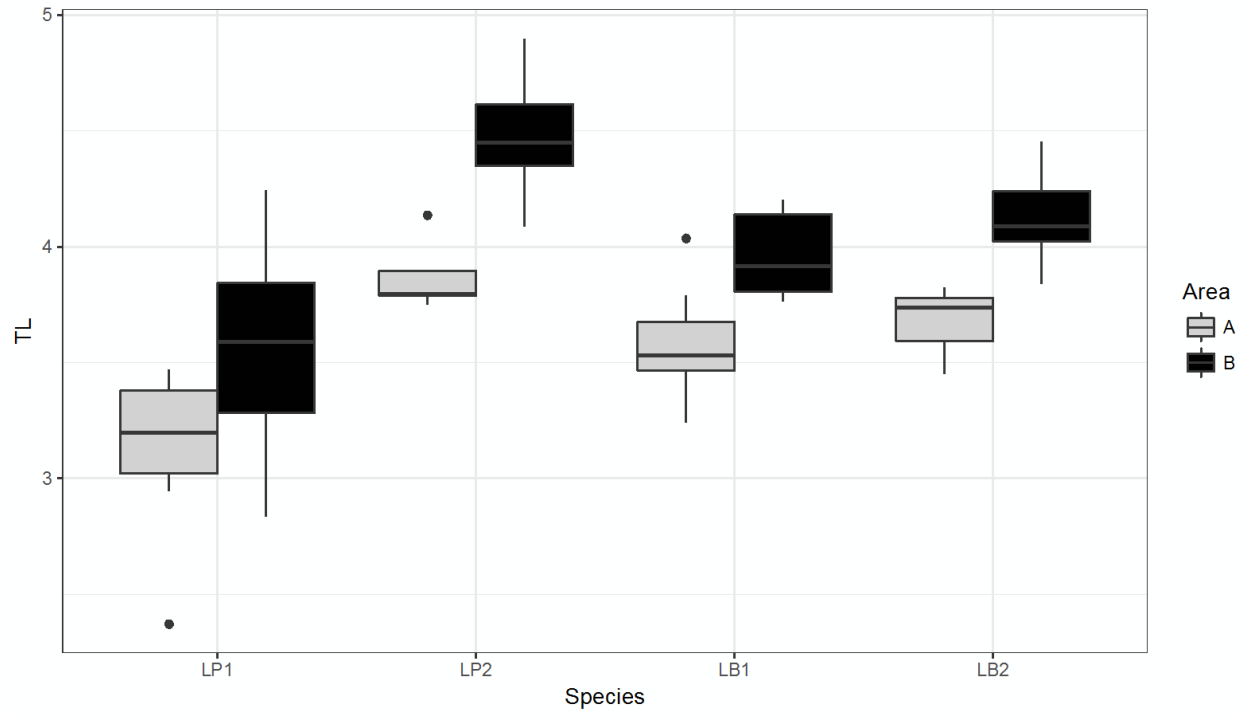
Figure 4
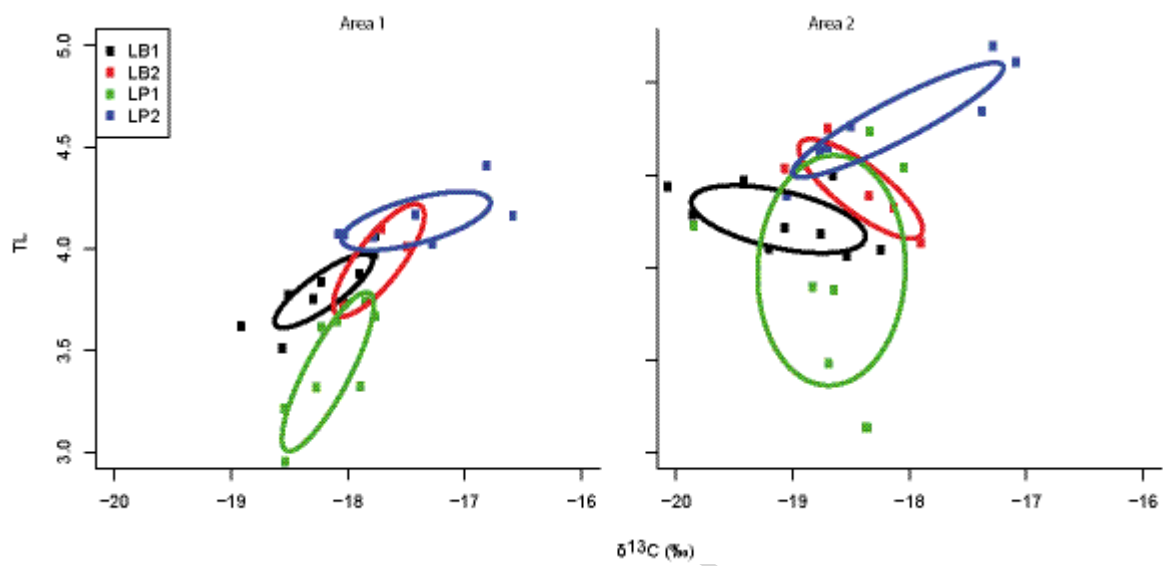
Table 1

| Species | Area | Size Class | Gut content analysis | | Stable isotope analysis |
			Mean (sd) prey number/ fish gut	Total number of taxa recorded	Nb of samples
Lophius budegassa	A	1	1.1 (0.3)	6	10
		2	1.2 (0.6)	18	3
	B	1	1.4 (1.2)	14	9
		2	1.4 (0.5)	8	5
Lophius piscatorius	A	1	1.3 (0.5)	14	8
		2	1.5 (1.6)	15	7
	B	1	1.3 (0.7)	9	6
		2	2.5 (2.3)	11	8

Table 2

Shoener's overlap index

Area A		*L. piscatorius* SC1	*L. piscatorius* SC2	*L. budegassa* SC1
L. piscatorius	SC2	0.39		
L. budegassa	SC1	0.37	0.18	
L. budegassa	SC2	0.27	**0.49**	0.21
Area B		*L. piscatorius* SC1	*L. piscatorius* SC2	*L. budegassa* SC1
L. piscatorius	SC2	0.05		
L. budegassa	SC1	**0.62**	0.05	
L. budegassa	SC2	0.27	0.32	0.35

SEAc overlap index

Area A		*L. piscatorius* SC1	*L. piscatorius* SC2	*L. budegassa* SC1
L. piscatorius	SC2	0.00		
L. budegassa	SC1	0.00	0.00	
L. budegassa	SC2	0.02	**0.07**	0.04
Area B		*L. piscatorius* SC1	*L. piscatorius* SC2	*L. budegassa* SC1
L. piscatorius	SC2	0.03		
L. budegassa	SC1	**0.21**	0.00	
L. budegassa	SC2	**0.18**	0.05	0.00