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Integrating prey monitoring and stable isotope analysis to assess the diet of *Octopus vulgaris* in Portuguese coastal waters

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ABSTRACT

This study explores the feeding ecology of *Octopus vulgaris* in the Cascais region through a combined approach of long-term prey monitoring and stable isotope analysis. Over several months, we worked with local fishermen to observe and record prey items found in octopus pots and those carried by octopuses at the time of capture. These field observations enabled the identification of key prey species, which were subsequently analysed isotopically to estimate their contribution to the octopus diet. The results show that Atrina fragilis is the main prey, making up about 70 %, followed by *Polybius henslowii* (18 %), with *Cymbium olla* and *Cepola macrophthalma* contributing smaller amounts. Our findings highlight the limitations of traditional stomach content analysis, which often underestimates soft-bodied or highly digested prey, and emphasise the importance of isotopic methods to provide a more comprehensive and long-term view of trophic interactions. This integrated approach enhances our understanding of O. vulgaris feeding strategies and has significant implications for ecological research and the sustainable management of fisheries in the region.

1. Introduction

1.1. The significance of octopus fisheries

Octopus fisheries are crucial to the economy and social fabric of the Gulf of Biscay and the Iberian coast. In 2024, landings in these regions reached approximately 7481 tonnes, with a preliminary value of over 55,820 thousand euros, emphasising their significant contribution to local livelihoods and the regional fishing industry, according to data from the European Market Observatory for Fisheries and Aquaculture Products. In Portugal, the total catch accounts for 45.5 % (3402 tonnes) and in value represents 48 % (26,843 thousand euros). The many small-scale vessels dedicated to octopus fishing highlight the industry’s role in supporting the socio-economic well-being of coastal communities.

1.2. Ecological value of the octopus in marine ecosystems

The common octopus, *O. vulgaris* Cuvier 1797, (*O. vulgaris* sensu stricto, Fee et al., 2024), is essential to coastal marine ecosystems, influencing their structure and function. It is a meroplanktonic species with benthic juveniles and adults. As an opportunistic predator, it is inclusively cannibalistic (Hernández-Urcera et al., 2019). It preys on populations of crustaceans, bivalves, and small fish, shaping community dynamics and trophic interactions. At the same time, it is an important prey for larger predators like big fish, marine mammals, and seabirds, placing it centrally in the food web. Its high mobility, short lifespan, and rapid growth make it a sensitive indicator of environmental changes, such as shifts in prey, habitat quality, and oceanographic conditions. By connecting benthic and pelagic zones through feeding and movement, octopuses aid in nutrient cycling and energy flow across different habitats. Understanding their ecological role is crucial for the sustainable management of both the species and the ecosystems they support.

1.3. Previous research on feeding ecology

Historically, research on the dietary habits of octopuses has mainly depended on stomach content analysis, which identifies prey through residual parts such as shells and bones. However, this method is labour-intensive and offers only a snapshot of recent feeding. It may underestimate dietary variety because cephalopods often tear their prey into small pieces, making identification difficult due to partial digestion (Cherel and Hobson, 2007). For instance, a study by Rosa et al. (2004) in

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the same region found that 67 % of the diet consisted of decapods, with significantly lower proportions of bivalves, fish, and other prey types, demonstrating the limitations of this approach.

Using ecological tracers, such as isotope signatures in muscle tissues and trace element analysis, proves beneficial for gaining a broader understanding of cephalopod feeding ecology. These methods provide integrated insights into long-term dietary habits and assist in clarifying population structure, individual movements, and migrations (Post, 2002).

Combined studies using multiple methods have not been previously conducted on the common octopus. Our research seeks to address this gap by analysing the species consumed by octopuses and examining the wider ecosystem to better understand the octopus's ecological role. Although there have been studies on different octopus species, such as *Octopus insularis*, comparing stable isotope results with stomach contents (Dantas et al., 2020; Urrutia-Olvera et al., 2021), these did not specifically identify the exact prey species, instead sampling areas containing various molluscs, crustaceans, and fish. Markaida (2023) also sampled prey items, in *Octopus maya*, but did not determine the stable isotopes. Our study's novelty lies in directly observing and confirming prey items consumed on-site before sampling, thereby improving accuracy in dietary insights.

2. Materials and methods

2.1. Local

Cascais, situated in Portugal, near the Tagus River estuary (the largest estuary in Europe), embodies a lively and ecologically diverse coastal environment. Characterised by a temperate climate and a variety of habitat types, this region reflects influences from both land and sea. The nearby Tagus River supplies nutrient-rich freshwater, organic material, and terrestrial runoff into the coastal ecosystem, promoting high productivity in planktonic and benthic communities. This interaction creates a complex mosaic of habitats, including rocky shores, sandy sediments, and benthic grounds that support a wide range of benthic and pelagic species. The coastal zone serves as an ecological transition zone, where nutrient inputs from rivers influence local primary productivity and shape the composition and structure of food webs. These inputs lead to a significant mixing of energy sources, including terrestrial organic matter and marine phytoplankton, resulting in a highly interconnected ecosystem with both spatial and temporal variability. This environment supports a diverse range of species with different feeding strategies, from filter-feeding to predation, reflecting a highly productive and dynamic zone with vital biogeochemical and ecological processes. Cascais has a fishing port with about 30 vessels dedicated to octopus fishing, where fishermen use pots and traps. This study only considers vessels that use pots (the majority) to avoid problems with baited traps. The pots are made of plastic with concrete inside, so they sink.

2.2. Field

We conducted field collections over several months in collaboration with local fishermen to identify the main prey species available and potentially consumed by *Octopus vulgaris* in the Cascais region. Interestingly, some octopuses were still actively feeding at the time of capture. We worked closely with fishermen, recording their observations and insights. Prey items were directly collected from the contents of the pots, including shells and soft tissues, as well as the octopuses themselves—specifically prey or remains carried or pulled from the pots during capture. When the species was sampled, we collected all animals in late spring for analysis.

We carefully removed and stored the soft tissues of molluscs and crustaceans, while fish muscle tissue was extracted from the dorsal regions. This approach allowed us to assess available prey items in their natural environment and what octopuses were actively consuming or transporting at the time of capture, providing a targeted pool of potential dietary sources for subsequent isotopic analysis. We did not count the number of pots and prey found, but we sampled the most common ones that appeared. Other species appeared only once or twice, raising doubts about whether the animals ate them or were merely nearby and captured inside when the fishers pulled up the pots.

2.3. Sampling and sample preparation

After collection, all samples were rinsed with Milli-Q water, then freeze-dried and ground into a uniform powder using a mortar and pestle. The samples were subsequently weighed and placed into tin capsules for isotopic analysis. Specific procedures included: a) measuring key biometric parameters of each octopus—total length, mantle length, weight, sex, and maturation stage; b) extracting muscle tissue from octopus arms; c) removing shells from molluscs and isolating soft tissues; d) dissecting decapods to obtain their muscular tissues; e) collecting dorsal muscle tissue from fish. These meticulous procedures are designed to ensure the accuracy and reliability of our isotope measurements, accurately reflecting the dietary sources.

2.4. Laboratory procedures and stable isotope analysis (SIA)

All samples were thoroughly rinsed with Milli-Q water and freeze-dried. The dried samples were ground into powders, weighed, and placed in tin capsules. The elemental and isotopic composition of carbon and nitrogen was measured using a Flash EA 1112 Series elemental analyser connected to a Thermo Delta V isotope ratio mass spectrometer via a Finnigan Conflo II interface. δ13 C and δ15 N values are expressed in parts per thousand (‰) relative to standards—PDB limestone for δ13 C and atmospheric N_2 for δ15n. The precision of δ13 C and δ15 N measurements was better than 0.2 ‰.

2.5. Data analysis

Prey contributions to O. vulgaris's diet were estimated using a Bayesian stable isotope mixing model (SIAR; Parnell et al., 2008). The model included four potential prey sources: bivalve, fish, gastropod, and decapod. Before running SIAR, an R package based on two main Bayesian statistical methods commonly used in stable isotope ecology, isotopic signatures were adjusted for trophic enrichment using fractional values of + 0.4 % ± 1.3 ‰ for δ13 C and + 3.4 % ± 0.13 ‰ for δ15 N, in accordance with Post (2002).

2.6. Trophic levels and ecological roles

Trophic levels for each species were estimated from their δ^{15}N values obtained through tissue stable isotope analysis. Since there were no direct baseline δ^{15}N measurements in this study, the δ^{15}N value of 5.1 ‰, determined at the exact location (Cascais) in another study for particulate organic matter (POM) (Vinagre et al., 2012), was used. Trophic levels were calculated using the following formula: TL = 1 + δ^{15}N (‰) - 5.1 (POM value), where $\delta^{15}N_{specie}$ refers to the nitrogen isotopic value measured from tissue samples, δ^{15}N baseline was set at 5.1 ‰, and Δn was assumed to be 3.4 ‰, representing the typical trophic enrichment factor for δ^{15}N in marine ecosystems. All δ^{15}N values used in the calculations were derived from muscle tissue samples analysed by isotope ratio mass spectrometry. These trophic level estimates helped characterise the ecological roles of each species within the local coastal food web.

3. Results

3.1. Biological data of the octopus

The sampled *Octopus vulgaris* individuals exhibited a gender distribution of approximately 60 % males and 40 % females. The biological parameters of the 15 analysed individuals are as follows: the mean weight was 1626 g (±756 g), with a mean total length of 82 cm (±15 cm), and a mantle length of 14 cm (±3 cm). These individuals represent a typical adult population, ensuring our findings are relevant for understanding their feeding ecology.

3.2. Prey species selection

Based on our field observations and the contents of the sampled octopus pots, four main prey species were identified as likely key components of *O. vulgaris's* diet in the Cascais region. The species selected were a bivalve *Atrina fragilis*, a gastropod *Cymbium olla*, a crustacean *Polybius henslowii,* and a fish *Cepola macrophthalma* that was observed in the mouths of several captured octopuses and within the pots. Sampled:

- 6 exemplars of *Atrina fragilis*. The shells measure around 22 cm in length, indicating mature individuals living in sandy sediments.
- 15 exemplars of *Cymbium olla* (gastropod) with average shell length of 10.2 cm.
- 10 exemplars of *Polybius henslowii* (decapod) with 2–3 cm carapace length.
- 6 exemplars of *Cepola macrophthalma* (fish) with 20–25 cm length, with an elongated body suited for burrowing in sandy and muddy substrates.

Monitoring and sampling these prey items enabled us to obtain relevant isotopic signatures, ensuring the accuracy and ecological relevance of our diet estimations.

3.3. Stable isotope analysis

δ13 C indicates the primary carbon sources in the ecosystem. It demonstrates a declining trend with increasing latitude (Martino et al., 2022). δ15 N values act as markers of trophic level, with higher values signifying higher trophic positions (Cherel and Hobson, 2007). The territorial isotopic signatures of prey and predator samples within the convex hull imply potential diet connections. The isotope signatures of Octopus vulgaris tissues and potential prey sources (*A. fragilis*, *C. olla*, *P. henslowii*, and *C. macrophthalma*) are shown in Fig. 1. To account for isotopic fractionation during trophic transfer, we applied trophic enrichment factors (TEFs) of + 0.4 ‰ (±1.3 ‰) for δ13 C and + 3.4 ‰ (±0.13 ‰) for δ15 N, following Post (2002). These TEFs were used to adjust prey isotope signatures before inputting data into the Bayesian mixing model (SIAR; Parnell et al., 2008), resulting in the isotope plot shown in Fig. 1. Model estimates indicate that approximately 70 % of the *O. vulgaris* diet derives from A. fragilis, with *P. henslowii* contributing around 18 %. The remaining inputs include *C. olla* and *C. macrophthalma*, accounting for 7 % and 5 % respectively.

3.4. Trophic levels and ecological roles

The estimated trophic levels, based on δ1 ⁵N values and the assumed baseline of 5.1 ‰, ranged from approximately 2.12–4.15. The A. fragilis, a filter-feeding bivalve, occupied a lower trophic position ~2.12. The *O. vulgaris* resulting in a trophic level of approximately 2.74. The benthic eel-like species *C. macrophthalma* and the pelagic *P. henslowii* had trophic levels near 2.82 and 3.03, respectively. The *C. olla* exhibited the highest corresponding to a trophic level above 4, indicating its position as a top predator within the ecosystem.

4. Discussion

4.1. The significance of field-based prey identification

Integrating field collection data and collaboration with local fishers was key to accurately identifying the main prey of *O. vulgaris* in Cascais. This method ensured that the isotopic analysis focused on ecologically relevant prey items, representing real dietary components rather than just literature or assumptions. By examining octopus pots and observing feeding behaviour directly, our study provided a realistic view of *O. vulgaris*'s diet. This combined approach strengthened the reliability of isotope-based dietary estimates and offered crucial ecological validation, deepening our understanding of the species' role in this estuarine-coastal ecosystem. Field-based prey identification underscores the value of combining ecological observations with isotopic analysis to achieve accurate and meaningful insights into predator-prey relationships.

4.2. Identification and Characterisation of key prey species

The prey species chosen for isotopic analysis—*A. fragilis*, *C. olla*, *P. henslowii*, and *C. macrophthalma*—were identified through direct field observations and by examining octopus pot contents. A. fragilis shells indicate mature individuals living in sandy sediments, aligning with their known ecological niche. *C. olla* gastropods are typically found in benthic coastal habitats. *P. henslowii*, a small crab is a common benthic

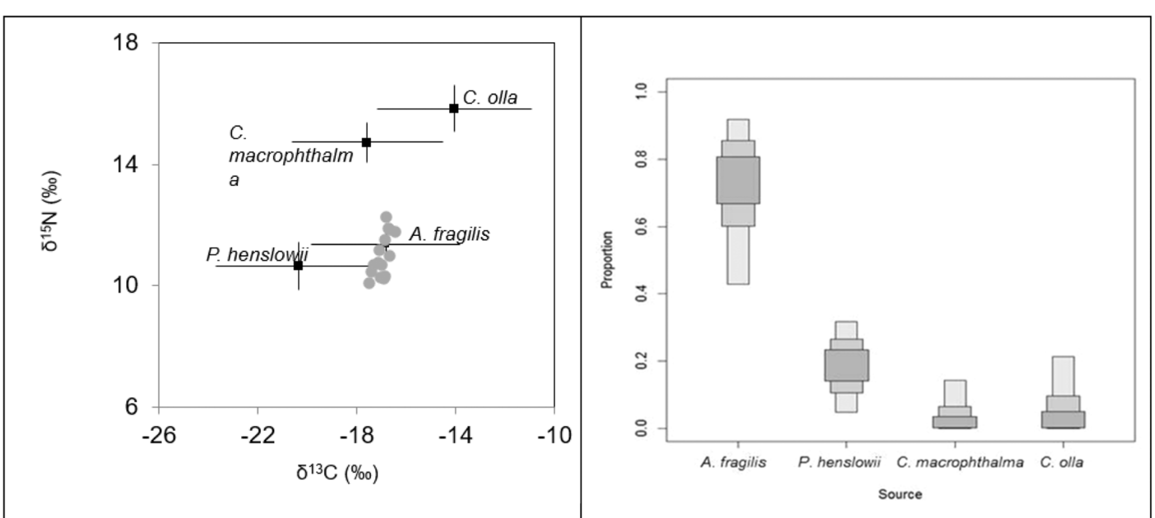

Fig. 1. – Fractionation values of + 0.4 % ± 1.3 for δ13 C and 3.4 % ± 0.13 for δ15 N were applied, following Post (2002) for the prey. Left: results of SIAR (95, 75, and 25 % credibility intervals) showing estimated contributions of food sources to the diet of Octopus vulgaris.

decapod in the region, while *C. macrophthalma* is an elongated burrowing fish that inhabits sandy-muddy substrates. These prey species cover various trophic levels and ecological niches, offering a broad view of *O. vulgaris*'s diet in this area. Their selection was crucial for interpreting isotopic signatures and understanding the diet composition within the local benthic ecosystem.

4.3. Interpretation of consumer diets using isotopic signatures and field observations

4.3.1. Bivalve

The credible intervals demonstrate the uncertainty in mixing models, but are corroborated by the bivalve *A. fragilis* as the main prey of the octopus. Its sturdy shells and size make it a frequent target, as octopuses can crack the shells to access the soft tissues inside. Its common presence in pots and field observations highlights its significance as a benthic prey resource. The $\delta^{15}N$ values with trophic levels around 2.12, indicate these organisms function as suspension-feeding consumers at lower to intermediate trophic levels. The $\delta^{13}C$ value suggests a connection to benthic and coastal areas. Since no other studies have reported similar data, $\delta^{15}N$ and $\delta^{13}C$ values cannot be compared with existing literature. This might be the first documented case of stable isotope analysis in this species.

4.3.2. Gastropod

C. olla are characterised by their inability to fully retract into their shells, leaving soft parts exposed and more vulnerable to predators. Their habitat among rocky and sandy substrates, coupled with their moderate size, makes them easily accessible prey. This animal showed the highest $\delta^{15}N$, indicating it occupies a top-predatory position with a trophic level above 4.0, consistent with a predator feeding on organisms lower in the food chain, likely a predator and necrophagous. The $\delta^{13}C$ value appears to be more associated with benthic and coastal zones, as indicated by its relatively high level.

To our knowledge, no existing studies have examined the $\delta^{15}N$ and $\delta^{13}C$ signatures for this species, making direct comparisons impossible. This may be the first report of stable isotope data for this organism.

4.3.3. Crustacean

The pelagic crab has a trophic level of about 3.03, indicating a diet primarily of plankton or small benthic invertebrates. Its $\delta^{15}N$ value is slightly lower than the 7.33 ‰ reported in literature but remains higher than other studies, which range from 5.8 ‰ to 6.2 ‰ (Table 1). This elevated $\delta^{15}N$ suggests the species occupies an intermediate trophic position, likely preying on higher-level organisms or influenced by terrestrial nitrogen sources from the nearby River Tagus. The $\delta^{13}C$ values indicate that our value is the highest compared to similar studies (Table 1). The $\delta^{13}C$ measured here exceeds typical values for similar species or habitats, which generally range from −17.8 ‰ to −19.7 ‰. This enriched $\delta^{13}C$ signature may reflect a stronger influence of pelagic-estuarine carbon sources associated with terrestrial input, especially in Cascais, where the proximity to the River Tagus could enhance terrestrial organic matter in the food web.

4.3.4. Fish

C. macrophthalma was found in the mouths of several caught octopuses and inside the pots. In the Bay of Marseille (Mediterranean), this benthic opportunist mainly preys on decapod larvae, calanoid copepods, and small amounts of gelatinous zooplankton (Chen et al., 2022a, 2022b). It is an important part of the diet for marine predators like *Tursiops truncatus* (Giménez et al., 2017), influencing the marine ecosystem. The $\delta^{13}C$ value measured is less depleted than other reported values, which range from about −17.53 ‰ to −21.1 ‰, mostly around −20 ‰. This relatively enriched $\delta^{13}C$ indicates a stronger influence of benthic or coastal carbon sources, possibly due to proximity to terrestrial inputs or sedimentary organic matter linked to river influence, such as from the nearby River Tagus. Typically, enriched $\delta^{13}C$ values are seen in organisms that rely on benthic autotrophs or sediment organic matter, contrasting with the more depleted pelagic planktonic signatures. Therefore, this higher $\delta^{13}C$ suggests the species mainly feeds in benthic habitats or consumes material influenced by terrestrial and coastal carbon pathways, reflecting its ecological niche and local environmental conditions. The $\delta^{15}N$ with trophic levels, indicating a low-to-moderate predatory level, consistent with a benthic omnivore or small predatory fish.

This species' $\delta^{15}N$ value is among the higher reported, ranging from 8.14 ‰ to 12.0 ‰. The elevated $\delta^{15}N$ suggests that the species occupies a relatively high trophic position, feeding on prey higher up the food chain or enriched in $\delta^{15}N$ due to their dietary sources. This may also reflect environmental influences, such as nitrogen input from terrestrial or human sources common in river-influenced coastal areas. Overall, the higher $\delta^{15}N$ indicates its significant role as a predator or top-level consumer in its ecological niche.

4.3.5. Octopus

The findings indicate that *O. vulgaris* is a dietary generalist, feeding on a wide array of prey, with approximately 70 % of their diet consisting of A. fragilis. Other prey, such as *P. henslowii*, *C. olla*, and *C. macrophthalma*, make smaller but still important contributions. Long-term isotopic analysis provides a broader view of dietary diversity than traditional stomach content analysis, which can underestimate soft-

Table 1
Our values and values found in literature.

N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Local	Author
Atrina fragilis				
6	-17.2 ± 0.4	8.0 ± 0.1	Our study	Our study
Cymbium olla				
	-14.5 ± 0.5	12.4 ± 0.5	Our study	Our study
Polybius henslowii				
10	-20.8 ± 0.74	7.2 ± 0.5	Our study	Our study
4	-17.8 ± 0.6	6.2 ± 0.2	Berlenga Island (Portugal)	Ceia et al. (2014)
6	-18,27	7.33	Ria Coruña (North Spain)	Carabel et al. (2006)
2	-19.13 ± 0.04	7.23 ± 0.46	Strait of Gibraltar	Sorell et al. (2017)
9	-19.7 ± 0.6	5.8 ± 0.2	Cascais (same place)	Vinagre et al. (2012)
Cepola macrophthalma				
6	-18.0 ± 0.4	11.3 ± 0.4	Our study	Our study
9	-17.53 ± 0.27	10.05 ± 0.40	Gulf of Cadiz (Spain, Atlantic)	Giménez et al. (2017)
5	-18.2 ± 0,3	12.0 ± 0.3	Bay of Biscay (Atlantic)	Chouvelon et al. (2012)
2	-20.4 ± 0.05	8.16 ± 0.04	Catalan Sea (Mediterranean)	Lloret-Lloret et al. (2020)
3	-20.42 ± 0.05	8.14 ± 0.04	Catalan Sea (Mediterranean)	López et al. (2016) Barría et al., (2018)
18	-20.5 ± 0.8	8.8 ± 0.6	Marseille (Mediterranean)	Chen et al. (2023)
9	-21.1 ± 2.5	9.3 ± 0.4	Marseille (Mediterranean)	Chen et al. (2022a), (2022b)
Octopus vulgaris				
15	-17.0 ± 0.3	11.02 ± 0.7	Our study	Our study
11	-16.10 ± 0.73	11.49 ± 0.98	Gulf of Cadiz (Spain, Atlantic)	Giménez et al. (2017)
5	-16.9 ± 0.6	11.1 ± 0.4	Bay of Biscay	Chouvelon et al. (2012)
3	-18.4 ± 1.37	8.62 ± 0.46	Catalan Sea (Mediterranean)	Barría et al., (2018)
4	-16.36	10.15	Ria Coruña (Iberian Coast)	Carabel et al. (2006)

bodied or highly digested prey. The prevalence of *A. fragilis* might reflect its abundance or preference, although the variety of prey sources suggests adaptable feeding behaviour. Additionally, individual prey choices result in varied foraging strategies, likely affected by factors like maturity or prey availability.

The $\delta^{13}C$ value for this species falls within the reported range of −16.10 ‰ to −18.4 ‰ (Table 1). This relatively enriched $\delta^{13}C$ signature suggests a strong influence from benthic or coastal carbon sources, possibly due to nearby terrestrial inputs or specific organic matter typical in sediment-rich environments. The less negative $\delta^{13}C$ value indicates that this species likely derives its carbon from benthic autotrophs or sediment-associated sources, rather than pelagic, planktonic origins, which usually have more negative values. In summary, the $\delta^{13}C$ value reflects the species' ecological niche and dependence on coastal carbon sources, shaped by local environmental conditions affecting its habitat.

4.3.5.1. O. vulgaris values of $\delta^{15}N$ and trophic level classifies it as an intermediate predator. The $\delta^{15}N$ value for this species is relatively high compared to other reported measurements, which range from 8.62 ‰ to 11.49 ‰. This elevated $\delta^{15}N$ suggests a higher trophic level, indicating a diet that includes prey from upper trophic levels or exposure to enriched nitrogen sources. Such enrichment could result from the species consuming higher-trophic organisms or benefiting from nutrient inputs typical of riverine or coastal ecosystems. Consequently, this $\delta^{15}N$ value supports the idea that the species serves as a predator or top consumer within its local food web, shaped by the ecological conditions of its environment.

A previous study in the same region by Rosa et al. (2004), using stomach content analysis, found that about 67 % of prey were decapods, while only 7 % were bivalves. This stark difference highlights a key limitation of stomach content methods: they only detect intact prey that can be identified during digestion. Cephalopods typically crush and extensively digest their prey, often leaving only fragments of shells or other hard parts, which results in underestimating prey types that are easily broken down or fragmented.

In contrast, stable isotope analysis offers a comprehensive view of the octopus diet over extended periods. Our results indicate that bivalves constitute a much larger portion of the diet than stomach content analysis suggests, highlighting the importance of using multiple methods for a fuller understanding of feeding ecology. This discrepancy underscores that relying solely on stomach content data can produce biased or incomplete insights into dietary habits, particularly for species like octopuses that rapidly fragment their prey.

4.4. Implications for ecosystem dynamics and resource management

This study emphasises the vital role of *A. fragilis* and benthic invertebrates in *O. vulgaris*'s diet, highlighting their significance in the local benthic food web of Cascais. The prominence of *A. fragilis* in the octopus's diet suggests it acts as a key energy link, connecting primary benthic production with higher predators. Additionally, the notable presence of crustaceans like *P. henslowii* points to a flexible foraging approach that involves multiple trophic pathways, demonstrating the species' opportunistic feeding habits within a dynamic estuarine-coastal environment.

These findings have wider ecological significance, highlighting the linkages among benthic invertebrate populations, predators like *O. vulgaris*, and the role of estuarine inputs, including terrestrial organic matter, in shaping food web structure and nutrient transfer. The elevated $\delta^{15}N$ signatures in various taxa indicate nutrient enrichment from rivers and human activities, which may influence trophic relationships, prey availability, and predator-prey interactions within this ecosystem.

Understanding these trophic connections is essential for effective ecosystem management and conservation efforts in the region. Since *O. vulgaris* holds ecological and economic significance, knowledge about its diet and trophic level can guide sustainable fishing and habitat preservation, particularly in systems strongly affected by estuarine influences. Additionally, highlighting the importance of benthic resources underscores the necessity of protecting benthic habitats and reducing human impacts that could disturb vital parts of the food web.

4.5. Limitations and future directions

Despite the valuable insights from this study, several limitations should be recognised. First, the spatial and temporal scope of sampling was limited, which could affect how representative the dietary and isotopic data are. The baseline isotopic signatures used in the mixing models, mainly derived from literature and limited local sampling, may not fully reflect the spatial variability of primary producers and basal resources within the estuarine-coastal system. Future research should involve systematic sampling of primary producers, particulate organic matter (POM), and other basal sources across different zones and seasons to improve baseline correction and diet estimations. Additionally, combining methods such as stomach content analysis, molecular diet markers, and stable isotope analysis of additional tissues would provide a more comprehensive understanding of trophic interactions. These approaches would help clarify short-term dietary changes and refine prey contribution estimates over various timescales. Future studies should focus on expanding sampling efforts, including a wider range of basal sources, employing advanced modelling techniques, and integrating multiple methodologies to achieve a thorough understanding of the food web dynamics in the region.

CRediT authorship contribution statement

Marques João: Writing – review & editing, Supervision, Methodology, Funding acquisition. **Sónia Seixas:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Alexandra Baeta:** Writing – original draft, Methodology, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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