



Mechanical properties and elemental composition of the beak in selected cephalopod species: patterns and variability

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ABSTRACT

Cephalopod beaks are remarkable organic structures that play a crucial role in the feeding ecology of these marine molluscs. This study investigates the mechanical properties, microstructure, and elemental composition of beaks from four commercially available cephalopod species: *Eledone cirrhosa*, *Sepia officinalis*, *Loligo vulgaris*, and *Sepioteuthis lessoniana*. Using nanoindentation, we measured the elastic modulus of the rostrum, revealing that lower beaks are stiffer than upper beaks across all species. Notably, *L. vulgaris* exhibited the highest stiffness. The study highlights significant intra- and interspecific variability in beak properties, suggesting ecological implications regarding diet and environmental factors. Scanning electron microscopy (SEM) showed a fibrous microstructure with nanoparticles of different sizes, while energy dispersive spectroscopy (EDS) identified carbon, oxygen, and nitrogen as primary elements, along with trace elements like silicon and calcium. These initial results suggest that the relationships between beak structure, composition, and biomechanical properties are likely to be complex and species-specific, underscoring the need for more comprehensive analyses to better understand beak function and its adaptive implications. This research provides new baseline data for comparative studies on cephalopod functional morphology and raises the potential of beaks as tools for ecological and environmental monitoring. We recommend that future studies incorporate larger and developmentally diverse samples to refine our understanding of cephalopod feeding adaptations and their interaction with changing marine environments.

1. Introduction

Found in all seas and oceans around the world from the surface to the abyss, cephalopods are renowned for their remarkable intelligence, camouflage abilities, and diversity. Even though this fascinating group of marine molluscs is widely known by its most popular representatives such as octopuses, squids and cuttlefish, over 800 species are found today with a wide diversity in size and lifestyle (Fig. 1A) (Anderson et al., 2021; Jereb et al., 2005; Jereb et al., 2010; Jereb et al., 2014). Due to their pivotal position in the trophic chain, being both important prey

and predators (Clarke, 1986; Boyle and Rodhouse, 2008), they hold a significant role in marine ecosystems.

Almost all cephalopod species are considered carnivorous predators, and some have been observed feeding on a large diversity of prey (Rodhouse et al., 1996; Cherel et al., 2005; Villanueva et al., 2017). As most Octopodiformes are benthic, living close to the sea floor, they are opportunistic and capable of feeding on hard prey (bivalves or crustaceans), whereas Decapodiformes are mostly pelagic, swimming higher up in the water column and having a softer diet composed largely of fishes (Nixon, 1987).

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The unique cephalopod feeding system is enclosed in the buccal mass and includes upper and lower beaks (Fig. 1B and C) surrounding a tongue-like radula (Messinger et al., 1999), all moved by a set of masticatory muscles (Boyle et al., 1979; Kear, 1994; Uyeno et al., 2005; Roscian et al., 2023). The well described shape diversity of cephalopod beaks is extensively used in taxonomy (Clarke, 1986; Xavier et al., 2018). However, a recent study found that beak shape is not only driven by phylogeny but also correlates with trophic level and habitat (Roscian et al., 2022), making it useful for further ecological studies.

In coleoid cephalopods, i.e. species with no external shell, the beak is fully organic, composed of a chitin-protein complex with no inclusion of minerals (Miserez et al., 2007; Miserez et al., 2008; Miserez et al., 2010; Tan et al., 2015). The beak grows throughout the entire life of the animal by the successive addition of lamellae (Perales-Raya et al., 2010; Arkhipkin et al., 2018; Guerra-Marrero et al., 2023). Lamellae are produced by a thin layer of cells called beccublasts, located between the insertion of the masticatory muscles and the beak (Dilly and Nixon, 1976). As a result, the oldest layers are found at the tip of the rostrum, while the most recently deposited layers form the more posterior sections (Perales-Raya et al., 2010; Liu et al., 2017).

Cephalopod beaks are among the stiffest fully organic materials on earth (Miserez et al., 2008). They display a stiffness gradient from the hard tip, incorporating a larger proportion of protein, to the more flexible posterior parts including a greater amount of water, while the chitin content stays constant (Miserez et al., 2008; Tan et al., 2015). This gradient correlates with the tanning pattern of the beaks that varies from one species to another and progresses with age. Adult specimens have a wider tanned area than juveniles, and the color of the chitin is hypothesized to be linked to the stiffness of the material (Miserez et al., 2008).

To date, beak mechanical properties have been measured in four species: *Dosidicus gigas* (Miserez et al., 2008), *Pareledone turqueti* and *Adelieledone polymorpha* (Matias et al., 2019), and *Sepia officinalis* [juveniles vs. adults; 28]. The commonly accepted hypothesis is that mechanical properties should be linked to prey toughness with the need for a reinforced rostrum to bite into harder prey such as crustaceans (Matias et al., 2019; Xavier et al., 2023). However, intraspecific variability is unknown, and the suggested link between diet and beak stiffness remains to be tested more broadly.

Trace elements, which are naturally occurring but increasingly

prevalent due to human activities, can be either essential or toxic at high concentrations (Jakimska et al., 2011; Sen and Peucker-Ehrenbrink, 2012). They accumulate in organisms and can increase in level through food webs, impacting marine species such as cephalopods (Xavier et al., 2023; Szykowska et al., 2018). Recent studies have expanded the analysis of these elements from cephalopod soft tissues (Bustamante et al., 2000; Pierce et al., 2008; Lischka et al., 2020; Seco et al., 2020) to cephalopod beaks, which are useful for ecotoxicological [(Xavier et al., 2016; Queirós et al., 2020), biogeographical (Fang et al., 2019; Northern et al., 2019), and ecological research (Matias et al., 2019)]. While mercury concentrations in beaks have been well-studied (Matias et al., 2019; Xavier et al., 2016; Queirós et al., 2020; Matias et al., 2020), research on other trace elements is less common. Techniques such as solution-based and laser ablation inductively coupled plasma mass spectrometry have been used to measure a broad range of elements, revealing variability in detection capabilities and beak section differences (Xavier et al., 2016; Queirós et al., 2020; Fang et al., 2019; Northern et al., 2019). Beak analyses offer insights into individual life stages, diet, and migration patterns, though they present challenges due to lower trace element concentrations compared to other tissues and methodological limitations. Understanding trace element distribution in beaks is, however, crucial for studying environmental contamination and managing cephalopod fisheries (Xavier et al., 2023).

The aim of this study is to explore the intra- and interspecific variability in the mechanical properties of cephalopod beaks, and to explore potential differences in structural characteristics linked to these properties. To do so, we chose four common commercially available species representing three main coleoid orders: *Eledone cirrhosa*, *Sepia officinalis*, *Loligo vulgaris* and *Sepioteuthis lessoniana* (Fig. 1). To stay consistent in our comparison, we chose the rostrum as our focus area. We determined mechanical properties of the rostrum using nano-indentation and explored the material structure and elemental composition using Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS).

2. Materials and methods

2.1. Sampling

Four species were considered in this study: *Eledone cirrhosa* (n = 10),

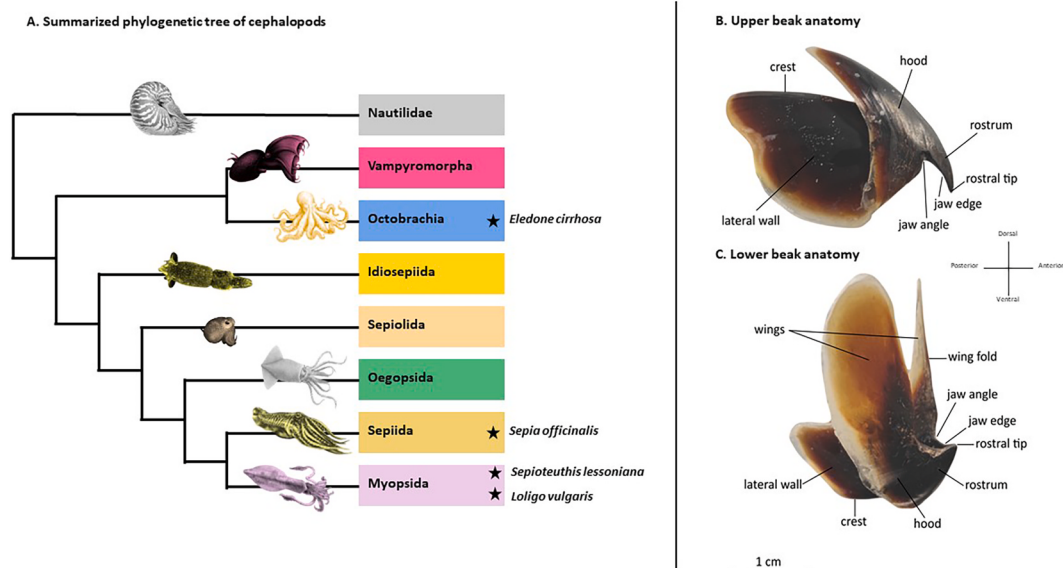


Fig. 1. Sampling and beak anatomy. A) Summarized phylogenetic tree of cephalopods based on Anderson and Lindgren (Anderson et al., 2021) with stars representing the species included in this study. Illustration explaining the anatomical nomenclature of the cephalopod B) upper and C) lower beak, using *Sepia officinalis* as a model species.

Sepia officinalis (n = 2), *Loligo vulgaris* (n = 8) and *Sepioteuthis lessoniana* (n = 2), representing three major cephalopod families – Octobranchia, Sepiida and Myopsida (Fig. 1). Species were selected based on both commercial availability and their ecological and morphological diversity. While accessibility was a practical factor, these species also represent distinct ecological niches (benthic vs. pelagic lifestyles), different feeding strategies, and a range of body sizes and beak morphologies. This combination allowed us to capture a broad spectrum of functional variation within cephalopods and to explore potential biomechanical and structural trends across taxonomic and ecological contexts. Due to their availability, *E. cirrhosa* and *L. vulgaris* were chosen to investigate intraspecific variability and therefore more specimens were procured. All specimens were obtained from local fishmongers in France and England (north Atlantic) and were adults. Full bodies were weighed immediately after purchase, and mantle length was measured (Supplementary Table 1). For each specimen, the buccal mass was dissected and both upper and lower beaks were extracted. The total beak length, from rostrum tip to posterior edge, was measured on each lower and upper beak. All samples were stored in the freezer prior to analysis. For comparative purposes, the upper beak of a juvenile *S. officinalis* (2 months old), previously analyzed in Souquet et al. (2023), was also imaged by SEM.

2.2. Nano-indentation

For each specimen, both the upper and lower fresh beaks were embedded separately in cold cure epoxy resin (Buehler, Germany). The samples were then polished to expose the sagittal section, using silicon carbide paper and aluminum oxide slurries with a final particle size of 0.05 μm , following a protocol from Moazen et al. (2015), and as used on cephalopod samples in Souquet et al. (2023). Nanoindentation was then performed at room temperature using an Anton Paar system (UNHT3, Anton Paar GmbH, Switzerland) with a Berkovich diamond tip [see e.g. (Ebenstein and Pruitt, 2006)]. To ensure reproducibility between species, the rostrum was chosen as the area of interest, and a line from the tip of the rostrum to the apex, representing the junction between the wall and the hood, was created (Fig. 2A) (Perales-Raya et al., 2010). Along this line, indentations were performed every 100 μm under force-controlled linear loading to a force of 50 mN at 100 mN/min,

followed by a 10s hold. The elastic modulus was calculated using the standard Oliver-Pharr method (Oliver et al., 1992). Here, the Poisson's ratio of the indented tissue and indenter tip were assumed to be 0.3 and 0.07 respectively, with the elastic modulus of the indenter tip being 1140 GPa (based on the manufacturer's data). Differences in elastic modulus between upper and lower beak and differences between species were tested using a pairwise PERMANOVA with 9999 permutations. Potential correlations between elastic modulus and size and shape parameters were investigated using linear regressions.

2.3. SEM images

Upper beaks were freeze-fractured along the sagittal line aiming at the center of the rostrum. Rostrum microstructures were characterized for each species by SEM (Zeiss SEM Gemini 360, Germany).

2.4. EDS analysis and mapping

Chemical composition scans were conducted using a Zeiss SEM Gemini 360 equipped with an EDS detector. Two types of samples were used for the chemical mapping. Bulk samples, the same as those for SEM images, were used to determine the macroscopic composition of the oral, central and aboral areas of the beak for each described species. The composition of those areas was calculated as an average of multiple measurements. For precise examination of the chemical composition of particles, thin samples were used. Samples were carefully extracted using a scalpel from freeze-fractured upper beak rostra, specifically targeting areas of interest. The extracted material was then deposited onto a copper mesh for analysis. To minimize the risk of beam-induced damage and signal overlap, the analyses were performed on thin sections of the samples in transmitted mode. This approach ensured accurate detection of the chemical composition by minimizing the volume where the EDS signal is generated. This method was applied to two upper beaks per species. It has to be noted that EDS is not capable of detecting hydrogen (H) so this is left out of the quantification (Piergiorgianni et al., 2024).

For the transmitted samples, elemental mapping was performed with an accelerating voltage of 7.5 kV and a dwell time of 53.2 ms. The pixel size of the generated maps was 9.8 nm. Quantification was carried out

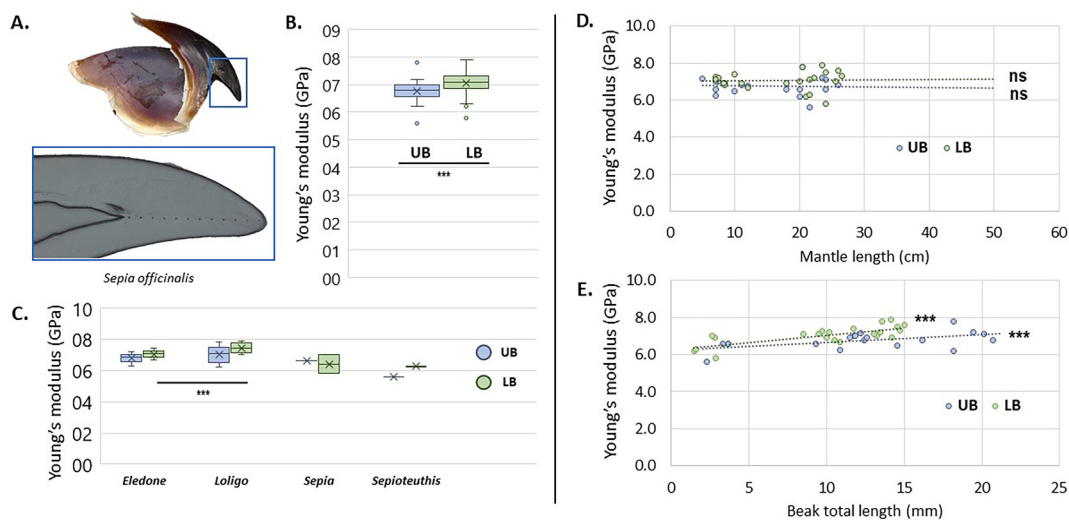


Fig. 2. Mechanical properties of the cephalopod beak rostrum. A) Illustration of the beak rostrum, using *Sepia officinalis* upper beak as a model, and of a sagittal section in the same orientation with visible nanoindentations. B) Boxplot comparing Young's modulus (in GPa) for the upper (UB) and lower (LB) beak for all specimens together. Stars show significant differences between UB and LB. C) Boxplot comparing Young's modulus (in GPa) for UB and LB in all species separately. Stars show significant differences between *Eledone cirrhosa* and *Loligo vulgaris*. D) Linear regression between mantle length (in cm) and Young's modulus (in GPa) for UB and LB separately. Ns = non-significant. E) Linear regression between beak total length (in mm) and Young's modulus (in GPa) for UB and LB separately. Stars indicate significant regression.

by analyzing spectra reconstructed from regions identified as particles or particle-free matrix areas, based on elemental intensity maps for each selected element line. For the freeze-fractured samples analyses were performed using point scans at an accelerating voltage of 7.5 kV, with an acquisition time of 120 s per point.

3. Results

3.1. Mechanical properties

The lower beak rostrum had a higher elastic modulus (LB mean = 7.1 GPa) than the upper beak (UB mean = 6.7 GPa), indicating stiffer material (Fig. 2B). This difference was observed across the sample as a whole (PERMANOVA, $F = 7.659$, $P = 0.0037$) but also in each species taken separately.

The mean standard deviation observed in the rostrum was 0.2 GPa showing homogeneous values across the rostrum. In the two species chosen to investigate intraspecific variation, the range at the intraspecific level was greater than 1 GPa indicating a high variability. However, a significant interspecific variation was observed between *Eledone cirrhosa* and *Loligo vulgaris* (PERMANOVA, $F = 7.788$, $P = 0.0022$), with the latter having higher modulus values (Fig. 2C). For the other species, the small sample size prevented assessment of significant differences. However, values for *Sepia* seemed closer to those for *E. cirrhosa*, and the Young's modulus of the beaks in *Sepioteuthis lessoniana* is lower than that of all other species.

There was no significant correlation between modulus and mantle size or body weight (Fig. 2D). A small but significant correlation was observed between rostrum length and elastic modulus with longer rostra being stiffer (Fig. 2E; Lower Beak: $R^2 = 0.5$, $P = 0.0003$; Upper Beak: $R^2 = 0.26$, $P = 0.022$). The slope of the regression for lower beaks was greater (6.41) than that for upper beaks (6.25).

3.2. SEM images

In all species, we saw fibrous material composing the beak (Fig. 3). All beaks studied displayed a general orientation of lines running from the edges of the beak, curving towards the posterior part and the center of the rostrum, and perpendicular to the expected direction of the growth lines. These probably correspond to the individual material fibers deposited by each beccublast, the cells secreting the beak, as described in Dilly and Nixon (1976). The non-fibrous and water repellent outer layer described by Miserez et al. (2008) was not observed in our samples. At high magnification (micrometric scale), different textures were observed, with areas that looked more fibrous, others that looked porous, and yet some that were smoother and appeared more solid (Fig. 4). No major consistent difference was observed between species in the general structure.

Under high magnification, small particles were observed in the aboral region of the rostrum in *Sepia officinalis* and all regions of the rostrum in *Sepioteuthis lessoniana* (Fig. 4). In *S. officinalis*, we observed mostly small particles with a diameter of 20–70 nm, and some particles with a diameter of 70–130 nm. In *S. lessoniana*, we observed 2 sets of particles with a diameter of 20–50 nm and 70–140 nm covering a similar area. Additionally, in this species we observed a few large particles with a diameter of 150–200 nm.

3.3. EDS analysis

The primary elements identified in the beak were Carbon (C), which constituted approximately half of the material, followed by Oxygen (O) and Nitrogen (N) (Fig. 5). Additionally, a small proportion of trace elements was present, varying among species. Specifically, trace elements represented about 1 % of the material in juvenile *Sepia*, approximately 2 % in *Eledone cirrhosa* and *Loligo vulgaris*, and about 4 % in *Sepioteuthis lessoniana* and adult *Sepia officinalis* (Fig. 5; Supplementary Table 2). These species contained particles with variable trace element compositions. The trace elements identified included Calcium (Ca), Phosphorus (P), Sulfur (S), Silicon (Si), Aluminium (Al), Potassium (K), Magnesium (Mg), and Titanium (Ti) (Fig. 6). Notably, there were differences in trace element distribution within different regions of the same rostrum, with no clear pattern emerging, indicating possible variability. Among the trace elements, the most prevalent across all species were Silicon (Si), Calcium (Ca), Phosphorus (P) and Sodium (Na).

Analysis of particles from *Sepia officinalis* and *Sepioteuthis lessoniana* revealed that the majority were rich in Carbon (C), Oxygen (O), Calcium (Ca), Nitrogen (N), and Phosphorus (P), while others also include Silicon (Si) (Fig. 6). Additionally, a particle from *S. officinalis* appeared to contain Titanium (Ti), Oxygen (O) Potassium (K) Chlorine (Cl) and Iron (Fe). Chemical maps (Fig. 6) and plots (Fig. 7) showed that certain elements are visibly correlated. Additionally, we evidenced distinct differences between specimens collected from similar areas of the beak.

4. Discussion

4.1. Lower vs. upper beak stiffness

Our results indicate that the rostrum of the lower beak is significantly stiffer than that of the upper beak, which contrasts with findings from Souquet et al. (2023) on *Sepia officinalis*. In that study, the upper beak exhibited higher stiffness values. This discrepancy can be attributed to several factors. First, our study included a larger number of specimens, allowing us to account for intraspecific variability that was not considered in Souquet et al. (2023), where only one specimen per group was analyzed. Moreover, we measured the entire length of the rostrum with multiple indentation points, providing a more comprehensive dataset

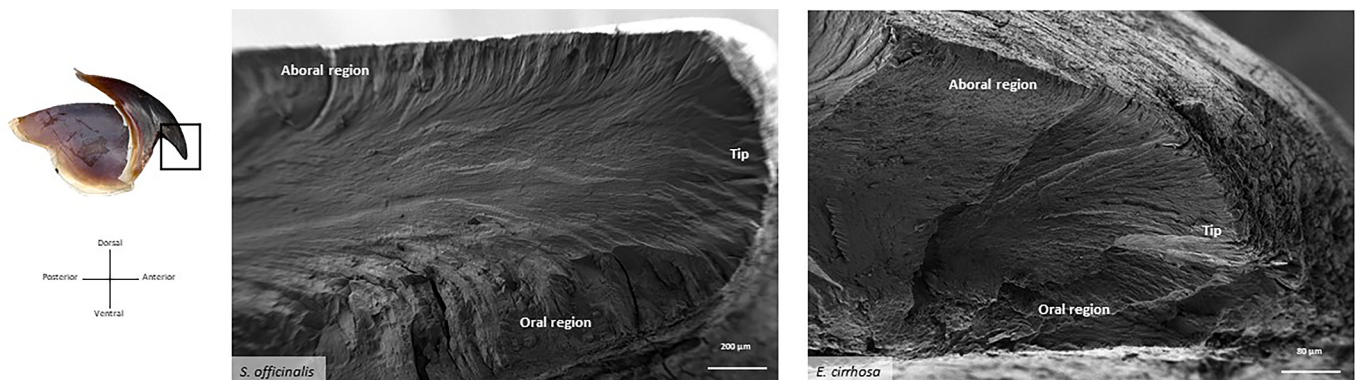


Fig. 3. SEM images of the macrostructure of the upper beak rostrum in a freeze-fractured sagittal plane of *Sepia officinalis* (left, scale bar: 200 μm) and *Eledone cirrhosa* (right, scale bar: 80 μm).

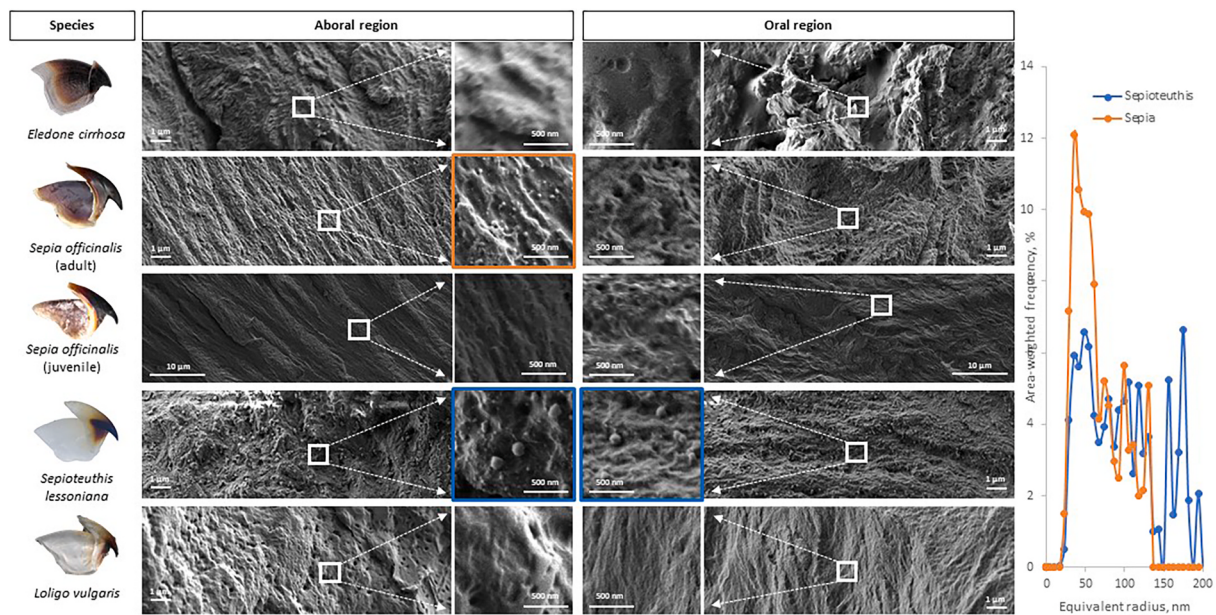


Fig. 4. SEM images of the microstructure of the upper beak rostrum in a freeze-fractured sagittal plane for all study species. Magnified regions are presented in the aboral and oral region of the upper beak rostrum. Orange outlines highlight the images where particles can be observed. Plot on the right shows size distribution of particles quantified from multiple areas. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

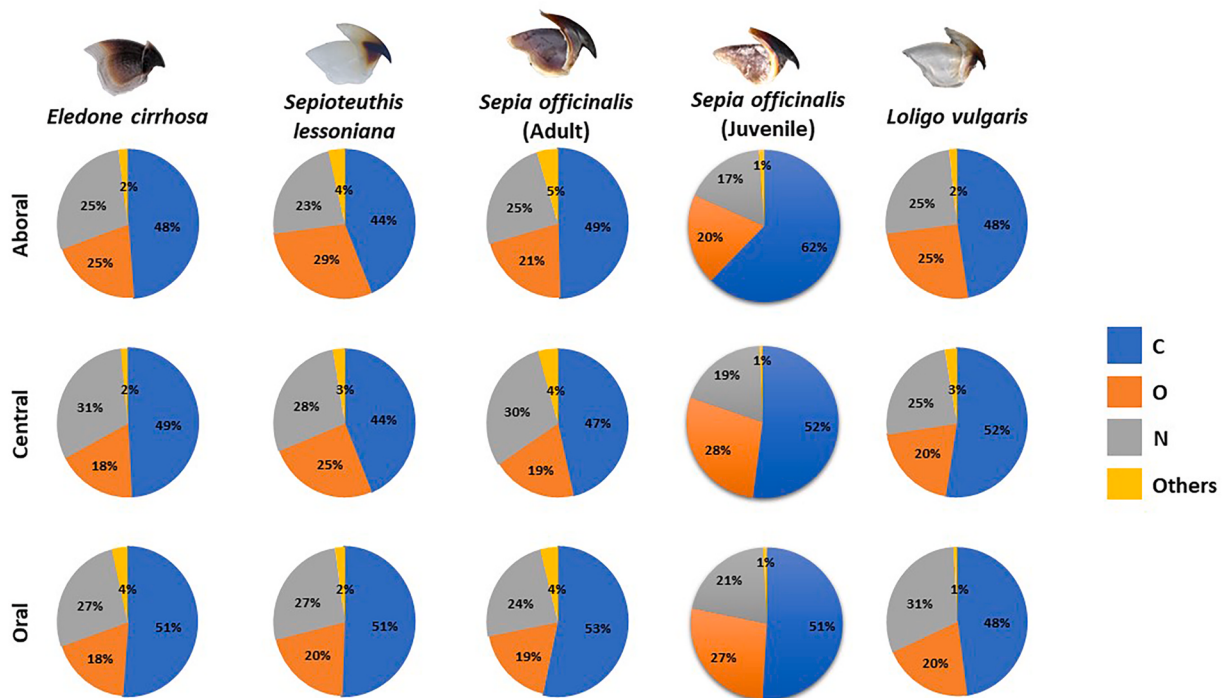


Fig. 5. SEM-EDS results showing the main elements present in the upper beak rostrum of all study species and of a 2-month-old juvenile of *Sepia officinalis*, and their proportions, in different regions of the rostrum.

compared to [Souquet et al. \(2023\)](#), which treated the rostrum as homogeneous and focused on a few specific zones, particularly the tip. This finding challenges our initial hypothesis that the upper beak, due to its generally more elongated shape in most species and its primary role in puncturing prey ([Wang et al., 2024](#)), would require greater mechanical stiffness to prevent damage during feeding. It suggests that our current understanding of the functional roles of the upper and lower beaks in prey capture and processing is incomplete. [Altman and Nixon \(1970\)](#) observed that animals missing their lower beak exhibited a greater

reduction in feeding efficiency compared to those missing their upper beak, highlighting the critical role of the lower beak in food acquisition. However, the underlying reason for the higher stiffness in the lower beak remains unclear. Further studies are necessary to determine whether this increased stiffness is an adaptation to greater mechanical stress during feeding or if it results from developmental processes.

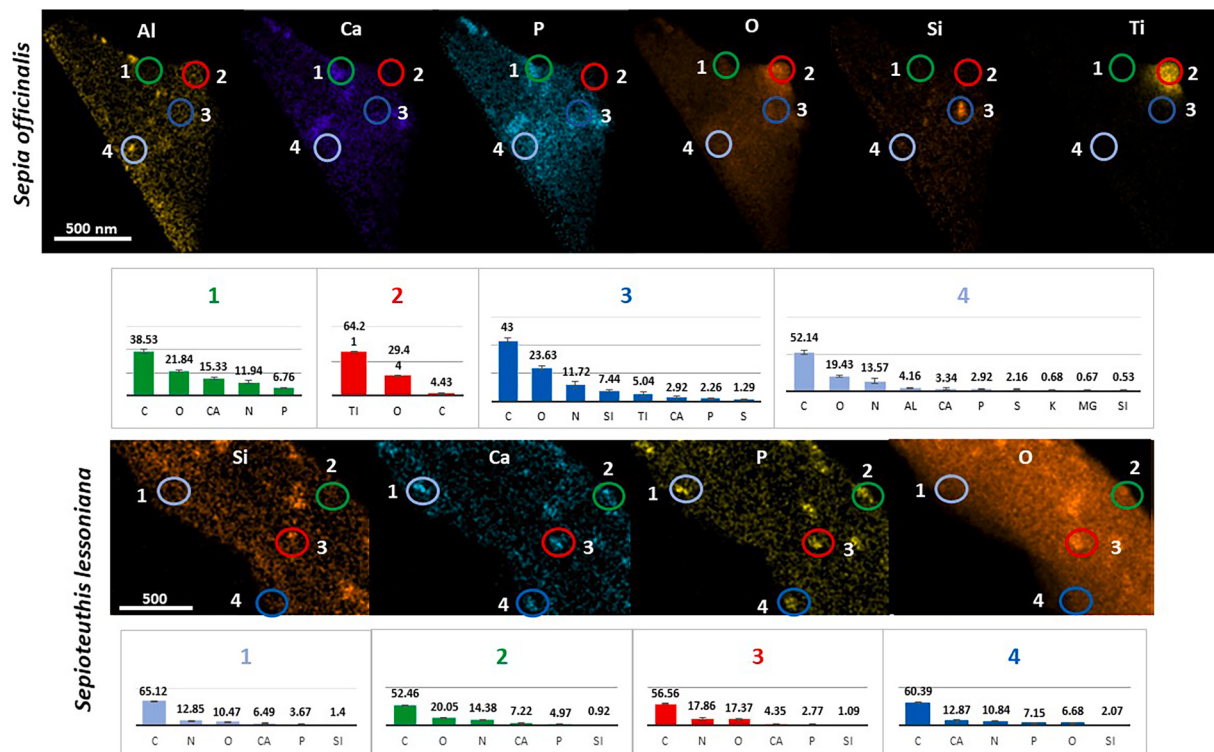


Fig. 6. SEM-EDS results showing the elements present in small target regions of thin samples extracted from the upper beak rostrum in the two species where particles were observed: *Sepia officinalis* and *Sepioteuthis lessoniana*. The images show the elemental mapping of the studied elements, with intense colours showing element rich regions. Each coloured circle represents an area of interest, and its detailed elemental composition is displayed in the corresponding histograms. Error bars illustrate the uncertainty for each element. Only elements present in the region are displayed in the histograms. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

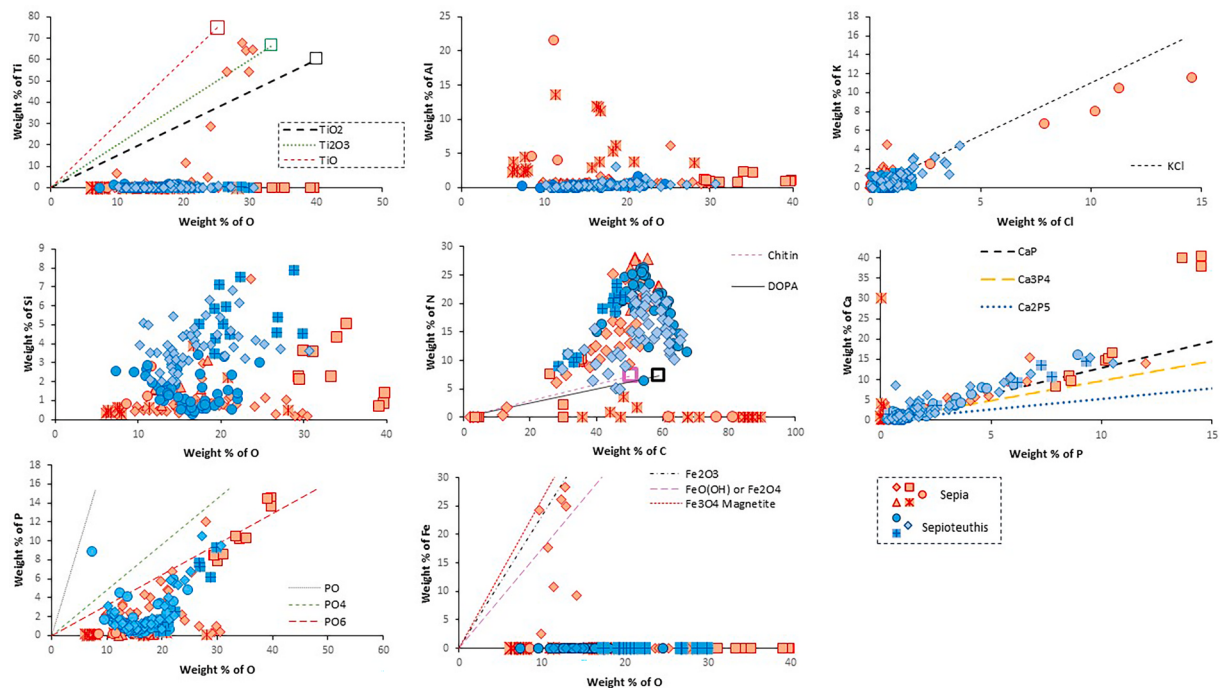


Fig. 7. SEM-EDS results showing the percentage weight (%) of the main elements observed in thin samples extracted from the upper beak rostrum *Sepia officinalis* and *Sepioteuthis lessoniana*. Each point represents a region of interest. Larger features were divided into multiple regions. Each marker shape represents a different sample extracted from the same beak.

4.2. Intra- and interspecific variability of beak stiffness

The inter- and intraspecific variation in beak stiffness observed in this study highlights important nuances in interpreting mechanical properties across cephalopod species. Previous research by [Matias et al. \(2019\)](#) found that *Pareledone turqueti* beaks had a Young's modulus of 4.99 GPa, while *Adelieledone polymorpha* exhibited a slightly lower Young's modulus of 4.69 GPa. These values are significantly lower than the stiffness values we measured here. In contrast, [Miserez et al. \(2008\)](#) found that the upper beak of *Dosidicus gigas*, when freeze-dried, had a Young's modulus close to 10 GPa. When including our data, the species can be ranked in terms of beak stiffness as follows: *Dosidicus gigas* (10 GPa) > *Loligo vulgaris* (7 GPa) > *Eledone cirrhosa* (6.8 GPa) > *Sepia officinalis* (6.6 GPa) > *Sepioteuthis lessoniana* (5.6 GPa) > *Pareledone turqueti* (4.99 GPa) > *Adelieledone polymorpha* (4.69 GPa).

However, our findings demonstrate the importance of considering both intraspecific variability and variability within a single beak. While interspecific variation is significant when the sample size is large enough, differences in stiffness between species often remain relatively small. This poses a risk of sampling bias in comparative studies with low sample sizes. For instance, in [Matias et al. \(2019\)](#), only one upper beak was sampled from each species, leading to the conclusion that *Adelieledone polymorpha* had a significantly lower stiffness than *Pareledone turqueti*. However, the average difference in modulus between these two species was only 0.3 GPa – smaller than the intraspecific variation observed in our study. This raises concerns about whether such small differences are representative of species-wide traits. We therefore recommend that future studies include larger sample sizes to avoid these biases. Hence in this study, we cannot conclude that *Sepioteuthis lessoniana* beaks possess weaker mechanical properties than the other species examined.

Additionally, [Miserez et al. \(2008\)](#) emphasizes the impact of tissue hydration on beak elasticity. In *Dosidicus gigas*, the upper beak's modulus dropped below 5 GPa in rehydrated specimens, compared to the much higher stiffness of freeze-dried beaks (10 GPa). Even slight methodological differences can result in varying tissue hydration levels, which in turn may account for some discrepancies between results across different studies. While maintaining constant experimental conditions within a single study allows for accurate comparisons, caution should be exercised when comparing absolute stiffness values across different studies. The difficulty in precisely controlling hydration levels may introduce significant variation in outcomes.

4.3. Stiffness, diet, size and tanning pattern

The observed differences in beak stiffness between *Eledone cirrhosa* and *Loligo vulgaris* reveal that *L. vulgaris* has a slightly stiffer rostrum, despite differences in diet that challenge our initial hypotheses. *L. vulgaris* primarily feeds on soft prey, like fish ([Jereb et al., 2014](#); [Vafidis et al., 2008](#)), whereas *E. cirrhosa* consumes harder prey, such as crustaceans ([Jereb et al., 2014](#); [Xavier et al., 2018](#); [Fanelli et al., 2013](#)). This discrepancy suggests that beak stiffness may not be directly correlated with prey toughness, as originally hypothesized. However, it is important to note that the exact mechanical properties of prey and the force required to penetrate different types of food remain largely unknown. Misconceptions may exist regarding the beak stiffness necessary for consuming different prey types, and further studies are needed to clarify this relationship. Moreover, it is the combination of beak shape, beak movement, and beak stiffness that will determine the overall efficiency of penetration, yet most of these parameters remain unknown.

An alternative hypothesis is that larger animals possess stiffer beaks, which could explain the high stiffness values observed in *Dosidicus gigas*. In our study, only beak size correlates with stiffness, albeit with a very shallow positive slope. It is uncertain whether this correlation would hold with the addition of more species, suggesting that size alone may not fully account for the observed differences in stiffness across species.

It is also possible that the observed variation in stiffness is not driven by functional differences but by developmental or plastic factors. Given the rostral structure of these species, they already have a significant mechanical advantage in processing various prey types. Their beak morphology presumably enables efficient stress distribution, which may suffice without the need for additional beak stiffness. Behavioral factors may further mitigate mechanical stress on the beak. For example, octopuses tend to manipulate prey with their arms and rely more on their suckers than their beaks when handling hard-shelled organisms like bivalves ([Nixon and Dilly, 1977](#); [Kier and Smith, 2002](#)). Additionally, octopuses are known to discard the harder parts of prey after exposing the flesh, unlike *Sepia officinalis*, which actively consumes larger prey, often as long as its body, and uses its beak to sever the central nervous system before cutting the prey into digestible pieces. *Sepia officinalis* ingests the entire prey, including hard parts, and leaves no remains, indicating that prey processing behaviors may reduce selective pressures on beak stiffness in some species.

Furthermore, the link between tanning pattern and stiffness, which has been proposed in the literature ([Miserez et al., 2007](#); [Miserez et al., 2008](#); [Miserez et al., 2010](#); [Tan et al., 2015](#); [Xavier et al., 2023](#)), remains unclear. When considering the rostra of the species in this study, there did not appear to be significant differences in tanning coloration between species, though this aspect is difficult to quantify visually. Although it is well established that within a given species, tanned parts of the beak are stiffer than untanned parts, and juvenile beaks (less tanned) are more elastic than those of tanned adults, a direct relationship between tanning intensity and stiffness across species has yet to be demonstrated. Superficially, the degree of tanning in the beaks of all the species examined here seemed similar, with fully tanned, dark rostra, and only the posterior parts varying in color. More detailed analyses and the development of methods to accurately quantify beak tanning are necessary. Until then, it is not advisable to infer the mechanical properties of beaks across species solely based on tanning intensity.

4.4. Particles and trace elements

Cephalopod beaks are primarily composed of chitin ($(C_8H_{13}O_5N)_n$), a polymer of N-acetyl-D-glucosamine, proteins (including chitin-binding and histidine-rich proteins), and water. The elemental composition of these proteins includes carbon (C), hydrogen (H), oxygen (O), and nitrogen (N), which align with the main elements found in the beaks in this study, though hydrogen was not measured. The recorded level of nitrogen was higher than expected for chitin. Additionally, two samples of *Sepia officinalis* showed high carbon content with minimal nitrogen levels, uncommon for proteins but characteristic of polymers.

In *Sepia officinalis* and *Sepioteuthis lessoniana*, particles rich in oxygen (O), calcium (Ca), and phosphorus (P) suggest the presence of a mineral phase that could correspond to hydroxyapatite $Ca_5(PO_4)_3(OH)$ or a similar calcium phosphate. Hydroxyapatite is a major component of bone, enamel, and dentin in vertebrates, making its presence in cephalopod beaks, which are not mineralized, particularly intriguing. With the exception of *Nautilus*, which has a calcitic ($CaCO_3$) component at the tip of its beak, cephalopods generally lack mineralized beaks, and the origin of these small mineral particles in the beaks we examined remains unclear. One hypothesis is that these particles are produced by the animal to reinforce the beak, a phenomenon observed in some invertebrates like crayfish where the mandibles are strengthened ([Bentov et al., 2012](#)). However, the cephalopod species in which we found these particles also tend to have less stiff beaks, warranting further investigation with a larger sample size. Alternatively, these particles might originate from the animal's diet or from seawater and bioaccumulate in the beak during growth, as suggested by the absence of such particles in juvenile *S. officinalis* which were fed exclusively on shrimp in a controlled seawater environment ([Souquet et al., 2023](#)).

Trace elements are a significant focus in cephalopod research due to their role as biological markers of marine pollution and indicators of

capture location (Xavier et al., 2023; Duarte et al., 2023). For example, *Onykia ingens* beaks contain up to 3.7 % trace elements, including 23 different elements, with sodium (Na), potassium (K), phosphorus (P), calcium (Ca), and zinc (Zn) constituting 99 % of this content (Northern et al., 2019). Similar elemental findings, also including iron (Fe) and magnesium (Mg), have been reported across various squid species (Miserez et al., 2007; Broomell et al., 2007). However, *Dosidicus gigas* beaks did not show detectable trace elements (Miserez et al., 2007). Additionally, elements like arsenic (As), bromium (Br), and selenium (Se) in *Octopus vulgaris* have been proposed as markers of capture locations and pollution levels (Duarte et al., 2023). Research on species like *Sthenoteuthis oualaniensis* and *Ommastrephes bartramii* has further demonstrated the potential of cephalopod beaks as indicators for ocean pollution due to the accumulation of elements such as vanadium (V) and uranium (U) (Fang et al., 2019; Ichihashi et al., 2001).

In this study, the only metals found were aluminium (Al) and iron (Fe) in small quantities, and titanium (Ti), notably concentrated in a specific area of *Sepia officinalis*, alongside a high presence of oxygen (O). The presence of titanium and aluminium may reflect bioaccumulation of marine pollution, with titanium dioxide nanoparticles, commonly found in sunscreen, being a significant pollutant in seawater (Shi et al., 2019). Surprisingly, however, Rowe et al. (2024) reported traces of titanium in fossil coleoids from the Cretaceous. These traces were localized on the soft tissues (oviductal glands, arm tips) and to a lesser extent on the beak. It is not clear whether this accumulation of titanium is the result of diagenetic processes or whether it occurred during the life of the animal. This coincidence suggests further exploration of the possible capacity of cephalopods to incorporate titanium and to track this element. Additionally, silicon (Si) rich particles, likely originating from the diet due to the widespread presence of silicon in marine ecosystems and zooplankton, were also detected in the samples.

Although nanoindentation and elemental analysis are methodologically distinct, their integration offers a more complete understanding of beak function. Trace elements may contribute to mechanical reinforcement – either actively through biological incorporation or passively via environmental exposure. In this context, mechanical stiffness and elemental diversity may not be independent. Future work, particularly involving high-resolution mapping (e.g. nano-CT, TEM), could explore correlations between specific particles or mineral inclusions and localized mechanical properties.

Comparable incorporation of metals has been observed in other invertebrate systems, such as polychaete jaws and mussel byssal threads (Harrington et al., 2018), where specific metal ions contribute to structural reinforcement. These analogies suggest potential convergent strategies in marine biomaterials, warranting further biochemical analysis of cephalopod beaks.

4.5. Limitations and scope of contribution

We acknowledge the limitations posed by the small sample sizes for *Sepia officinalis* and *Sepioteuthis lessoniana* ($n = 2$), which constrain the strength of species-specific conclusions. However, this study was not designed to provide exhaustive statistical certainty across all taxa but rather to establish a foundational dataset and explore under-investigated questions surrounding cephalopod beak structure and composition. By combining nanoindentation, SEM imaging, and EDS elemental mapping, we provide a multifaceted view of cephalopod beak morphology and function – spanning material stiffness, internal architecture, and trace elemental presence. This integrative approach allows us to highlight key inter- and intraspecific trends while identifying intriguing patterns of potential biomineralization. Our findings serve as a springboard for future, more comprehensive investigations and underline the value of cephalopod beaks as models for studying biological materials and environmental interactions. In this exploratory context, even data derived from smaller samples contribute meaningfully to the broader understanding of cephalopod biology and functional

adaptation.

Additionally, due to incomplete data on sex and maturity stage for several specimens, we were unable to assess the potential influence of these biological variables on beak stiffness or elemental composition. This remains an important limitation, as physiological changes during growth and reproduction may affect material properties, and future studies should aim to include these factors in the analysis.

5. Conclusion

This study provides new insights into the mechanical properties and structural characteristics of cephalopod beaks, revealing significant intra- and interspecific variability. The observed differences in stiffness between upper and lower beaks, as well as among species, underscore the complexity of beak function and its potential links to diet and environmental factors. Notably, whereas lower beaks are generally stiffer than upper beaks, the relationship between beak stiffness and prey toughness is not straightforward, suggesting that other factors such as developmental processes may play a role. Our findings emphasize the importance of considering both intraspecific variability and methodological consistency in comparative studies of mechanical properties. Further research with larger sample sizes and refined analytical techniques will be crucial to deepen our understanding of cephalopod beak mechanics and their broader ecological implications.

CRediT authorship contribution statement

Anastasiia Maliuk: Writing – review & editing, Visualization, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Dominik Dziedzic:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Arsalan Marghoub:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Isabelle Rouget:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Anthony Herrel:** Writing – review & editing, Resources, Methodology, Conceptualization. **Susan Evans:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Mehran Moazen:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Louise Souquet:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Ethics

This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility

This article has no additional data.

Declaration of AI use

We have not used AI-assisted technologies in creating this article.

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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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2025.107472>.

Data availability

Data will be made available on request.

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