Echoes in the Chambers: Exploring the Ecology and Evolution of Mesophotic Zone Scavengers in Chambered Cephalopods

Job Lukas Veloso

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Peter Ward, Chair
Billie Swalla
Samuel Wasser

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Abstract

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Job Lukas Veloso

Chair of the Supervisory Committee:
Peter Ward

Department of Biology

Cephalopods are ecologically and evolutionarily significant marine invertebrates, yet major gaps remain in our understanding of their trophic ecology and physiological adaptations, particularly among deep-living and morphologically conserved lineages such as nautiloids. This dissertation investigates the ecological and metabolic dimensions of phragmocone-bearing cephalopods through a multi-scalar isotopic approach. In Chapter 1, stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) of multiple *Nautilus* and *Allonautilus* populations across the Pacific reveal strong geographic structuring of isotopic niches, with region-specific variations in baseline carbon sources and trophic levels. Chapter 2 focuses on mesophotic scavengers, including nautiloids and other reef slope taxa, and applies nitrogen-based trophic level estimates to show convergence in foraging roles across distinct geographic locales in Fiji and Papua New Guinea. Chapter 3 compares metabolic signatures of extant nautiloids, sepiids, and extinct ammonoids using C_{meta}, a carbon-isotope-based proxy for metabolic rate. Results show that modern nautiloids exhibit lower metabolic signals than both extinct ammonites and extant cuttlefish, consistent with their slow-growing, low-energy lifestyles. Together, these findings illuminate the trophic ecology, niche dynamics, and physiological diversity of chambered cephalopods across evolutionary time, enhancing our understanding of how environmental constraints shape deep-sea and mesophotic life.

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Introduction

Cephalopods have long captivated scientists and the public alike—not just for their intelligence and adaptability, but for the remarkable range of habitats they occupy. From shallow coastal waters to the dim, steep slopes of coral reefs, these invertebrates play key roles in marine ecosystems. While much attention has been given to squid and octopuses—the fast-growing, short-lived coleoids that dominate many oceanic food webs—their shelled cousins, the nautiloids, remain far more mysterious.

Modern nautiloids, represented by the genera *Nautilus* and *Allonautilus*, inhabit the fore-reef slopes of the tropical Indo-Pacific, often hundreds of meters below the surface. Their biology is strikingly different from that of their coleoid relatives: they grow slowly, reproduce late, and exhibit limited dispersal across deep ocean barriers. These traits, combined with their distinctive external shells, set nautiloids apart as living fossils—survivors of ancient lineages that date back more than 500 million years. Yet despite their evolutionary significance, we still know little about how nautiloids interact with their environment or how they have persisted in deep and often resource-limited habitats.

This dissertation brings together three lines of inquiry that explore the ecology and physiology of these chambered cephalopods. Using stable isotope analysis, I examine how nautiloids and other deep-living cephalopods acquire resources, occupy ecological niches, and vary in metabolic intensity. The goal is to connect what we observe today with broader patterns in cephalopod evolution and adaptation.

In C**hapter 1**, I take a broad geographic view—comparing stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) across multiple *Nautilus* and *Allonautilus* populations from the Pacific. These isotopic signatures reveal distinct ecological profiles shaped more by local environmental conditions than by species identity. Some populations show narrow, highly specialized niches, while others appear more flexible in their foraging strategies, hinting at how these animals persist in such varied underwater landscapes.

Chapter 2 zooms in on a particular ecological setting: the mesophotic reef slopes of Fiji and Papua New Guinea. Here, I explore the trophic structure of deep-sea scavengers, including nautiloids and other benthic foragers, using nitrogen isotope values to estimate their relative positions in the food web. Across sites and species, a shared scavenging strategy emerges—one that may reflect convergent responses to the challenges of living in low-light, low-resource environments.

Finally, **Chapter 3** steps back in time, placing nautiloids within a broader evolutionary framework. Using a carbon-isotope-based metric known as C_{meta}, I compare inferred metabolic signals across modern nautiloids, modern cuttlefish, and extinct ammonoids. The results underscore just how physiologically distinct nautiloids are: their low metabolic signals contrast sharply with the higher-energy lifestyles of both their extinct and extant relatives, reinforcing their identity as slow-paced survivors of deep time.

Taken together, these chapters paint a more complete picture of chambered cephalopods—linking their trophic behavior, ecological roles, and metabolic constraints to both environmental conditions and evolutionary history. In doing so, this work contributes to our understanding of how ancient marine lineages persist and adapt in a changing ocean.

Chapter 1: Ecological Dynamics and Isotopic Niches of Pacific Nautiloids: Insights from Stable Isotope Analyses

Job Lukas Veloso*[1], Gregory Barord[2], Frederick Dooley[3], and Peter Ward[1]

jlveloso@uw.edu

[1]Department of Biology, University of Washington, Seattle, WA 98995, USA

[2]Department of Marine Science, Central Campus, Des Moines, Iowa, USA

[3]School of Biology, Everett Community College, Everett, WA, USA

Abstract

Modern nautiloids are fore-reef slope dwelling cephalopods with distinct morphological and phylogenetic characteristics. We examined their habitat and trophic ecology across the Pacific using stable isotope analyses (SIA) of tissue samples from multiple populations. Unlike many cephalopods, *Nautilus* species exhibit scavenging behavior rather than active predation, with $\delta^{15}N$ and $\delta^{13}C$ values reflecting their unique ecological niches. Our findings, representing a comprehensive comparison of *Nautilus* populations, reveal significant dietary variation and niche differentiation. *N. samoaensis* and *N. pompilius* from the Bismarck Sea show the highest $\delta^{15}N$ values, indicating nitrogen enrichment, while *N. stenomphalus* from the Great Barrier Reef (GBR) displays the lowest $\delta^{13}C$ values, suggesting distinct carbon sources. These traits highlight the ecological adaptability and evolutionary divergence of *Nautilus* species in deep-sea ecosystems, contributing to their persistence in diverse marine environments.

Keywords: Stable isotopes, isotopic niche, mesophotic ecology, nautiloids

Significance Statement

This study provides insights into the ecological roles and “big-picture” dietary habits of nautiloid species across Pacific populations by using stable isotope analysis. By revealing significant variations in carbon and nitrogen isotopic values among different populations, the study enhances our understanding of how regional factors influence trophic interactions and evolution in deep-sea ecosystems. These findings can be beneficial for conservation efforts, as they help identify habitat preferences and potential environmental impacts on nautiloids, contributing to the broader knowledge of marine biodiversity and ecosystem dynamics.

Introduction

Cephalopods are integral components of marine ecosystems, thriving in environments ranging from shallow coastal waters to the deep sea. Recent studies suggest that the ecological roles of certain cephalopods, particularly predatory coleoids like octopuses and large squid, may be undergoing significant shifts due to environmental changes such as rising ocean temperatures and altered predator-prey dynamics (1–4). These transformations highlight broader ecological changes in the world’s oceans, and may similarly affect nautiloids, the longest-lived of all modern cephalopods.

Extant nautiloids, comprising *Nautilus* (seven species) and *Allonautilus* (two species), inhabit deep fore-reef slopes of different areas in the Indo-Pacific (Figure 1) at depths of 100–800 meters (5). They forage continuously for benthic food sources, exhibiting scavenging rather than active predation (5–8). The life-history traits of living nautiloids, including their late reproductive maturity, long lifespan, low fecundity, and limited dispersal ability (9–11) separate them from other cephalopods. Additionally, their inability to migrate beyond depths of 800 meters, due to shell implosion (12), isolates populations geographically, resulting in limited gene flow across deep oceanic barriers. This life history strategy and limited dispersal capability has likely driven evolutionary divergence, as nautiloid populations in different regions exhibit significant morphological and genetic differentiation, even within the same species (13, 14). New species have recently been described, and the number of recognized species has doubled over the past century (15). As a result, morphological diversity, previously attributed to a few species, now possibly indicates a much richer and more complex evolutionary history for this group than currently accepted.

Understanding the ecology of specific nautiloid populations or taxa can further illuminate the possible degree of ecological differentiation among geographically isolated populations. Direct observations of nautiloid feeding behavior are rare and have only been documented a few times (16), owing to their relatively deep habitats. Baited Remote Underwater Video Systems (BRUVS) and gut content analyses (17) have provided some insights into their scavenging behavior, yet dietary variation between different populations remains largely unexplored (8, 18). Stable isotope analysis (SIA) has emerged as a powerful tool for uncovering the ecological roles of organisms in their natural habitat (19–21). Typically, examining the isotopic signatures of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) can provide insights into an organism's foraging habitat and trophic position, respectively(19, 22). Isotopic niches, defined by the stable isotope ratios in an organism's tissues, offer a unique framework for understanding ecological interactions, resource use, and environmental adaptation. Although recent studies have begun to explore isotope values in some nautiloid species (8, 23, 24), no comprehensive study has yet compared the isotopic niches of multiple nautiloid populations or species. Additionally, SIA studies on marine taxa and communities has grown, albeit most are regional (20, 21); with a limited number of global-scale SIA studies of marine species (25–28)

Here, we investigate carbon and nitrogen isotope ratios in nautiloid populations across the Pacific (Table 1), characterizing the isotopic niches of these organisms. Our findings reveal previously undocumented dietary variation and provide new insights into the ecological dynamics of nautiloids in deep-sea ecosystems. This study advances our understanding of nautiloid feeding ecology and offers a foundation for future research on their role within oceanic food webs.

Results

Values of $\delta^{13}C$

We observed a significant difference in $\delta^{13}C$ values among nautiloid populations ($F_{6,60} = 14.58$, $p < 0.001$). The Mele Bay (Vanuatu) population of *Nautilus vanuatuensis* exhibited the heaviest mean $\delta^{13}C$ values (–13.18 ± 0.37‰), while the Great Barrier Reef population of *N. stenomphalus* showed the lightest (–17.20 ± 1.70‰). Although these represent the extremes across all sampled populations, they are not directly comparable at the species level due to their geographic endemism. Nevertheless, when comparing species overall, we detected a significant difference in $\delta^{13}C$ values among taxa ($F_{4,76} = 26.04$, $p < 0.001$).

Pearson correlation analysis revealed a statistically significant but biologically negligible correlation between $\delta^{13}C$ and shell diameter in the GBR population ($r^2 = 0.0029$, $p < 0.001$), and slightly stronger correlations in the Bohol Sea ($r^2 = 0.44$, $p < 0.001$) and Mele Bay ($r^2 = 0.47$, $p < 0.001$). Although statistically significant, the strength of these correlations suggests limited biological relevance. Notably, a loess curve revealed a slight dip in $\delta^{13}C$ in intermediate-sized individuals followed by an increase in larger nautiloids.

Values of $\delta^{15}N$

There was significant difference among the different nautiloid populations ($F_{7,73} = 67.07$, $p<0.001$). The values were highest in *Nautilus* found in the pooled Papua New Guinea (Kavieng and Ndrova) populations (15.88 ± 0.5 ‰) and lowest in the Fiji *Nautilus* population (10.71± 0.42‰). In terms of species of *Nautilus*, there was significant difference among the different species ($F_{4,76} = 24.34$, $p<0.001$). The values were highest in *Nautilus samoaensis* (15.6 ± 0.32‰) and lowest in *Nautilus vitiensis* (10.71± 0.42‰).

We found a significant positive correlation between $\delta^{15}N$ and shell width in the GBR ($r2=0.05$, $p < 0.001$) Bohol Sea ($r2=0.23$, $p < 0.001$) and Mele Bay ($r2=0.4$, $p < 0.001$), as illustrated in Figure 5. Our data indicates that larger adult nautiluses tend to have higher levels of Nitrogen.

Isotopic niches of *Nautilus* spp.

The isotopic niche represents a quantitative approximation of an organism's trophic ecology, defined by the distribution of their Carbon and Nitrogen isotopic ratios. This multidimensional space reflects the range of assimilated resources. Isotopic niches differ significantly among regions, with considerable overlap between some sites (e.g., Beqa Harbor and Pago Pago), while other locations, such as the Bismarck Sea and Bohol Sea, show clear separation. Standard ellipse analysis (29) revealed that the narrowest niche was observed in American Samoa (TA = 0.24, SEAc = 0.3), based on both the convex hull area (TA) and the small-sample corrected standard ellipse area (SEAc), whereas the largest niche was found in the Great Barrier Reef (TA = 10.29, SEAc = 3.1). However, the results for American Samoa were based on only five specimens, which may have led to an underestimation of the niche size.

When observed in a species perspective (Figure 3), isotopic niches varied across *Nautilus* species and *A. scrobiculatus,* with notable differentiation in niche width and centroid position. *N. pompilius* populations, in regions where they are present, exhibited variability based on geographic origin (e.g., Philippines vs. Papua New Guinea), while *N. stenomphalus* and *N. vanuatuensis* have more constrained niches.

Discussion

Isotopic analyses reveal distinct ecological patterns among nautiloid populations across the Pacific, highlighting both unique and overlapping niche adaptations influenced by regional biological, oceanographic, and ecological factors. Our findings reveal distinctions between populations of nautiloids, although there remains slight overlap in isotopic niche spaces across the three major clades (14): the South Pacific clade, including *N. samoaensis* from American Samoa, *N. vitiensis* from Fiji and *N. vanuatuensis* from Vanuatu; the Coral Sea clade, comprising populations from the Great Barrier Reef and Papua New; and the Indo-Pacific clade, which includes *N. pompilius* from the Philippines. This is consistent with the hypothesis here that despite genetic differences, different populations and different taxa are likely occupying similar ecological niches in their respective areas.

With regards to isotopic niche space, geographic location, rather than species identity, emerges as a better determinant of isotopic niche differentiation among nautiloid populations. Distinct regional environments, such as the Bismarck Sea or the GBR, shape localized trophic dynamics and resource use. For instance, *N. stenomphalus* in the GBR exhibits a notably broad isotopic niche (Figure 2), possibly reflecting a diversity of food sources from an expansive coral reef system. Conversely, the narrower isotopic niche of *N. vanuatuensis* in Port Villa, Vanuatu suggests limited dietary resources, highlighting the constraints imposed by regional food web structures. The substantial overlap among some locations suggests shared resource use or comparable baseline isotopic values, while the distinct separation of others, such as the Bismarck Sea and Suva, indicates localized differences in food web structure or primary productivity(30). The isotopic niche overlap between *A. scrobiculatus* and *N. pompilius* in PNG suggests that these genera, despite their morphological and behavioral differences, occupy similar trophic positions and rely on comparable food resources in this region. This species-level variation highlights both interspecific and intraspecific trophic diversity, with *N. pompilius* (PNG) exhibiting geographic structuring and *A. scrobiculatus* occupying a broader isotopic niche (Figure 3), potentially reflecting opportunistic foraging. This overlap indicates that environmental factors, such as prey availability and habitat structure, may play a stronger role in shaping their foraging ecology than genus-level distinctions. When analyzed collectively, *A. scrobiculatus* and *N.pompilius* (PNG) exhibit a broader isotopic niche (larger standard ellipse area) compared to when populations are grouped geographically, reflecting greater dietary and habitat diversity across their entire range. This pattern suggests that niche breadth expands at the genus level due to the integration of isotopic variability from different local environments, rather than fundamental differences in resource use between the two genera. Consequently, geographic context appears to be a key driver of isotopic niche width, with localized ecological conditions constraining niche space more than phylogenetic identity. These findings underscore the critical role of spatial environmental factors in driving niche specialization, revealing that ecological context often supersedes species-level traits in shaping trophic strategies. These findings also provide insights to the ecological flexibility of nautiloids, emphasizing their capacity to adapt to heterogeneous marine environments—a key consideration amid ongoing oceanic change.

One unexpected finding was the differentiation of carbon isotope results of *N. stenomphalus* from the Great Barrier Reef (GBR) compared to other populations of *Nautilus*. In the GBR, *N. stenomphalus* exhibited a broader isotopic niche. This could suggest a more diverse diet, possibly reflecting greater prey availability or ecological flexibility (31). We hypothesize that the wider range of $\delta^{13}C$, particularly lighter, values in this area may be due to a combination of biological and oceanographic factors. The extensive coral reef ecosystems in the GBR require substantial amounts of dissolved inorganic carbon (DIC) for coral growth (32, 33). The high productivity of corals can lead to low $\delta^{13}C$ values in the available carbon sources in the lower depths (34). As a result, nautiloids feeding on detritus within this system might exhibit correspondingly lower $\delta^{13}C$ values because of this. Additionally, pelagic subsidies are recognized as the primary carbon inputs to reef predators in the GBR, emphasizing the interconnectedness of pelagic and reef ecosystems (35). Similar patterns have been observed in other reef systems, underscoring the complexity of food web interactions in these environments (36). Conversely, the narrower niche of *N. vanuatuensis* from Port Vila may indicate limited prey resources, which could have significant implications for the resilience of this population to ecological change (37).

In mesophotic environments, where food resources are limited, organisms typically exhibit higher $\delta^{15}N$ values, reflecting adaptations to resource scarcity(38). The elevated nitrogen isotope values observed in *N. pompilius* from the Bismarck Sea and *N. samoaensis* could result from a combination of oceanographic and biological processes that affect nutrient cycling and primary productivity. One potential explanation is the presence of nitrogen-fixing bacteria in waters between 200-1000 meters in the Bismarck Sea, which have been shown to influence nitrogen isotope values in plankton, leading to higher $\delta^{15}N$ at the base of the food web (39). This phenomenon may also be occurring in American Samoa, where we observed similarly high $\delta^{15}N$ values.

Moreover, the relationship between shell diameter and nitrogen isotopic values in *N. pompilius* from the Bohol Sea aligns with a well-documented pattern in most cephalopods, where $\delta^{15}N$ values tend to increase with growth (22, 28, 40–44). This trend is generally attributed to ontogenetic shifts in diet, as larger individuals often occupy higher trophic positions, consuming prey with higher $\delta^{15}N$ signatures (22, 45, 46). However, recent work by Ward et al. (2023) reported a contrasting pattern in some nautiloid species, where $\delta^{15}N$ values decreased across successive septa within the phragmocone. This unexpected decline, which has previously only been observed in vampire squids (28), suggests that early septal shell stages may reflect metabolic inputs from yolk-derived nutrients (47) rather than external dietary sources.

In our study, only organic tissues from live-caught *N. pompilius* individuals were analyzed, providing a snapshot of their ecological behavior in the wild at their present (caught) state. The observed increase in $\delta^{15}N$ values with shell diameter suggests a dietary shift as individuals transition from juvenile to adult stages, likely reflecting a change in prey selection or foraging depth. While $\delta^{13}C$ values of *N. pompilius* from Bohol Sea did not show a direct linear relationship with shell diameter as seen in Figure 4. This suggests a potential trend of isotopic variation as adult nautiluses mature. This pattern may indicate shifts in feeding habitat or dietary preferences, potentially influenced by factors such as changes in locomotion, predation risk, or environmental resource availability.

As cephalopods mature, they often undergo ontogenetic shifts in their feeding habitats and dietary preferences, which are influenced by factors such as locomotion, predation risk, and environmental resource availability (45). Juvenile cephalopods tend to inhabit different ecological niches compared to adults(45, 48, 49), leading to variations in prey selection and feeding strategies. The possible shift in habitat use and prey consumption can result in changes to their isotopic signatures, as seen in the $\delta^{13}C$ and $\delta^{15}N$ values, which reflect the types of carbon sources available in different feeding habitats. Previous studies have documented such ontogenetic dietary shifts in a variety of cephalopod species, including the common cuttlefish (*Sepia esculenta*), where changes in trophic ecology and migratory patterns were observed through stable isotope analysis(45). These shifts in both habitat and diet are commonly associated with changes in isotopic signatures, indicating the complex relationship between growth, feeding behavior, and the ecological roles that cephalopods occupy at different life stages.

In conclusion, this study enhances the understanding of Carbon-Nitrogen isotopic signatures among *Nautilus* populations across the Indo-Pacific. This highlights the uniqueness of each population, which may support the hypothesis of incipient speciation among isolated populations in the area. The unexpected differentiation of carbon isotope values in the GBR and Fiji populations highlights the complex interplay between biological processes, nutrient cycling, and oceanographic conditions that shape isotopic signatures in these regions. Additionally, the elevated nitrogen isotope values in certain populations, such as those from the Bismarck Sea, suggest the influence of unique oceanographic features, including the potential role of nitrogen-fixing bacteria.

Materials and Methods

Sample collections

Tissues were collected from different nautiloid populations across the Pacific for stable isotope analyses. In these sites, weighted traps were deployed between 200 and 400 m depths using a similar method to that used in Vandepas 2016. The weighted traps were made of metal interlinks and approximately measured 2 m x 1 m with a double entry. Raw chicken and canned tuna were used as bait. From each of these sites, captured nautiloids were nonlethally sampled for both shell and tissue. Soft tissue samples (~ 500 mg) were collected by snipping the tips of a single nautiloid tentacle. The soft tissue samples were then placed in small vials with 95% ethanol. Additionally, apertural shell snips were obtained and placed in dry vials in air. All samples were returned to the University of Washington, Seattle, WA, USA, for processing.

Bulk stable isotopes analysis of δ13C and δ15N

Tissue samples were placed in an oven to dry at approximately 60°C for 48 hours. Dried tissues were each sectioned into smaller pieces (~0.4 mg). Samples were weighed using a microbalance and wrapped in tin capsules. The concentration and isotopic composition of carbon and nitrogen in the samples were measured with a Costech™ ECS 4010 Elemental Analyzer coupled to a Thermo Finnigan™ MAT253 continuous flow isotope ratio mass spectrometer in IsoLab at the University of Washington. Combustion was carried out at 1000°C with a 10 mL pulse of O_2. The result of the combustion are gases that are passed through a reduced copper column maintained at 700°C. A magnesium perchlorate trap was then used to remove water from the gas stream, after which the gases were separated via gas chromatography and fed into the mass spectrometer via a Thermo Finnigan Conflo III.

Raw isotopic data were corrected using a two-point calibration with three in-house standards: two glutamic acids and dried salmon, which are calibrated against international reference materials NBS19, LSVEC, IAEA-N-1, USGS32, USGS-40 and USGS-41. Nitrogen isotopic data are reported in delta notation relative to air. Carbon isotopic data are reported in delta notation relative to Vienna Peedee Belemnite (VPDB).

Data analyses

Linear regression was used to assess the relationship between $\delta^{13}C$ and $\delta^{15}N$ and their correlation with shell diameter to detect potential shifts through ontogeny. Our analyses involved ANOVA or Kruskal–Wallis H tests, followed by pairwise multiple comparisons using Tukey's HSD test or Mann–Whitney U test (50). All tests were conducted with a significance level of $\alpha = 0.05$. We used recent metrics based on a Bayesian framework (Stable Isotope Bayesian Ellipses in R: SIBER (29) to analyze stable isotopic niche widths in different groups.

Statistical analysis, computations, and graphs were conducted utilizing R (version 4.3.2), PAST 4.15(51), and MS Excel (version 16.81).

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Tables and Figures.

Table 1. Summary of Carbon and Nitrogen isotope values categorized in species and locality.

Species of *Nautilus*	Geographic area	Mean $\delta^{15}N$	SD	Mean $\delta^{13}C$	SD	n
N. stenomphalus	Great Barrier Reef, Australia	11.95	0.75	-17.20	1.74	28
N. vitiensis	Beqa and Suva Harbors, Fiji	10.88	0.81	-14.88	2.01	11
N. pompilius	Bohol Sea, Philippines	12.66	0.76	-14.52	0.434	26
N. vanuatuensis	Mele Bay, Vanuatu	11.41	0.36	-13.18	0.37	7
N. samoaensis	American Samoa	15.60	0.32	-14.66	0.30	5
N. pompilius	Bismarck Sea, Papua New Guinea	15.88	0.46	-13.46	0.45	18
A. scrobiculatus	Bismarck Sea, Papua New Guinea	15.4	1.03	-13.53	1.07	5

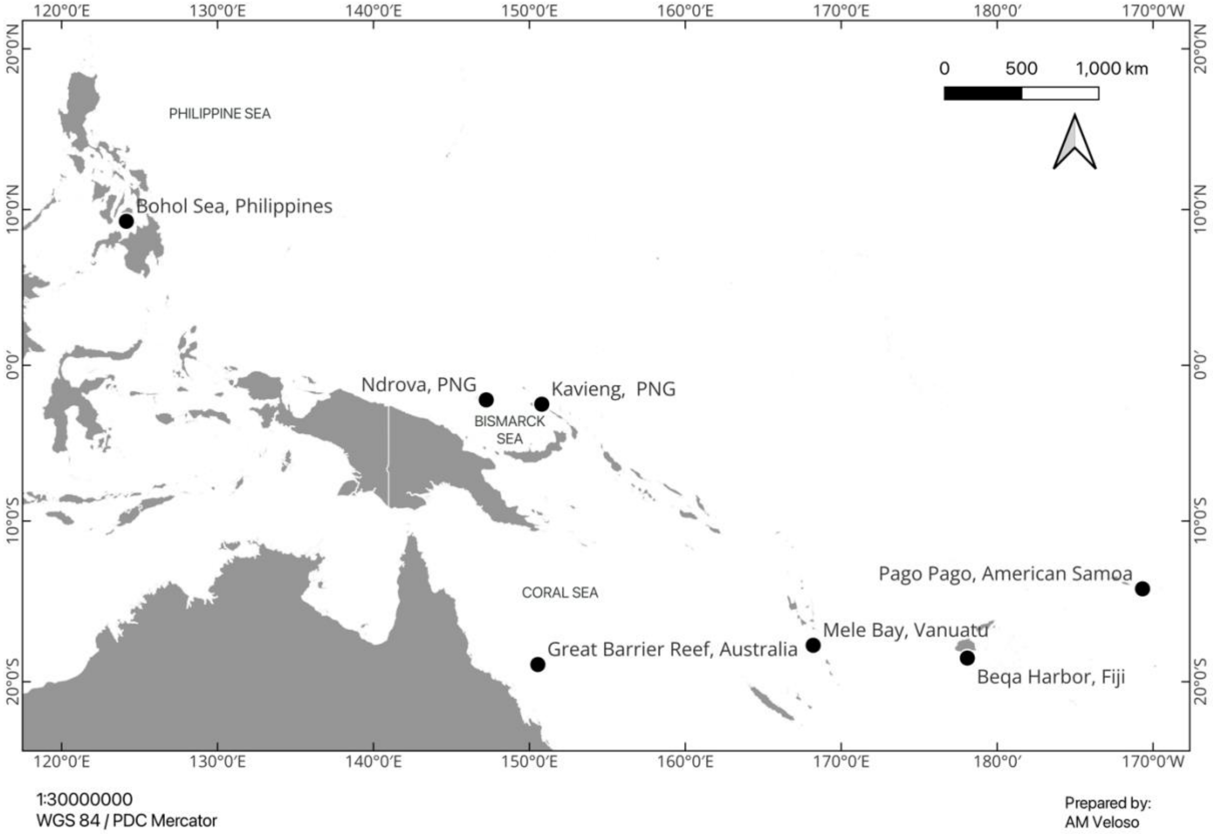


Figure 1. Nautiloid populations across multiple sites in the Indo-Pacific region, including the Philippines, Vanuatu, the Great Barrier Reef of Australia, Fiji, and American Samoa during the summer months of 2011 and 2012. Additionally, we collected samples from Kavieng and Ndrova in Papua New Guinea during May 2022. Available in QGIS.

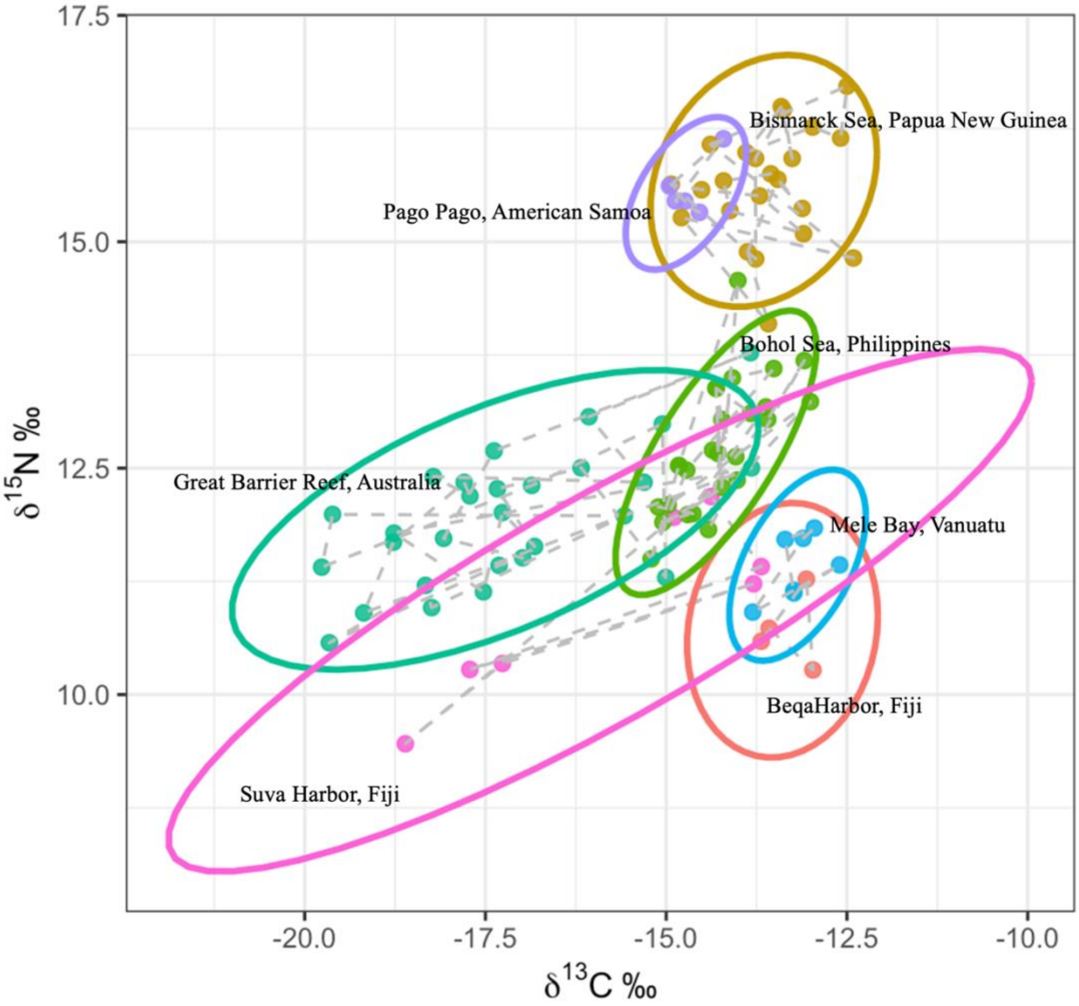


Figure 2. Stable isotope ellipses of $\delta^{15}N$ and $\delta^{13}C$ values for nautiloid populations around the Pacific Ocean. The standard ellipse represents the core isotopic niche of each nautiloid population; the convex hulls contain all data points from each nautiloid population.

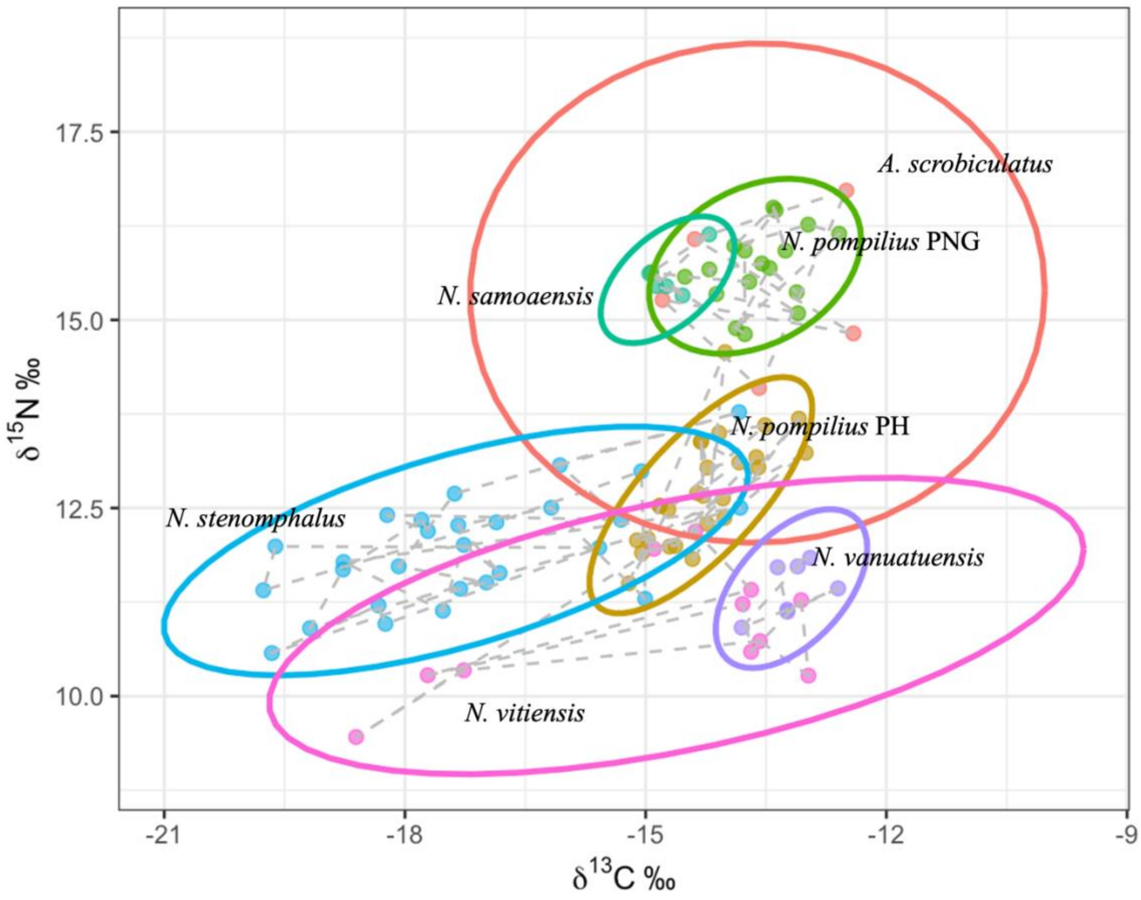


Figure 3. Isotopic niches of the nautiloid species presented in this study. Solid lines represent the ellipse area. The dotted lines represent the convex hull area.

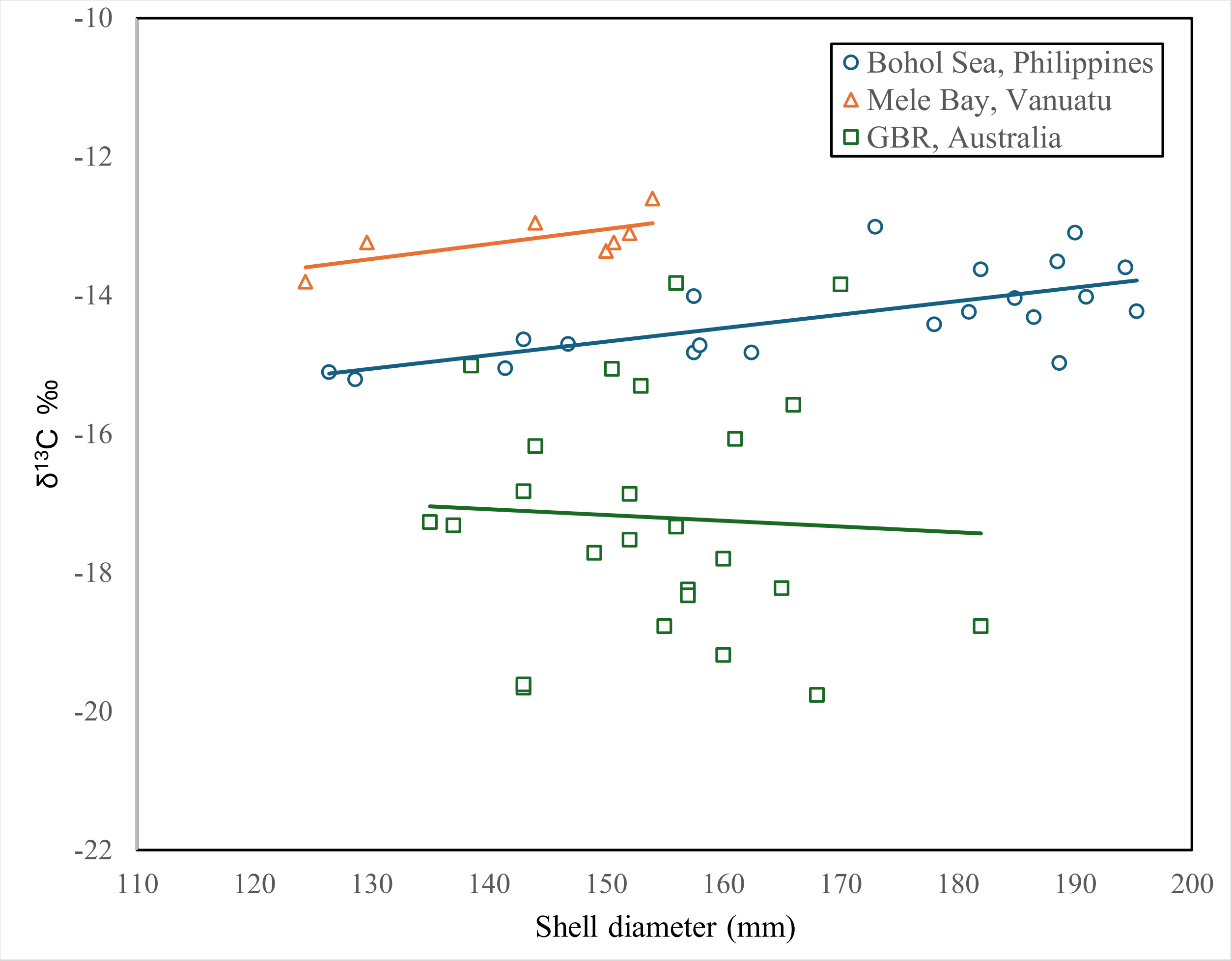


Figure 4. Relationship between shell diameter and δ^{13}C for *Nautilus* populations from the Bohol Sea (Philippines), Mele Bay (Vanuatu), and the Great Barrier Reef (Australia). Each symbol represents an individual specimen, with trend lines indicating population-specific patterns. The Vanuatu population exhibits the highest δ^{13}C values, while the GBR population has the lowest, suggesting regional differences in carbon source utilization or habitat preference.

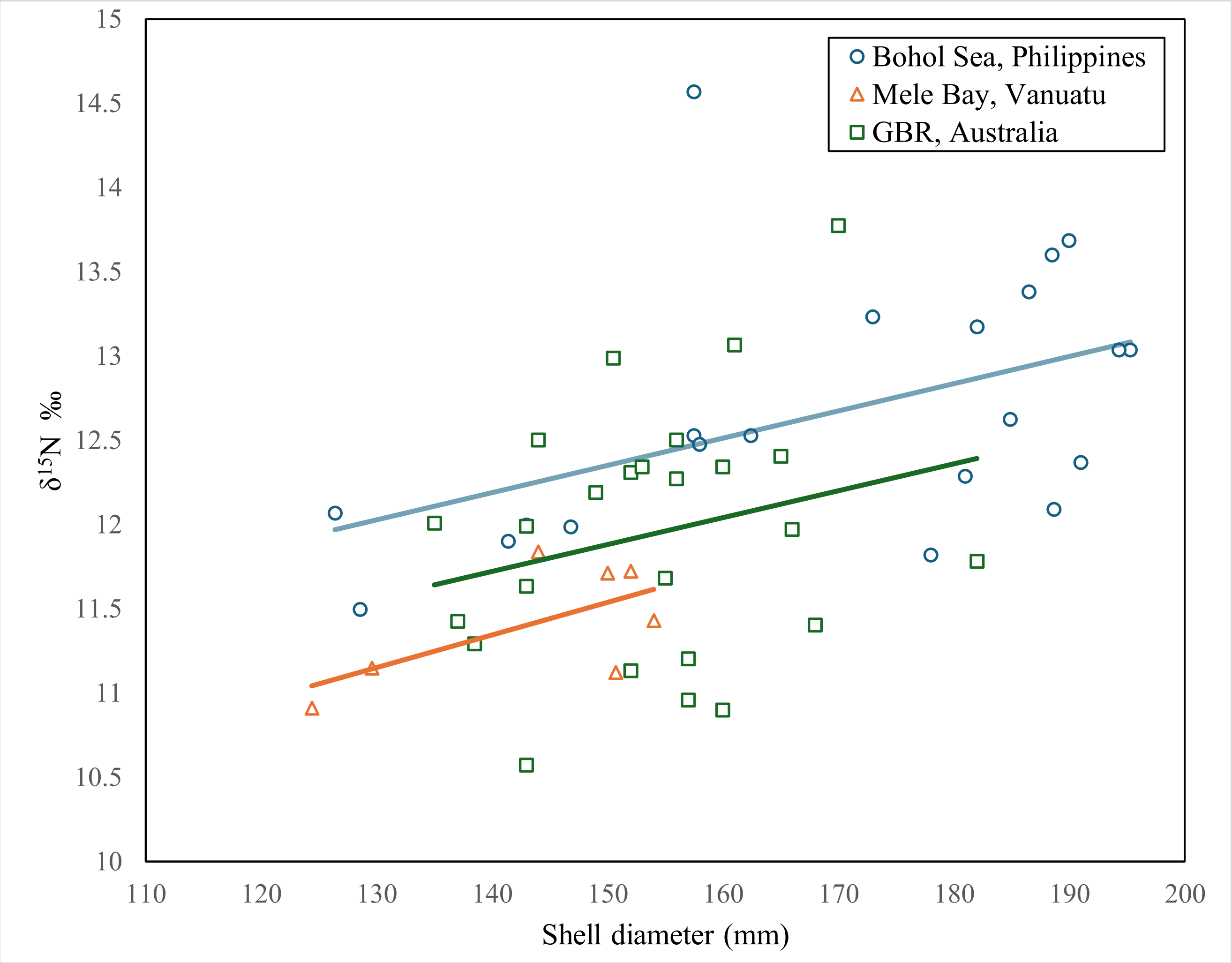


Figure 5. Relationship between shell diameter and δ^{15}N for *Nautilus* populations from the Bohol Sea (Philippines), Mele Bay (Vanuatu), and the Great Barrier Reef (Australia). Each symbol represents an individual specimen, with trend lines indicating the direction and strength of the relationship. The Bohol Sea population shows relatively high δ^{15}N values and the steepest slope, while the Vanuatu population has the lowest δ^{15}N values and the shallowest slope, suggesting regional differences in trophic position or nitrogen baselines.

Supplemental Data

Table 1. Isotopic niche metrics for the different nautiloid populations across the Pacific

Location & Species	**TA**	**SEA**	**SEAc**
Great Barrier Reef, Australia (*N. stenomphalus*)	10.2949	2.9422	3.0554
Panglao, Philippines (*N. pompilius*, PH)	3.0359	0.9405	0.9796
Bismarck Sea (*N. pompilius*, PNG)	2.1577	0.8332	0.8853
Beqa Harbor, Fiji (*N. vitiensis*)	0.5783	0.4884	0.6512
Port Vila, Vanuatu (*N. vanuatuensis*)	0.4911	0.3533	0.4240
Pago Pago, American Samoa (*N. samoaensis*)	0.2387	0.2279	0.3038

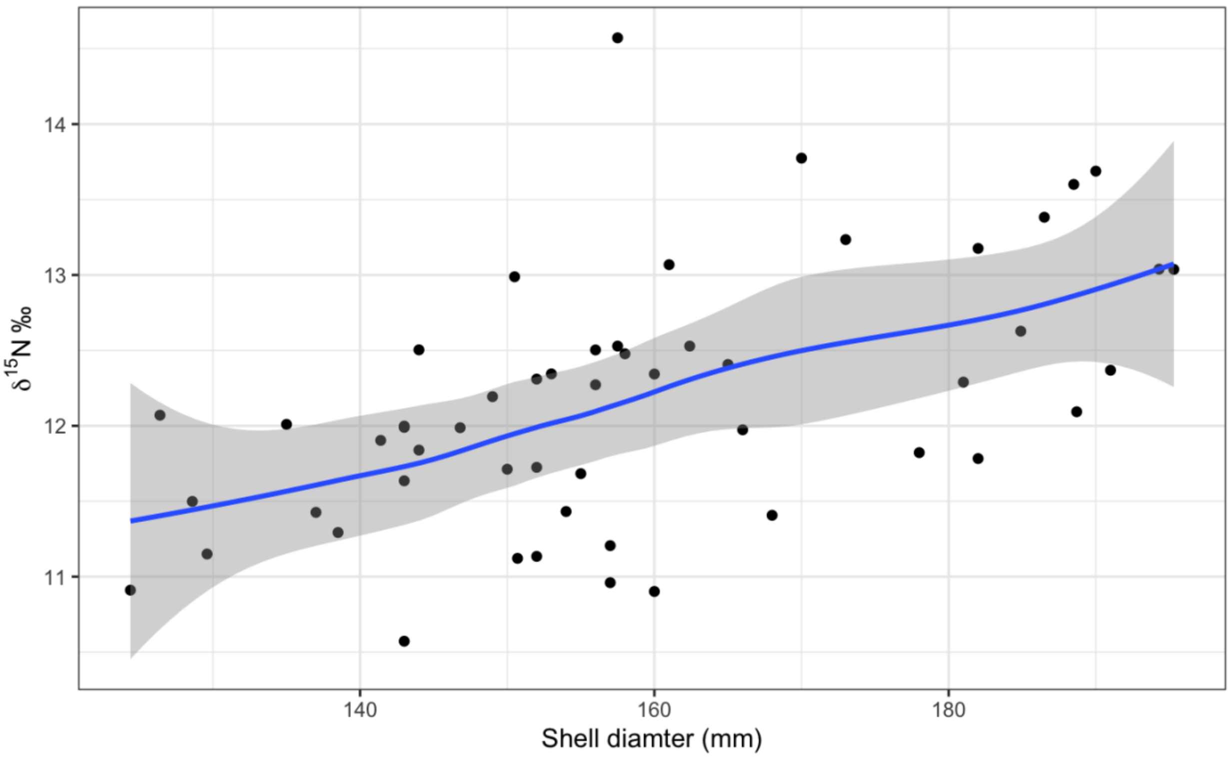


Figure 1. δ^{15}N vs shell diameter of all nautiloid specimens. Note that the shell diameter increases as the δ^{15}N also increases. Solid line indicates fitted LOESS curve, standard errors in grey. Confidence interval is 95%.

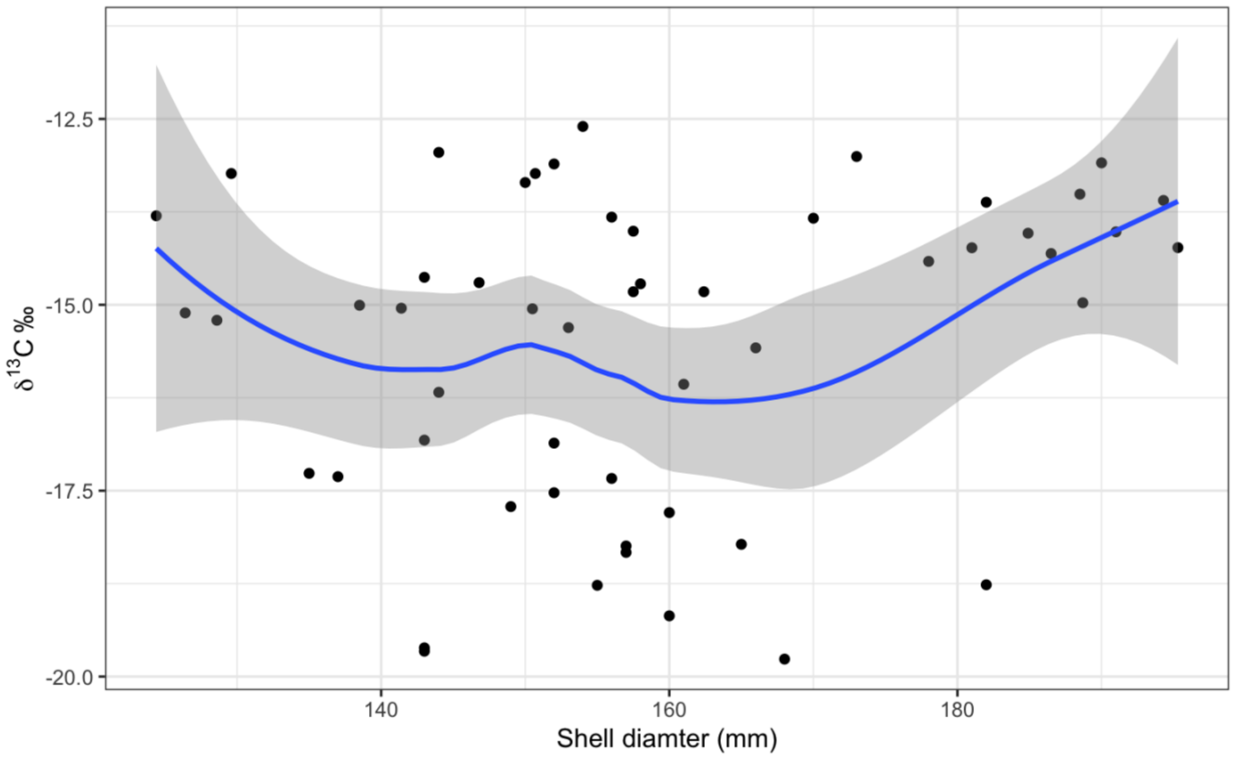
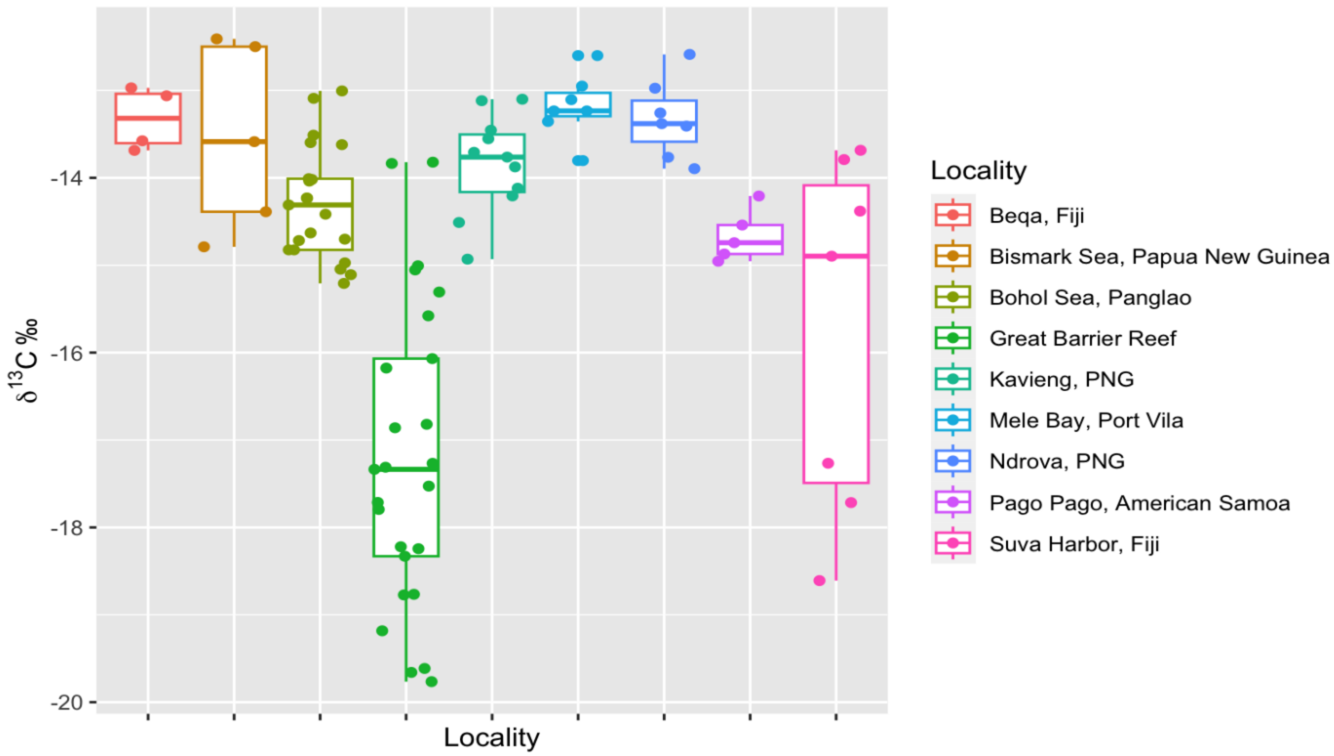
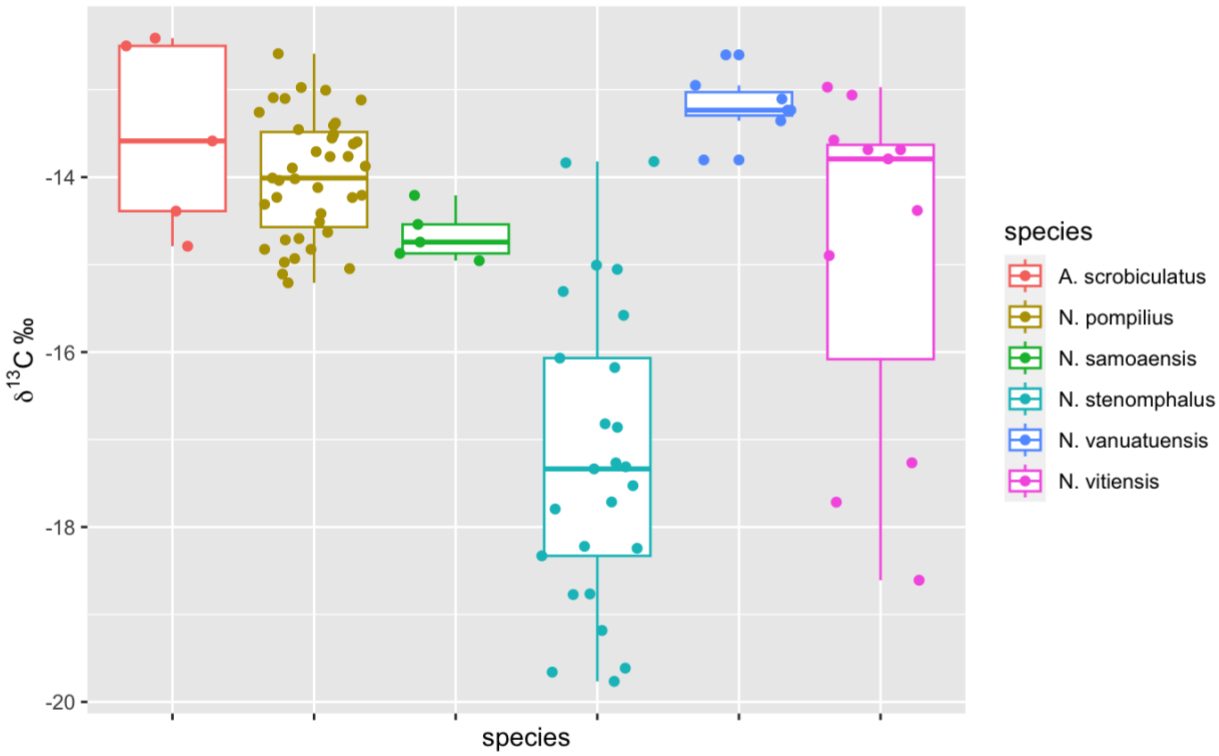


Figure 2. δ^{13}C vs shell diameter of all nautiloid specimens. Solid line indicates fitted LOESS curve, standard errors in grey. Confidence interval is 95%.

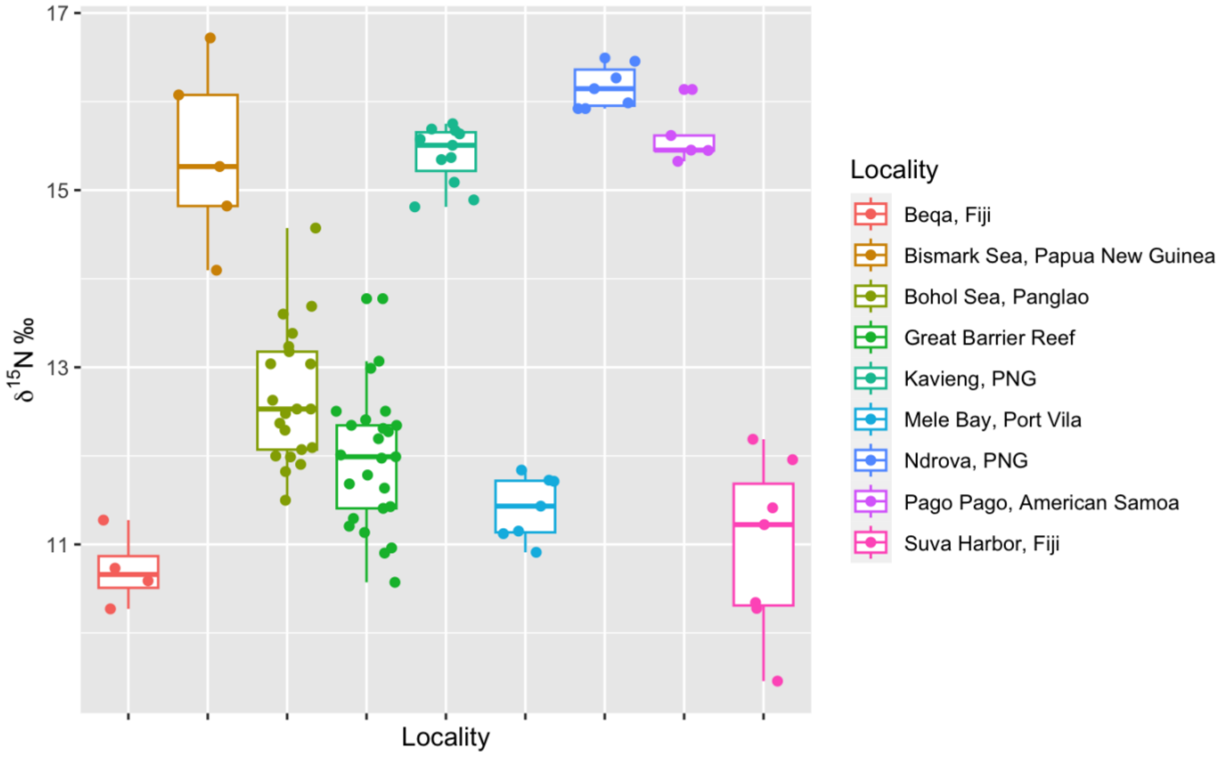


$\delta^{13}C$ ‰
-14
-16
-18
-20
Locality
Locality
Beqa, Fiji
Bismark Sea, Papua New Guinea
Bohol Sea, Panglao
Great Barrier Reef
Kavieng, PNG
Mele Bay, Port Vila
Ndrova, PNG
Pago Pago, American Samoa
Suva Harbor, Fiji



$\delta^{13}C$ ‰
-14
-16
-18
-20
species
species
A. scrobiculatus
N. pompilius
N. samoaensis
N. stenomphalus
N. vanuatuensis
N. vitiensis

Figure 3. Boxplots of carbon isotope values. The Y axis shows the δ^{13}C value. The X-axis shows the different nautiloid populations (above) and nautiloid species (below) observed in this study



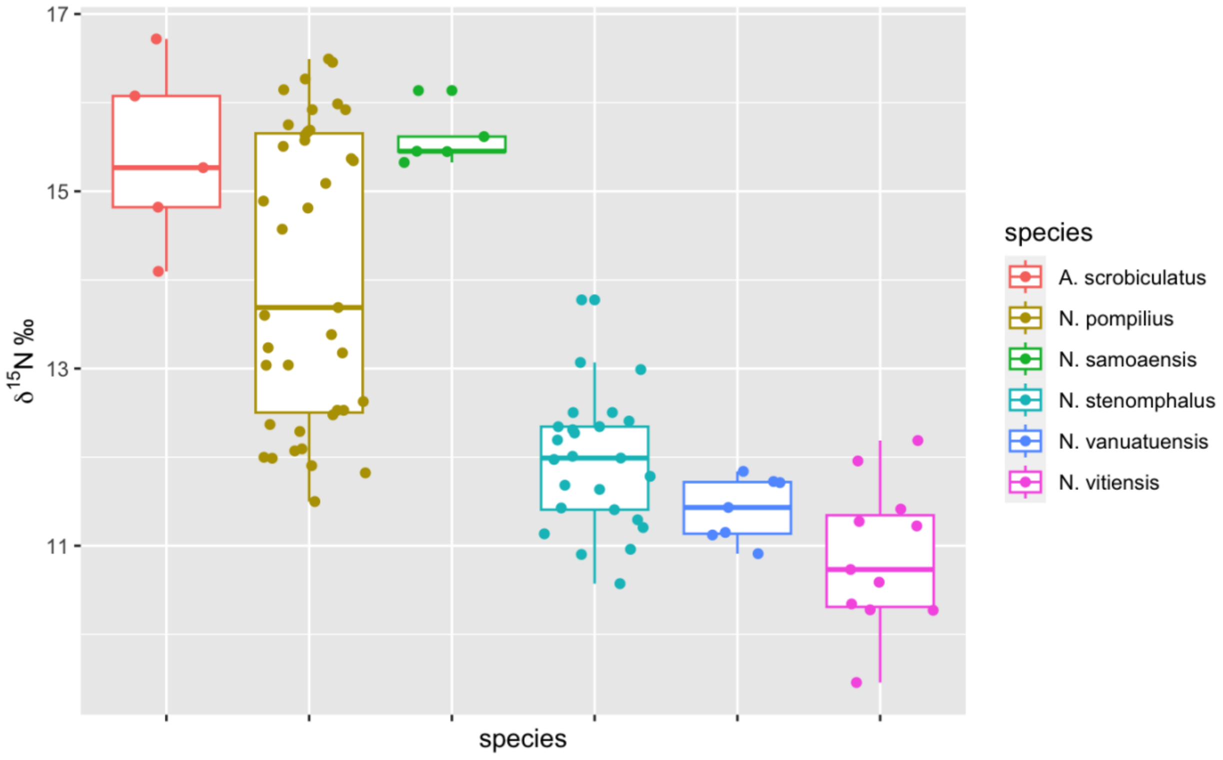


Figure 4. Boxplots of nitrogen isotope values. The y-axis shows the δ^{15}N values. The x-axis shows the different nautiloid populations (above) and nautiloid species (below) observed in this study. Among sites, *Nautilus* from Papua New Guinea and American Samoa showed the highest δ^{15}N values while Fiji and Vanuatu showed the lowest values.

Chapter 2: Trophic level estimates of Mesophotic zone scavengers on fore reef slopes of Fiji and New Ireland, Papua New Guinea.

Job Lukas Veloso*[1], Gregory Barord[2], and Peter Ward[1]

*jlveloso@uw.edu

[1]Department of Biology, University of Washington, Seattle, WA 98995, USA

[2]Department of Marine Science, Central Campus, Des Moines, Iowa, USA

Abstract

Mesophotic reef slopes remain among the least studied marine ecosystems, despite their potential to harbor diverse and functionally important species assemblages. In this study, we investigate the trophic structure of scavenger communities from two mesophotic fore reef ecosystems in the Indo-Pacific: Suva Harbor, Fiji, and Kavieng, Papua New Guinea (PNG). Using bulk stable isotope analysis (SIA) of $\delta^{13}C$ and $\delta^{15}N$, along with compound-specific amino acid isotope analysis (CS-AAIA), we quantify food chain length and estimate the relative trophic positions (RTP) of key mesophotic scavengers, including two co-occurring nautiloid species in Kavieng (*Allonautilus scrobiculatus* and *Nautilus pompilius*) and *Nautilus vitiensis* in Fiji. Bayesian modeling using the SIBER framework reveals longer food chain length and greater isotopic niche breadth in Kavieng compared to Fiji, suggesting higher trophic complexity. Despite sympatry, *A. scrobiculatus* and *N. pompilius* exhibit similar trophic positions (RTP = 3.5 and 3.1, respectively), indicating potential dietary overlap. Comparisons with shrimp populations from Kavieng, Fiji, and the San Juan Islands (USA)—the latter representing a nautiloid-free temperate ecosystem—revealed no significant differences in $\delta^{15}N$ values, suggesting functional consistency across biogeographic regions. These findings highlight the role of nautiloids and benthic scavengers in mesophotic nutrient cycling and underscore the utility of isotopic tools for understanding deep reef food webs in data-limited systems.

Introduction

Marine ecosystems are complex networks of interdependent organisms, where energy flow and nutrient cycling are governed by intricate food webs. Understanding the structure and dynamics of marine ecosystems, as well as investigating their food webs, can be an effective tool in conservation and management, comprehending the dynamics of marine communities, and predicting their responses to environmental changes. Moreover, ecosystem-based management is increasingly used in marine conservation and natural resource management worldwide (Barbier et al. 2008; Leslie 2018). Among the most poorly studied are deeper ecosystems, including mesophotic fore reef slopes in many parts of the world. These ecosystems are home to a diverse array of marine organisms. Among these organisms are nautiloids (class Cephalopoda, subclass Nautiloidea), which have a significant role as scavengers in the mesophotic fore reef slopes in certain areas of the Indo-Pacific (Ward 1987; Barord et al. 2021).

These ancient creatures may offer valuable insights into mesophotic marine ecosystems due to their longevity, relatively large size, and stable position in the food chain. While substantial funding has been allocated to studying coral reefs—recently driven largely by concerns over climate change and ocean warming—mesophotic habitats downslope from active reef regions remain among the most poorly studied marine ecosystems. Mesophotic ecosystems are often buffered from direct physical disturbances, such as strong wave action and sedimentation, which can adversely affect shallower reefs. This stability contributes to the long-term resilience of these ecosystems, allowing them to maintain biodiversity and structural complexity (Hinderstein et al. 2010; Eyal et al. 2021). The ecological roles of mesophotic zones are still being explored, and ongoing research aims to better understand their dynamics and the implications for conservation strategies (Hernandez-Agreda et al. 2022).

Stable isotope analysis (SIA) has become a fundamental tool in assessing trophic relationships and energy flow in marine ecosystems. By analyzing the ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes, researchers can infer trophic levels and food web structures. Carbon isotopes provide insights into primary production sources, while nitrogen isotopes indicate trophic positioning. Previous studies have applied SIA to examine reef-associated fishes and benthic organisms, but mesophotic food webs, particularly those involving scavengers, remain underexplored. Given their reliance on detrital inputs and carrion, mesophotic scavengers may occupy unique trophic niches that differ from their shallow-water counterparts.

Here, we focus on comparing the food webs and aspects of trophic ecology of two distinct nautiloid populations found in Fiji and Kavieng, Papua New Guinea (PNG). Both regions harbor unique marine habitats with distinct environmental characteristics and species assemblages, making them ideal case studies for investigating the differences in nautiloid trophic ecology. Moreover, Kavieng, PNG is one of only two documented populations with sympatric species of modern nautiloids—*Allonautilus scrobiculatus* and *Nautilus pompilius* (Ward and Saunders 1997; Ward et al. in review) (Figure 1A)—while Fiji hosts only one species, *Nautilus vitiensis* (Figure 1B). Despite their ecological significance, there is a limited understanding of the interactions between these sympatric species.

This research presents the first-ever relative trophic position (RTP) estimates for the two sympatric nautiloids in Kavieng. While previous studies have used stable isotopes to explore aspects of modern nautiloid ecology (Tajika et al. 2022; Ward et al. 2023), none have explicitly compared nautiloid-inhabited food webs across different geographical regions. This study aims to fill that gap by quantifying food chain lengths and trophic positions in nautiloid-dominated ecosystems using stable isotope analysis. Specifically, we compare a population with one nautiloid species (Fiji) to a population with two species (and two genera) offshore of Kavieng, PNG.

Using stable isotope analysis, we aim to quantify the trophic positions of mesophotic scavengers and assess potential differences in energy flow between these two locations. Specifically, we ask: (1) Do mesophotic scavengers in Fiji and Papua New Guinea occupy similar trophic positions? (2) How do site-specific environmental factors influence stable isotope signatures? (3) What are the broader implications for nutrient cycling in mesophotic ecosystems? Addressing these questions will contribute to a more comprehensive understanding of deep reef food webs and the ecological roles of scavengers in mesophotic ecosystems.

Materials and Methods:

Collection of samples

In both ecosystems, nautiloids and associated benthic marine invertebrates and fish were captured together using locally deployed fishing traps in Kavieng, Papua New Guinea, and Suva Harbor, Fiji. Only soft tissue samples were collected through non-lethal sampling, and all specimens were subsequently preserved in 90% ethanol for further analysis.

Bulk stable isotopes analysis of δ13C and δ15N

Tissue samples were placed in an oven to dry at approximately 60°C for 48 hours. The dried tissues were each sectioned into smaller pieces (~0.4 mg). The samples were weighed using a microbalance and wrapped in tin capsules. The concentration and isotopic composition of carbon and nitrogen in the samples were measured with a Costech™ ECS 4010 Elemental Analyzer coupled to a Thermo Finnigan™ MAT253 continuous flow isotope ratio mass spectrometer in IsoLab at the University of Washington. Combustion was carried out at 1000°C with a 10 mL pulse of O_2. The result of the combustion are gases that are passed through a reduced copper column maintained at 700°C. A magnesium perchlorate trap was then used to remove water from the gas stream, after which the gases were separated via gas chromatography and fed into the mass spectrometer via a Thermo Finnigan Conflo III.

Raw isotopic data were corrected using a two-point calibration[100] with three in-house standards: two glutamic acids and dried salmon, which are calibrated against international reference materials NBS19, LSVEC, IAEA-N-1, USGS32, USGS-40 and USGS-41. Nitrogen isotopic data are reported in delta notation relative to air. Carbon isotopic data are reported in delta notation relative to Vienna Peedee Belemnite (VPDB).

Compound-specific amino acid isotope analysis

In addition to bulk stable isotope analysis (SIA), Compound-specific amino acid isotope analysis (CS-AAIA) was also performed for *A. scrobiculatus* and *N. pompilius* samples from Kavieng, PNG. The tissues were desiccated in an oven at approximately 60°C for 48 hours. The dried tissues were individually powdered and placed in glass vials. The powdered samples were weighed using a microbalance.

δ15N values of amino acids can be used to to evaluate trophic position, similarly to conventional or bulk SIA. However, this method uniquely identifies the δ15N of basal resources, enabling direct estimations of trophic position without the need for distinct baseline samples of δ15N(Chikaraishi et al. 2007, 2010; Murphy et al. 2020)(Pethybridge et al. 2018).

CS-AAIA was performed on a Thermo Trace GC 1310 gas chromatograph coupled to a Thermo Scientific Delta V Advantage isotope-ratio mass spectrometer via a GC IsoLink II combustion interface. CS-AAIA of samples were performed at the Stable Isotope Facility in the University of California-Davis.

The relative trophic position (RTP) was calculated using this equation(Chikaraishi et al. 2010):

$$\mathbf{RTP= (\delta^{15} N_{Glu} - \delta^{15} N_{Phe} - 3.4) / 7.6 + 1}$$

Where RTP denotes the relative trophic level, δ^{15} N_{Glu} and δ^{15} N_{Phe} are the nitrogen isotopic compositions of glutamic acid and phenylalanine, respectively. In the process of metabolism, glutamic acid undergoes a systematic increase in 15N concentration due to the breaking of C–N bonds. Conversely, phenylalanine experiences minimal enrichment in 15N during metabolism since tyrosine formation involves adding a hydroxyl group instead of breaking C–N bonds(Ohkouchi et al. 2013)(Chikaraishi et al. 2007).

Data Analysis

To assess trophic structure and estimate food chain length in nautiloid-inhabited communities, we employed the Stable Isotope Bayesian Ellipses in R (SIBER) framework (Jackson et al. 2011). SIBER allows for the visualization and quantification of isotopic niche widths and overlaps using Bayesian inference, making it particularly useful for ecological studies where sample sizes may be limited or variability among groups is high. This approach provides robust, probabilistic estimates of isotopic niche area (standard ellipse area, SEA) and other metrics of trophic structure, such as Layman's metrics and food chain length (FCL), with credible intervals that reflect uncertainty.

Food chain length was estimated using the $\delta^{15}N$ range metric derived from the Bayesian posterior distributions, which provides a measure of vertical trophic structure within each community. Total area (TA) and ellipse-based niche widths were also computed to assess isotopic diversity. These metrics help reveal the degree of trophic complexity and potential dietary overlap between coexisting species.

To compare differences in isotopic values ($\delta^{15}N$ and $\delta^{13}C$) among organismal groups within each community, we performed one-way analysis of variance (ANOVA). All statistical analyses and data visualization were conducted using R (version 4.3.2), with additional comparisons and plots created using PAST 4.15 (Hammer 2001) and Microsoft Excel (version 16.81).

Results:

Food Web Comparison

Our analyses revealed distinct differences in food web structure between the two study sites. The Kavieng community exhibited a larger total area (TA = 18.2) and a longer food chain length (FCL = 4.1) compared to Fiji, indicating a broader trophic niche and greater trophic diversity. Additionally, there was greater variability in the $\delta^{15}N$ values of nautiloids in Fiji than in Kavieng, suggesting differences in nitrogen sources or dietary variation among individuals (Figure 2).

The results indicate a clear difference in food chain length (FCL) between Suva Harbor, Fiji, and Kavieng, Papua New Guinea (PNG). The median FCL estimate is substantially higher in Kavieng, suggesting a more complex trophic structure with additional predator-prey interactions. The broader credible intervals in Kavieng further suggest greater variability in trophic positions, potentially reflecting a diverse assemblage of top predators or a more dynamic ecosystem. In contrast, the food chain in Suva Harbor appears shorter and more constrained, with a lower median estimate and narrower credible intervals. This pattern may indicate a simpler trophic system, potentially driven by differences in ecosystem productivity, species diversity, or the presence of key top predators. The observed variation in FCL between these locations underscores the potential influence of local environmental conditions and ecological dynamics in shaping trophic structure across marine ecosystems. These results suggest that the food web in Kavieng is more trophically complex, potentially reflecting greater predator diversity, resource availability, or environmental stability. In contrast, the shorter food chain in Suva Harbor may indicate a more simplified trophic structure, possibly due to lower ecosystem productivity or differences in top predator abundance.

In the Fiji community, there was no significant difference in either carbon or nitrogen isotopic values among animal groups captured in the traps. In contrast, the Kavieng community demonstrated significant differences in both $\delta^{13}C$ and $\delta^{15}N$ values among animal groups, suggesting greater trophic segregation among species in this region (Figure 3).

Relative Trophic Position of *Allonautilus* and *Nautilus*

Both nautiloid species from Kavieng exhibited an estimated trophic level of approximately three, positioning them within the mid-trophic range of their respective food webs. There was a slight difference in relative trophic position (RTP), with *A. scrobiculatus* at RTP = 3.5 and N. pompilius at RTP = 3.1. Additionally, isotopic analysis revealed no significant difference in $\delta^{13}C$ or $\delta^{15}N$ values between *A. scrobiculatus* and *N. pompilius* in Kavieng, suggesting similar dietary sources and trophic positioning.

Difference in shrimp

Stable nitrogen isotope ($\delta^{15}N$) values did not differ significantly among shrimp populations from Kavieng, Fiji, and the San Juan Islands ($F_{3,13} = 3.183$, $p = 0.060$). While the boxplots suggest some variation in median values, the overall overlap in interquartile ranges and the non-significant test results indicate no strong differentiation among these populations. The intraclass correlation coefficient (ICC = 0.34) suggests moderate within-group variance, while the Bayes factor (1.349) indicates no strong evidence for either equal or unequal means. Additionally, Welch's F test for unequal variances ($F_{6.451} = 3.25$, $p = 0.096$) further supports the lack of a strong difference.

Discussion

Partial Food Webs of Fiji and Kavieng

The food chain lengths (FCL) observed in both Fiji and Kavieng, Papua New Guinea, were relatively short. The productivity of these ecosystems is often constrained by nutrient availability and light penetration, resulting in a simplified trophic structure. The presence of similar scavenging communities, primarily composed of scavenging invertebrates, is evidenced by their carbon and nitrogen (CN) isotopic interactions. Deep-sea ecosystems often support lower primary productivity than shallower environments (Perkins et al. 2014), which can lead to a more direct transfer of energy from primary producers to higher trophic levels, thereby shortening the food chain.

Differences in food chain length (FCL) between Kavieng, PNG and Suva Harbor, Fiji suggest variation in trophic complexity that may reflect underlying ecological and environmental gradients. The longer FCL observed in Kavieng is consistent with studies showing that ecosystems with higher biodiversity and resource availability tend to support more trophic levels (Post, 2002; McHugh et al., 2011). In marine environments, FCL can also be influenced by habitat heterogeneity, predator presence, and productivity (Hussey et al., 2014), all of which may differ between these two Indo-Pacific sites. The broader credible intervals observed in Kavieng further suggest variability in consumer trophic positions, potentially driven by a more diverse predator community or greater isotopic niche breadth (Layman et al., 2007). Conversely, the shorter and more constrained FCL in Suva Harbor could reflect a simpler trophic structure, possibly shaped by anthropogenic pressures or lower prey diversity (Pauly & Palomares, 2005). These findings align with growing evidence that FCL is a sensitive indicator of ecological structure and function, particularly in data-limited tropical marine systems (Jennings & Warr, 2003; Navarro et al., 2011). As such, stable isotope-based estimates of FCL provide valuable insight into how local environmental conditions shape food web architecture across the Indo-Pacific.

Despite being geographically distinct, both the Fijian and Kavieng ecosystems share notable similarities, particularly in the trophic interactions between nautiloid species and other scavengers, such as deep-sea shrimp. Both regions are characterized by steep underwater slopes and nutrient-rich waters, providing ideal habitats for these organisms. Within these environments, nautiloids and shrimp appear to occupy similar ecological roles, potentially engaging in a sympatric relationship where they share resources without directly competing.

Both nautiloids and shrimp are scavengers, primarily consuming organic matter, including carrion that sinks to the seafloor. Their similar nitrogen isotopic values (Figure 3) suggest that they occupy comparable trophic levels, potentially feeding on similar food sources. However, the ability of these species to coexist within the same ecological niche may be facilitated by resource partitioning. This could occur through temporal differences in feeding activity or slight variations in dietary preferences. The carbon isotopic values (Figure 3), particularly in Fiji, suggest distinct carbon sources for shrimp and nautiloids, further supporting the notion that direct competition between these species may be minimal. Overall, our findings indicate a stable coexistence between these scavengers, highlighting the ecological balance that maintains biodiversity in these deep-sea environments.

The Kavieng community exhibited slightly higher δ15N and δ13C values compared to Fiji, suggesting differences in basal resource composition. These disparities may be influenced by variations in environmental conditions, prey availability, or dietary preferences. One potential explanation is the presence of nitrogen-fixing bacteria in the Bismarck Sea (Benavides et al. 2015), which could elevate baseline δ15N levels at the base of the food web, subsequently affecting all trophic levels. Further investigations into prey composition, habitat structure, and interspecies interactions are necessary to better understand these isotopic differences.

Relative Trophic Levels of *Allonautilus scrobiculatus* and *Nautilus pompilius* in Kavieng, Papua New Guinea

Both nautiloid genera from Kavieng exhibit relatively high nitrogen isotopic values (Figure 3), with a mean of 15.88 ± 0.5‰. Such values are typically associated with higher trophic levels and have been documented in apex predators such as large marine vertebrates (Borisova et al. 2020).

However, our compound-specific amino acid isotopic analysis (CS-AAIA) indicates that *A. scrobiculatus* and *N. pompilius* occupy an estimated trophic level of approximately 3, consistent with scavengers rather than true apex predators.

While both species share a trophic level of 3, expressing trophic positions in decimal fractions provides a more nuanced perspective. Our analysis estimates a trophic position of 3.5 for A. scrobiculatus and 3.1 for N. pompilius. This differentiation suggests that these species consume a mix of prey items and organic detritus, a characteristic trait of scavengers (Barord et al. 2021). Despite the lack of direct evidence for active predation, their intermediate trophic positioning reflects their role in recycling organic matter within the ecosystem.

The sympatric occurrence of A. scrobiculatus and N. pompilius in Kavieng provides valuable insights into niche partitioning among nautiloids. Both species were captured using the same trapping methods, and their CN isotopic values suggest potential dietary overlap. This overlap raises the possibility of interspecific competition for resources. However, several ecological mechanisms may facilitate their coexistence, including slight differences in depth preferences, habitat selection, or temporal variations in feeding and reproductive cycles. These factors could mitigate direct competition and allow for stable sympatry.

Notably, this study represents the first investigation into the niche overlap of two nautiloid genera within the same ecosystem. While the principle of competitive exclusion suggests that complete niche overlap is unlikely (Golikov et al. 2019a, 2019b), our findings highlight the potential for sympatric species to share resources under specific ecological conditions. However, given that only four mature A. scrobiculatus specimens were sampled, our conclusions remain preliminary. Further stable isotope analysis (SIA) incorporating ontogenetic dietary shifts is necessary to refine our understanding of inter- and intraspecific competition within nautiloid communities.

Other scavenger groups

In this study, we also examined shrimp from an ecosystem—San Juan Islands—that lacks nautiloids, in contrast to our tropical sites. Fiji is home to a single nautiloid species (*Nautilus pompilius*), while Kavieng, Papua New Guinea, supports two co-occurring genera (*Nautilus* and *Allonautilus*), providing a gradient of nautiloid presence. By including a region without nautiloids, we aimed to explore whether shrimp maintain consistent ecological roles in ecosystems with varying scavenger diversity and trophic architecture.

Comparing shrimp from ecologically distinct regions—such as the temperate San Juan Islands and the tropical reefs of Kavieng and Fiji—provides an opportunity to investigate whether consistent ecological roles are maintained across large environmental gradients. Latitude-driven differences in ocean productivity, nutrient cycling, and food web complexity often lead to expectations of divergent trophic positions among comparable taxa (Longhurst & Harrison, 1989; Post, 2002). Shrimp, as benthic omnivores and scavengers, typically occupy intermediate trophic levels, but the extent to which their trophic ecology remains consistent across biogeographic boundaries remains unclear. Our analysis of $\delta^{15}N$ values showed no significant differences among shrimp populations from these regions ($F_{3,13} = 3.183$, $p = 0.060$), suggesting that shrimp may fulfill ecologically similar roles regardless of latitude.

This isotopic similarity could reflect convergent feeding behavior or commonality in prey type across regions. Shrimp from both temperate and tropical environments may feed on similar mixtures of detritus, benthic invertebrates, and organic matter, leading to overlapping trophic signals despite geographic separation (Fry, 2006; Newsome et al., 2007). Furthermore, it is possible that regional differences in baseline $\delta^{15}N$ values offset potential differences in trophic level, particularly in nearshore ecosystems where nitrogen sources are shaped by complex local dynamics (McMahon et al., 2013). While food web composition and primary producers differ substantially between tropical coral reef slopes and temperate rocky shorelines, shrimp may access basal resources that occupy similar positions in the nitrogen cycle, thus reducing isotopic contrast.

The moderate intraclass correlation coefficient (ICC = 0.34) supports the notion that while there is some group-level structuring, individual-level variation predominates. This suggests that shrimp exhibit trophic plasticity—a capacity to adjust feeding behavior based on local availability of resources—which may buffer against strong environmental or biogeographic signals in their isotopic signatures. Such plasticity has been observed in other benthic invertebrates and is a key factor in their ecological resilience (Vander Zanden & Rasmussen, 1999; Jennings et al., 2008).

Although the observed p-value approaches conventional significance thresholds, the Bayes factor (1.349) provides no strong evidence for either equality or inequality in means, reinforcing the interpretation of similarity rather than difference. Future studies incorporating compound-specific stable isotope analysis (CSIA) could help disentangle whether the apparent isotopic similarity is due to comparable trophic positions or offsetting baseline values. Additionally, paired $\delta^{13}C$ data would offer insights into whether shrimp across these regions rely on distinct basal carbon sources while occupying similar trophic levels.

Conclusion

This study provides new insights into the trophic structure and scavenger interactions within fore reef slope ecosystems of the tropical Indo-Pacific. Our isotopic analyses reveal that both Fiji and Kavieng support relatively short food chains, with nautiloids and shrimp playing important roles in nutrient recycling and mid-trophic energy transfer. The co-occurrence of *Allonautilus scrobiculatus* and *Nautilus pompilius* in Kavieng represents the first documented case of sympatric nautiloid genera with similar trophic positions, suggesting potential niche overlap mitigated by behavioral or habitat partitioning. Despite differences in nautiloid diversity, isotopic patterns among shrimp populations from Kavieng, Fiji, and the San Juan Islands suggest functional consistency across tropical and temperate ecosystems. This convergence points to the potential trophic stability of benthic scavenger guilds, even across large biogeographic gradients.

By integrating $\delta^{15}N$ and $\delta^{13}C$ values, as well as compound-specific isotope data, our study underscores the value of stable isotope tools for revealing hidden structure in deep reef food webs. Future research should expand spatial sampling, investigate ontogenetic dietary shifts, and incorporate finer-resolution isotopic tracers to better understand trophic interactions in mesophotic ecosystems. Ultimately, these insights will improve our understanding of how scavenger dynamics and food web architecture respond to environmental variation in data-limited marine systems.

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Figures

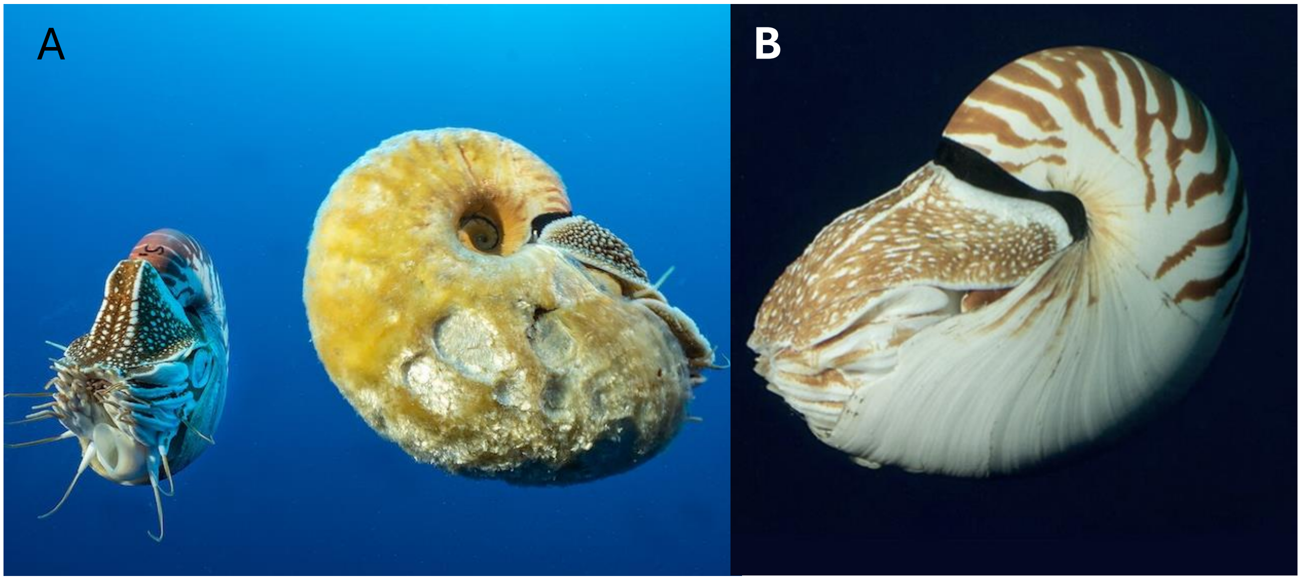

Figure 1. (A) *Nautilus pompilius* (left) and *Allonautilus scrobiculatus* (right) in Kavieng, PNG. Photo by P. Ward (B) *Nautilus vitiensis* from Fiji, photo by G.J. Barord.

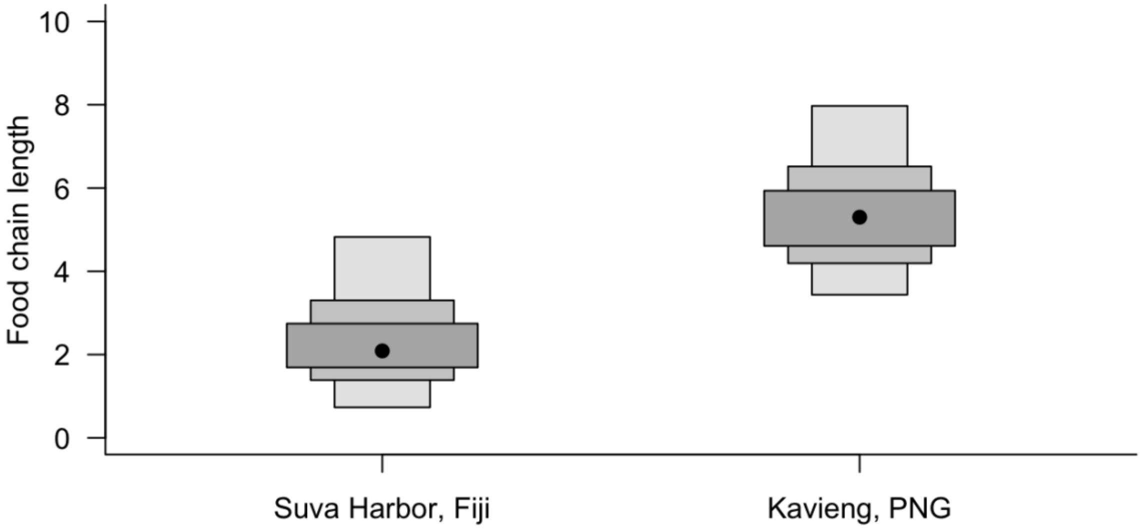

Figure 2. Estimated food chain length (FCL) for Suva Harbor, Fiji, and Kavieng, Papua New Guinea (PNG). The y-axis represents food chain length, inferred from nitrogen isotope ($\delta^{15}N$) values, where higher values indicate longer trophic pathways. The x-axis denotes the two study locations. Median FCL estimates (black dots) indicate a significantly longer food chain in Kavieng (approximately 5–6) compared to Suva Harbor (approximately 2–3). The dark gray boxes represent the interquartile range (50% credible interval), while the lighter gray shading extends to the 95% credible interval.

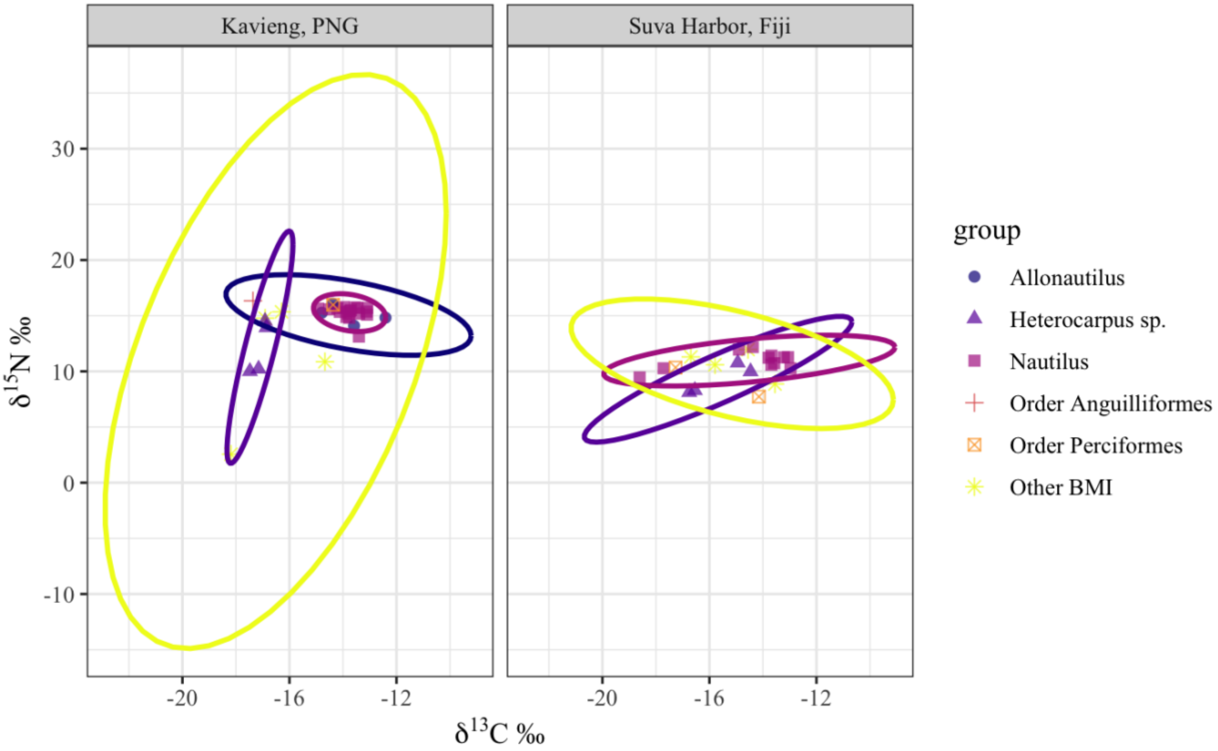

Figure 3. Carbon and nitrogen isotope values of organisms from two nautiloid-inhabited ecosystems in the Indo-Pacific. The four organismal groups are represented as follows: 1. *Allonautilus scrobiculatus* (red), 2. Benthic Marine Invertebrates (BMI) (green), 3. Fish (blue), and 4. *Nautilus pompilius* (purple).

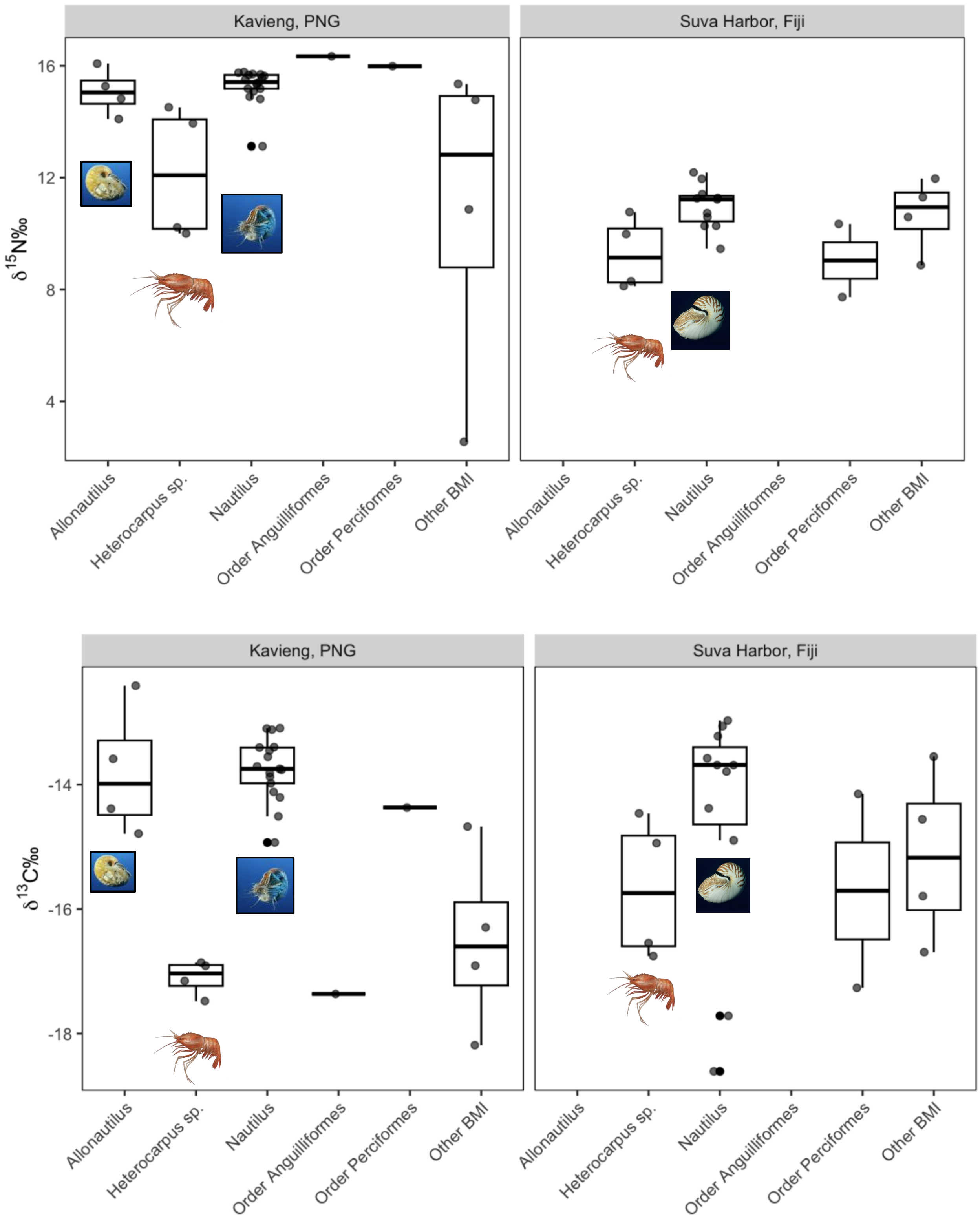

Figure 4. δ^{15}N values of different animal groups from both communities, along with δ^{13}C values, illustrating trophic structuring in each ecosystem.

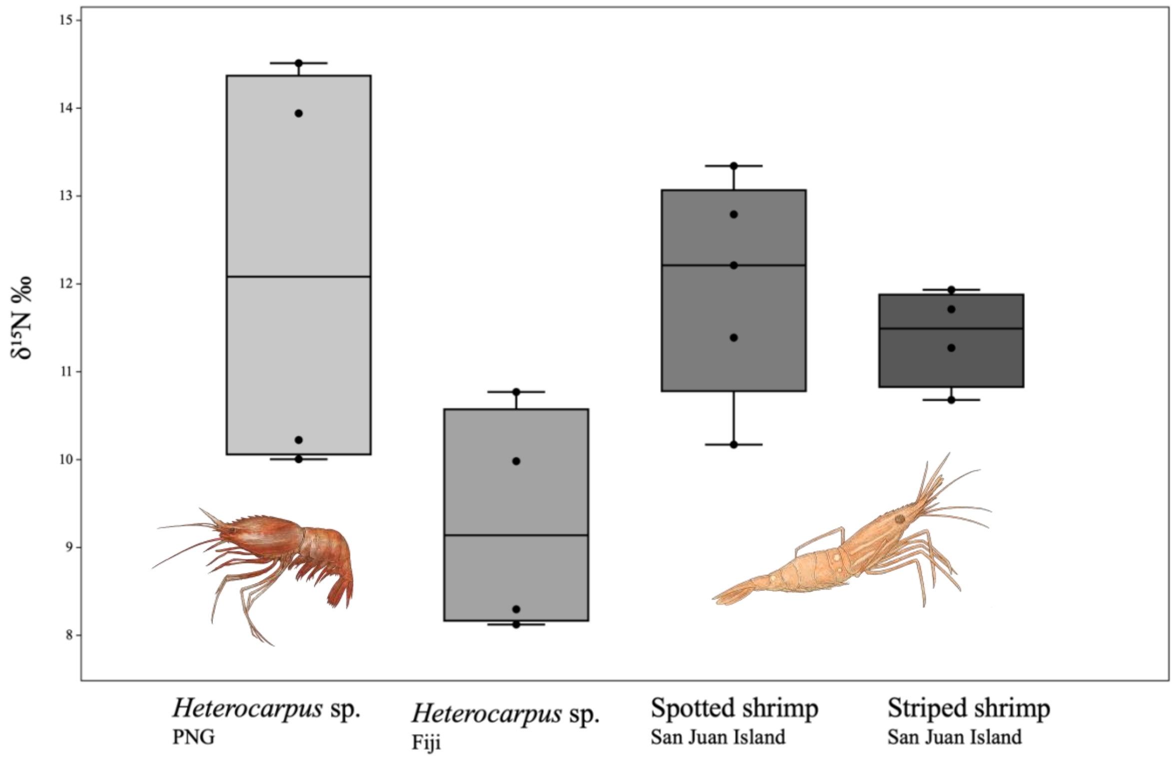

Figure 5. Relative trophic positions of various marine organisms, adapted from Ohkuchi et al. (2014), incorporating new data on *Allonautilus scrobiculatus* and *Nautilus pompilius* from Kavieng (Kav) and *Sepia latimanus* from the Philippines (PI).

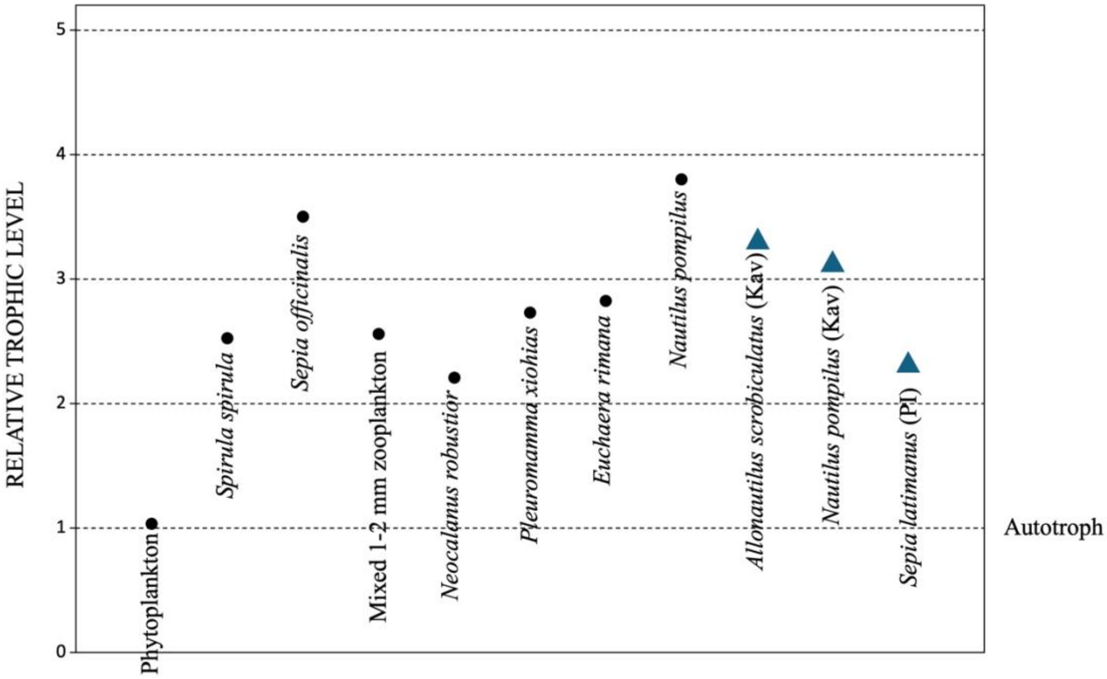

Figure 6. Nitrogen isotope ($\delta^{15}N$) values for shrimp collected from three distinct marine ecosystems: Kavieng (Papua New Guinea), Fiji, and the San Juan Islands (Washington, USA). The boxplots display the median, interquartile range (IQR), and overall spread of $\delta^{15}N$ values within each group. Despite visual variation, statistical analysis ($F_{3,13} = 3.183$, $p = 0.060$) indicates no significant differences in $\delta^{15}N$ values among groups, suggesting comparable trophic positioning across tropical and temperate regions.

Chapter 3: Metabolic Comparisons of Phragmocone-Bearing Cephalopods: Insights from C_{meta} into the Evolutionary and Ecological Divergence of Sepiids, Nautiloids, and Ammonites

Job Lukas Veloso*[1]and Peter Ward[1]

jlveloso@uw.edu

[1]Department of Biology, University of Washington, Seattle, WA 98995, USA

Abstract

Cephalopods exhibit a wide range of ecological and physiological adaptations, yet their metabolic strategies are often difficult to reconstruct, especially in extinct forms. Here, we use stable carbon isotopic signatures from biogenic carbonates to calculate C_{meta}, a proxy for the proportion of metabolically derived carbon incorporated into shell material, to compare metabolic profiles across modern sepiids and nautiloids, fossil nautiloids, and extinct ammonites. Our results reveal consistently high and variable C_{meta} values in sepiids and ammonites, reflecting active, high-energy lifestyles. In contrast, both modern and fossil nautiloids exhibit lower and more constrained C_{meta} values, indicative of a conservative metabolic strategy that has remained stable since the Paleozoic. These findings demonstrate the utility of C_{meta} as a quantitative proxy for reconstructing metabolic physiology and provide new insight into the evolutionary divergence and ecological resilience of shelled cephalopods.

Introduction

Cephalopods have undergone significant evolutionary and ecological diversification, yet core aspects of their physiology—particularly metabolic rate—continue to shape their ecological roles. Among extant forms, nautiloids and sepiids offer contrasting lifestyles: the former are slow-moving, deep-water scavengers, while the latter are active, short-lived predators that occupy a wide range of marine habitats. Extinct ammonites, which dominated Mesozoic marine ecosystems, exhibited ecological roles that overlap with both of these extant groups. Comparative assessments of their metabolic traits offer valuable insights into the evolutionary constraints and trajectories that have shaped cephalopod success and extinction.

Sepiids are ecologically versatile and highly mobile predators distributed from shallow coastal habitats to deeper continental shelves (Reid et al., 2005; Neige, 2021; Neige, 2003; Sherard, 2010). They use rapid jet propulsion and precise buoyancy control via their internal cuttlebone to maneuver between benthic and pelagic zones (Denton & Gilpin-Brown, 1961; O'Dor & Webber, 1986). These energetic behaviors correlate with elevated metabolic activity, supported by high C_{meta} values inferred from isotopic analyses (Chung et al., 2022). In contrast, modern nautiloids occupy mesophotic reef slopes, where they engage in opportunistic scavenging and grow at markedly slower rates (Saunders & Landman, 2009; Ward, 1987). Their external phragmocone shells impose mobility constraints and reflect a conservative, low-energy lifestyle that appears to have persisted across geological timescales (Guerra, 2023; Tajika et al., 2023).

Ammonites, though extinct, offer a vital comparative lens. Their morphological and ecological diversity suggests they spanned a broad spectrum of marine niches, from fast-moving pelagic hunters to shelf-dwelling species (Westermann, 1996; Klug et al., 2015). Isotopic analyses have provided compelling evidence that ammonites had higher metabolic rates than modern nautiloids, supporting the hypothesis that they were more physiologically comparable to modern coleoids, such as sepiids (Chung et al., 2022; Tajika et al., 2023). Conversely, extinct nautiloids exhibit metabolic signatures that closely mirror those of living nautiloids, underscoring their long-term metabolic conservatism (Tajika et al., 2023). These contrasting trends highlight how divergent life strategies may have influenced the survival or extinction of major cephalopod lineages.

Biogenic carbonates, such as those found in nautiloid shells, preserve durable isotopic signatures that reflect both organismal metabolism and ecological context (Chung et al., 2022; Gillooly et al., 2005; McConnaughey & Gillikin, 2008). Additionally, in sepiids, phragmocone morphology has been shown to represent functional adaptations for shell strength and buoyancy control, illustrating how structural innovations are closely tied to ecological and physiological demands in coleoid evolution (Ward et al., 2022). The carbon isotope–based metric C_{meta} provides a tool for estimating metabolic rate across both extant and extinct taxa (Chung et al., 2022; Tajika et al., 2023). Here, we apply stable isotope analyses of biogenic carbonates to quantify and compare C_{meta} values across modern nautiloids and sepiids, extending the comparison to include several fossil nautiloid species and ammonoids. These data allow us to assess patterns of metabolic convergence and divergence through deep time, offering insights into how physiology has influenced the ecological roles and evolutionary paths of phragmocone-bearing cephalopods.

Materials and Methods

Sampling of biogenic carbonates

Biogenic carbonate samples were collected by carefully sectioning the phragmocone segments of both sepiids and modern nautiloids. Specifically, cuttlebones were sampled systematically from early septa to the terminal loculus, while nautiloids were sampled at their conchs' septa (Figure) .

All sepiid cuttlebone and nautiloid shell specimens sampled in this study are part of a collection from the Ward Lab at the University of Washington.

Bulk stable isotopes analysis of δ^{13}C(carb) and δ^{18}O

Carbonate shell samples larger than 1mm^2 were ground into powder using a mortar and pestle. The powdered material were all individually weighed to ~0.1 mg samples in an aluminum weigh boat and placed into individual glass vessels and stored in aluminum trays. The Isotopic analyses (δ^{13}C, δ^{18}O) of the powdered biogenic carbonate samples were performed with a Kiel III Carbonate Device coupled to a Finnigan Delta Plus isotope ratio mass spectrometer. The system used 100% phosphoric acid (specific gravity 1.90 – 1.92) for the digestion of carbonates and the purification of the carbon dioxide product. The resulting gases were then passed into a Finnigan Delta Plus isotope ratio mass spectrometer (IRMS). The measured values of all samples were corrected to the VPDB scale with the use of internal reference materials that have been calibrated to and span a similar range of NBS19, NBS18, L-SVEC, and IAEA-603. All analyses were performed in IsoLab at the University of Washington.

Bulk Stable Isotope Analysis of $\delta^{15}N$ from Shell Carbonates

Biogenic carbonate samples (e.g., shell septa) were cleaned of adhering sediment and ground into fine powder using an agate mortar and pestle. For nitrogen isotope analysis, carbonate-bound organic nitrogen was extracted following decarbonation or thermal combustion protocols, depending on preservation and material volume. Approximately 0.4 mg of each powdered sample was weighed using a microbalance and encapsulated in tin capsules.

Isotopic measurements of nitrogen were performed using a Costech™ ECS 4010 Elemental Analyzer coupled to a Thermo Finnigan™ MAT253 isotope ratio mass spectrometer via a Thermo Finnigan Conflo III interface at the University of Washington's IsoLab. Combustion occurred at 1000°C with a 10 mL pulse of oxygen. The resulting gases passed through a reduced copper column at 700°C, followed by dehydration with a magnesium perchlorate trap. The gas stream was then separated chromatographically before being analyzed by the mass spectrometer.

Nitrogen isotope values ($\delta^{15}N$) were corrected using a two-point calibration with in-house standards (e.g., glutamic acids and dried salmon), which are themselves calibrated against international reference materials including IAEA-N-1, USGS32, USGS40, and USGS41. All $\delta^{15}N$ values are reported in delta notation (‰) relative to atmospheric N_2 (AIR).

Calculating Metabolic Proxy

According to a two-component mixing model (McConnaughey & Gillikin, 2008; Schwarcz et al., 1998; Solomon et al., 2006), the δ13C value of biogenic carbonates is a weighted average of the isotopic composition of carbon from two main sources: DIC and dietary carbon. Thus, in this study, I will use the following equation from Chung et al. (2021) to calculate for respired Carbon(meta):

$$\mathbf{\delta 13C = Cmeta \times \delta 13Cdiet + (1 - Cmeta) \times \delta^{13}CDIC} + \boldsymbol{\varepsilon},$$

where δ13Cdiet and δ13CDIC are the average δ13C values of the dietary carbon and DIC of seawater, respectively, and the term ε is the total net isotopic fractionation from the sources to biogenic carbonate. In this study, ε was set to 2.7 for aragonite. Additionally, dietary carbon and DIC of seawater values will be extracted from published data (Table 1). While δ13Ccarb values are all gathered from this current study.

Data analysis

Our analyses involved ANOVA or Kruskal–Wallis H test, followed by pairwise multiple comparisons using Tukey's HSD test or Mann–Whitney U test (Zar 2010). All tests were conducted with a significance level of α = 0.05.

Statistical analyses, computations, and graphs were illustrated using R (version 4.3.2), PAST 4.15(Hammer 2001), and MS Excel (version 16.81).

Results

Comparison of C_{meta} in phargmocone-bearing cephalopods

Sepiids, both from shallow-water species (e.g., *S. latimanus*, *S. pharaonis*) and deep-water taxa (e.g., *S. hedleyi*, *S. australis*), exhibit high and variable C_{meta} values, indicative of a metabolically active lifestyle. In contrast, extant nautiloids (e.g., *N. pompilius*, *A. scrobiculatus*) display significantly lower and more constrained C_{meta} values (Figure 1). This pattern supports the distinction between energy-demanding, actively foraging sepiids and energy-conserving, scavenging nautiloids. Even deep-dwelling sepiids exhibit higher metabolic carbon incorporation than their nautiloid counterparts.

The violin plot presented in Figure 2 illustrates variations in metabolic carbon fraction (C_{meta}) across multiple species of nautiloid cephalopods from four distinct geological intervals: the Late Cretaceous, Eocene, Miocene, and Recent. Notably, specimens from the Eocene exhibit the highest and most variable C_{meta} values, predominantly ranging between approximately 0.6 and 0.9. In contrast, Late Cretaceous species display intermediate metabolic values, typically between 0.3 and 0.6, with moderate intraspecific variability.

Specimens from the Miocene interval reveal substantially reduced metabolic values, primarily between 0.2 and 0.4, indicative of a distinct downward shift in metabolic demands. Finally, the Extant species (*N. pompilius* and *A. scrobiculatus*) showed the lowest C_{meta} values (~0.1–0.2) with remarkably tight distributions, underscoring a clear metabolic conservatism among modern nautiloids. These observed patterns indicate significant shifts in metabolic strategies across geological time, suggesting a transition from higher-energy lifestyles in older fossil taxa toward more energetically conservative and stable metabolic adaptations in contemporary nautiloids.

C_{meta} values varied markedly between ammonoids and nautiloids from the Late Cretaceous (Figure 3). Ammonoids (*Diplomoceras* sp. and *Canadoceras yokoyamai*) exhibited consistently higher C_{meta} values, with median values around 0.45–0.60 and distributions extending above 0.65 in *C. yokoyamai*. In contrast, nautiloids from the same period displayed lower C_{meta} values ranging mostly from 0.25 to 0.45. This clear difference in metabolic carbon incorporation suggests that ammonoids possessed higher metabolic rates than their nautiloid counterparts, consistent with prior interpretations of ammonoid physiology and life history as more active and potentially more energetically demanding. The overlap in stratigraphic age but divergence in C_{meta} values underscores fundamental physiological differences between these two groups during the Late Cretaceous.

Relationship of $\delta^{18}O$ and C_{meta}

A strong and highly significant negative relationship was observed (Figure 4A) between $\delta^{18}O$ values and C_{meta} in fossil nautiloids (Ordinary Least Squares regression: $r = -0.81$, $r^2 = 0.65$, $p < 2 \times 10^{-37}$). The regression slope was –0.094 (± 0.0055 SE), with a 95% bootstrapped confidence interval ranging from –0.106 to –0.081 (N = 1999 resamples), indicating a consistent and robust trend across the dataset. The intercept was estimated at 0.283 (± 0.0094 SE). These results suggest that lower $\delta^{18}O$ values—often associated with warmer water temperatures or increased physiological influence—are strongly correlated with higher incorporation of metabolically derived carbon into shell carbonate, supporting the hypothesis that C_{meta} reflects environmentally modulated metabolic activity in these extinct cephalopods.

For extant nautiloids, no statistically significant relationship was observed (Figure 4B) between $\delta^{18}O$ values and C_{meta} (Ordinary Least Squares regression: $r = –0.16$, $r^2 = 0.025$, $p = 0.248$). The regression slope was –0.018 (± 0.0151 SE), with 95% bootstrapped confidence intervals overlapping zero (–0.049 to 0.016), indicating a lack of consistent trend across the dataset. The intercept was 0.192 (± 0.0139 SE). These findings suggest that, unlike in fossil cephalopods, $\delta^{18}O$ variation in extant nautiloids does not strongly predict shifts in the proportion of metabolically derived carbon, likely reflecting their occupancy of relatively stable thermal environments and/or physiological buffering of carbonate chemistry.

In sepiids, a statistically significant negative correlation was observed (Figure 4C) between $\delta^{18}O$ values and C_{meta} (Ordinary Least Squares regression: $r = –0.33$, $r^2 = 0.108$, $p < 0.001$). The regression slope was –0.019 (± 0.0048 SE), with 95% bootstrapped confidence intervals ranging from –0.029 to –0.010, indicating a consistent trend of increasing metabolic carbon incorporation at lower $\delta^{18}O$ values. The intercept was 0.298 (± 0.0065 SE). Although the effect size was moderate, these findings suggest that temperature or physiological processes linked to $\delta^{18}O$ may influence metabolic activity in sepiids, supporting the potential of C_{meta} as an isotopic proxy for metabolic variability in modern coleoid cephalopods.

Among the cephalopod groups analyzed, ammonites exhibited a statistically significant negative relationship between $\delta^{18}O$ values and C_{meta} despite having the smallest sample size in the dataset. The regression yielded a slope of –0.019 (± 0.0048 SE) with 95% bootstrapped confidence intervals from –0.029 to –0.010, and an intercept of 0.298 (± 0.0065 SE). The correlation coefficient was $r = -0.33$ ($r^2 = 0.108$), with a highly significant p-value ($p = 0.00015$). These results suggest that even with limited data, lower $\delta^{18}O$ values—typically associated with warmer conditions or reduced physiological buffering—are linked to increased metabolic carbon incorporation in ammonite shells. This pattern reinforces the interpretation of ammonites as metabolically active taxa with environmental sensitivity captured in their shell isotopic composition.

Taken together, these results demonstrate a consistent negative relationship between $\delta^{18}O$ and C_{meta} across extinct and extant cephalopod groups, with varying degrees of strength. Fossil nautiloids and ammonoids showed the strongest correlations, likely reflecting broader environmental gradients and reduced physiological buffering in shell formation. Sepiids displayed a moderate trend, consistent with some physiological control but still responsive to environmental conditions. In contrast, extant nautiloids exhibited only a weak association, likely due to their more constrained $\delta^{18}O$ range, reflecting the relatively uniform thermal environments they inhabit today. These findings suggest that $\delta^{18}O$–C_{meta} relationships may serve as useful proxies for inferring relative metabolic intensity across both evolutionary and ecological contexts, though the strength and resolution of these proxies depend on environmental variability, taxon-specific physiology, and the quality of isotopic preservation.

Sepiid metabolism through ontogeny

C_{meta} values measured across ontogeny in pooled sepiid specimens show a trend of increasing metabolic activity from early to mid-development, with peak C_{meta} values reached in subadult stages (Figure 5). This rise likely reflects increased activity, growth, and foraging demand. A subsequent plateau or slight decline in late ontogeny suggests a metabolic shift toward energy conservation or reproductive investment.

$\delta^{15}N$ and metabolism in extant nautiloids

We found that in extant nautiloids, $\delta^{15}N$ values were significantly positively correlated with C_{meta} (Ordinary Least Squares regression: $r = 0.42$, $r^2 = 0.176$, $p = 0.0051$), suggesting a moderate association between trophic position and metabolic carbon incorporation. The regression slope was 0.0143 (± 0.0048 SE), with 95% bootstrapped confidence intervals ranging from 0.0042 to 0.0223, while the intercept was 0.121 (± 0.055 SE). These results indicate that individuals with higher $\delta^{15}N$ values—typically interpreted as occupying higher trophic positions—also exhibit higher C_{meta} values, consistent with the hypothesis that dietary quality or trophic position may influence metabolic activity in nautiloids.

Discussion

Our results demonstrate distinct metabolic strategies among phragmocone-bearing cephalopods, with important implications for their ecological roles and evolutionary fates. Sepiids exhibit consistently higher and more variable C_{meta} values than nautiloids, reflecting elevated metabolic demands associated with active predation, mobility, and short life cycles. This aligns with physiological and isotopic data from modern coleoids (Denton & Gilpin-Brown, 1961; O'Dor & Webber, 1986; Chung et al., 2022). By contrast, nautiloids maintain lower and less variable C_{meta} values, indicative of a conservative, energy-efficient strategy (Guerra, 2006; Tajika et al., 2023). This metabolic conservatism possibly contributed to their survival across multiple extinction events, though it may have also limited their ecological plasticity compared to more metabolically flexible coleoids.

A similar physiological divide is evident between ammonoids and nautiloids in the Late Cretaceous. Ammonoids such as *Diplomoceras* and *Canadoceras* exhibited significantly higher C_{meta} values than co-occurring nautiloids, suggesting higher aerobic scope and metabolic intensity. These values are consistent with ammonoids' fast growth, mobility, and broad ecological versatility—traits that parallel those of modern sepiids (Ritterbush et al., 2014). The persistence of these differences under shared environmental conditions underscores intrinsic physiological contrasts between the groups (Tajika et al., 2023). These differences likely contributed to their contrasting evolutionary fates: while high energetic demands may have increased ammonoids' vulnerability to extinction during late Cretaceous oceanic stress, nautiloids' low metabolic demands may have buffered them through environmental perturbation.

Our dataset also reveals a longer-term trend of metabolic decline in nautiloids from the Eocene to the present, as reflected in decreasing C_{meta} values. Eocene taxa exhibit higher and more variable values—possibly driven by warmer global temperatures (Hollis et al., 2019) and broader ecological niches—while modern species like *N. pompilius* show consistently low values, consistent with deep-water, low-energy lifestyles. This supports the view that metabolic suppression and physiological stasis have possibly underpinned nautiloid persistence through deep time, even as these traits may have constrained ecological diversification.

The negative relationship between $\delta^{18}O$ and C_{meta} observed across fossil nautiloids, ammonoids, and sepiids suggests a broad physiological link between environmental temperature and metabolic carbon incorporation. Lower $\delta^{18}O$ values—typically interpreted as indicators of warmer water or greater physiological influence—coincide with higher C_{meta}, suggesting that metabolic activity scales with thermal or energetic conditions. This trend is strongest in ammonoids and fossil nautiloids, where limited physiological buffering may allow environmental signals to be recorded more directly in shell carbonate. Sepiids show a similar but weaker trend, likely reflecting their ability to regulate internal chemistry. Extant nautiloids show little relationship between $\delta^{18}O$ and C_{meta}, likely due to their narrow thermal niche and greater physiological control. Overall, the $\delta^{18}O$–C_{meta} relationship provides further support for using C_{meta} as a proxy for both metabolic rate and environmental sensitivity in extinct taxa.

Additionally, we observed a positive relationship between $\delta^{15}N$ and C_{meta} in extant nautiloids, suggesting a possible link between trophic position and metabolic activity. Individuals with higher $\delta^{15}N$ values—indicative of feeding at higher trophic levels(Ward et al., 2023)—also showed increased metabolic carbon incorporation. This may reflect a coupling of dietary quality and metabolic demand, where scavengers consuming protein-rich prey exhibit elevated aerobic activity. Alternatively, the relationship may be behavioral, with more metabolically active individuals engaging in broader or deeper foraging. While correlative, this finding demonstrates the potential of stable isotope proxies to jointly inform reconstructions of trophic ecology and physiological function.

Altogether, these findings highlight C_{meta} as a powerful proxy for reconstructing the metabolic physiology of extinct cephalopods and for elucidating the evolutionary consequences of divergent energetic strategies. From high-energy ammonoids and sepiids to the conservative nautiloids, metabolic flexibility—or the lack thereof—emerges as a key axis shaping ecological opportunity, extinction risk, and evolutionary resilience across deep time.

Conclusion

This study demonstrates the value of C_{meta} as a robust proxy for reconstructing metabolic strategies across modern and extinct phragmocone-bearing cephalopods. Our comparisons reveal striking physiological divergence: sepiids and ammonoids exhibit consistently higher C_{meta} values, indicative of elevated metabolic rates linked to active lifestyles, rapid growth, and ecological versatility. In contrast, nautiloids maintain low and relatively stable C_{meta} values over deep time, reflecting a conservative, energy-efficient strategy. This metabolic conservatism likely underpins their evolutionary persistence but may also constrain ecological flexibility.

Correlations between C_{meta} and both $\delta^{18}O$ and $\delta^{15}N$ support the idea that metabolic intensity is shaped by a combination of environmental temperature, trophic position, and life history traits. The $\delta^{18}O$–C_{meta} relationship highlights how warmer or more variable habitats may foster higher metabolic carbon incorporation, while the $\delta^{15}N$ trend suggests a potential link between dietary quality and metabolic demand.

Taken together, these findings underscore the importance of metabolic physiology in shaping the evolutionary trajectories and extinction vulnerabilities of cephalopods. They also establish C_{meta} as a powerful, quantifiable tool for integrating physiological, ecological, and evolutionary information from both modern and fossil records—opening new avenues for paleoecological reconstruction and comparative biology across marine invertebrates.

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Figures, Tables, and Captions

Table 1. Estimated δ^{13}C DIC Values by Geologic Period

Period	Approximate δ^{13}C DIC (‰, VPDB)	Reference
Late Cretaceous	+1.0‰ to +2.0‰	Tajika at al., 2023; Arthur et al., 1985
Paleocene	+1.0‰ to +1.5‰	Zachos et al., 2001
Eocene	+1.5‰ to +2.0‰	Zachos et al., 2008
Oligocene–Miocene	+1.0‰ to +1.5‰	Cramer et al., 2009
Modern (Recent)	~+1.0‰	Gruber et al., 1999.

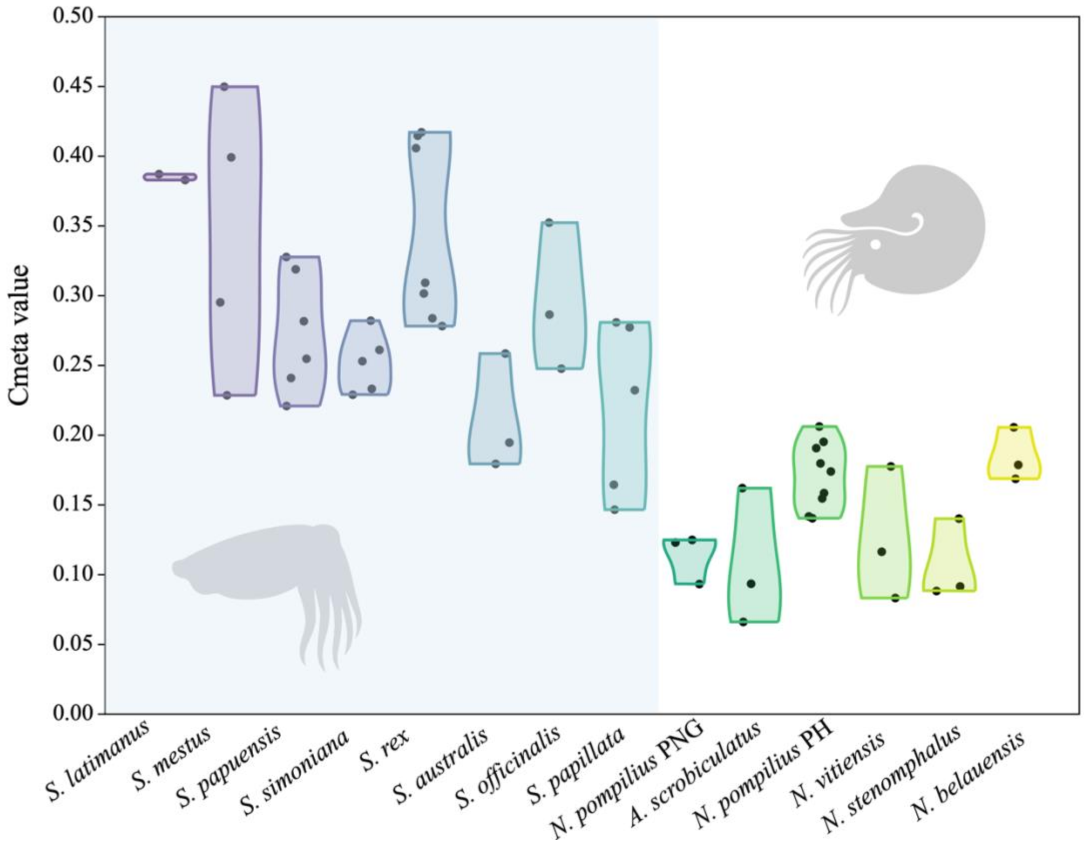


Figure 1. Violin plots showing C_{meta} values for modern sepiids (*Sepia* spp.) and nautiloids (*Nautilus* and *Allonautilus* spp.). C_{meta} is a proxy for the proportion of metabolically derived carbon incorporated into shell carbonate and is used here to infer relative metabolic activity. Sepiids (left, blue background) exhibit consistently higher C_{meta} values compared to nautiloids (right, white background), reflecting their more active, energetically demanding lifestyles. Each dot represents an individual shell sample; plot width indicates the distribution density. Species are arranged taxonomically, with notable interspecific variability within both groups.

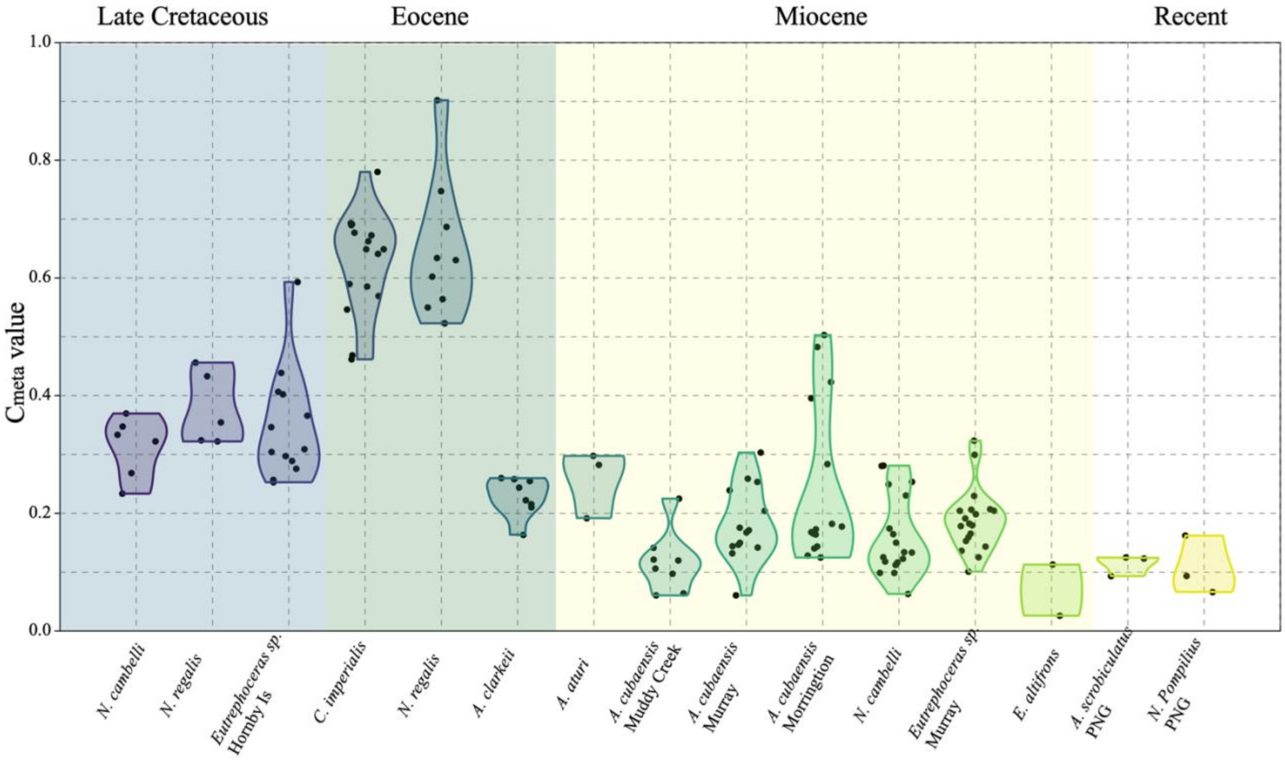


Figure 2. Violin plots showing C_{meta} values for fossil and extant nautiloid species spanning the Late Cretaceous to the Recent. C_{meta} represents the proportion of metabolically derived carbon incorporated into shell carbonate and serves as a proxy for metabolic rate. Species are organized chronologically by geologic period: Late Cretaceous (blue), Eocene (teal), Miocene (green), and Recent (yellow). Eocene taxa exhibit the highest and most variable C_{meta} values, while Recent nautiloids (*Allonautilus scrobiculatus*, *Nautilus pompilius*) display consistently low values. This temporal trend suggests a long-term decline in metabolic intensity among nautiloids, potentially reflecting physiological conservatism and ecological shifts into deeper, more stable marine environments. Each dot represents an individual septal sample; violin plot width indicates the density distribution of C_{meta} values within each taxon.

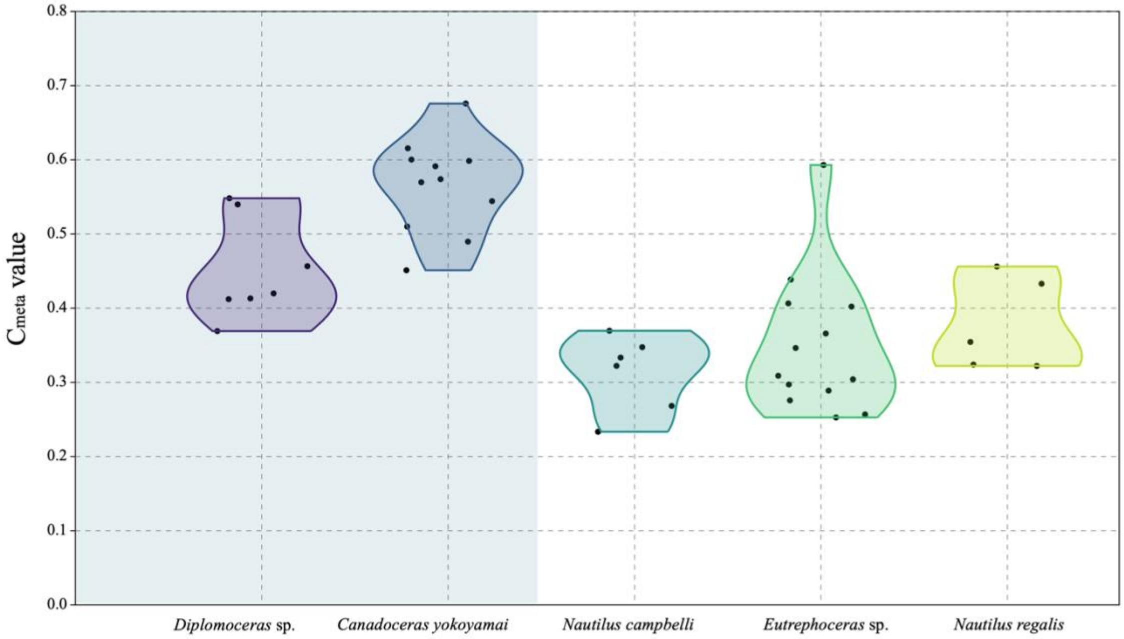
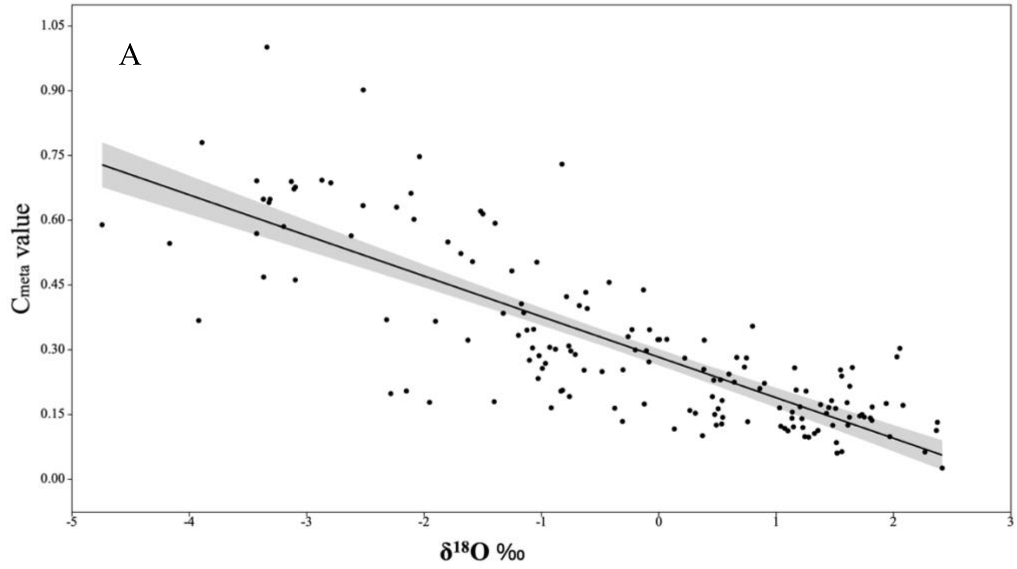


Figure 3. Violin plots comparing C_{meta} values—a proxy for the proportion of metabolically derived carbon in shell carbonate—between Late Cretaceous ammonoids (Diplomoceras sp., Canadoceras yokoyamai) and co-occurring nautiloids (Nautilus campbelli, Eutrephoceras sp., Nautilus regalis). Ammonoids exhibit higher median and overall C_{meta} values, suggesting elevated metabolic rates relative to nautiloids. These results support interpretations of ammonoids as more active, energetically demanding organisms and highlight fundamental physiological differences between the groups during the Late Cretaceous. Each dot represents an individual septal sample; violin width indicates the distribution density.


A
1.05
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0.75
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C_{meta} value
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-3
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-1
0
1
2
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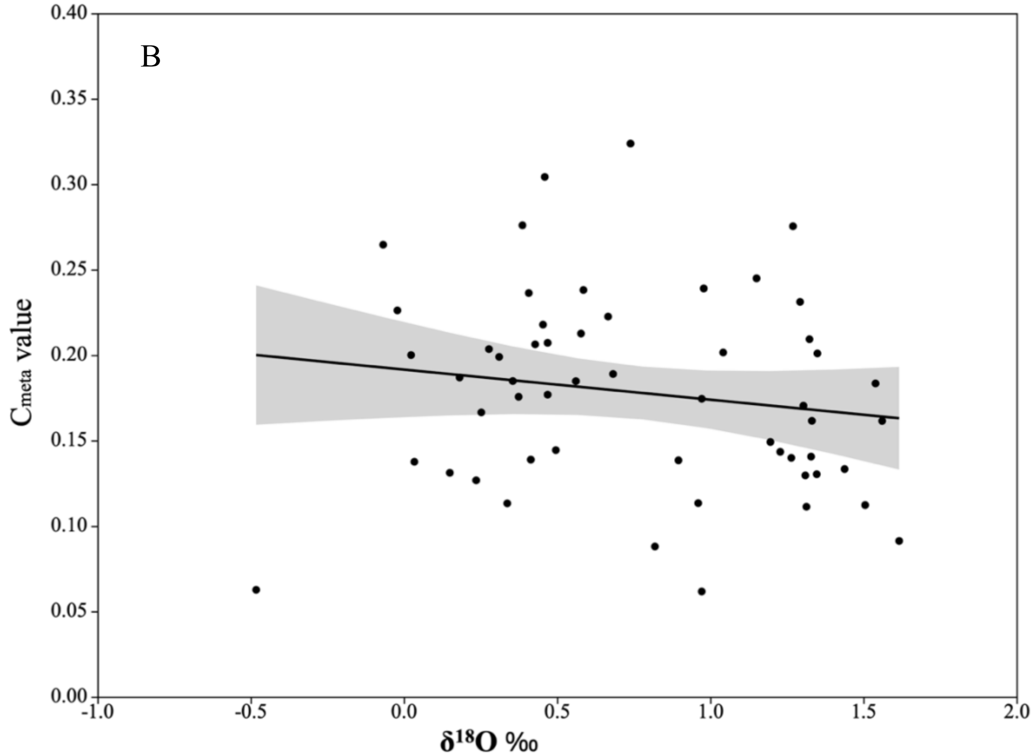
δ18O ‰


B
0.40
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0.30
0.25
0.20
0.15
0.10
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C_{meta} value
-1.0
-0.5
0.0
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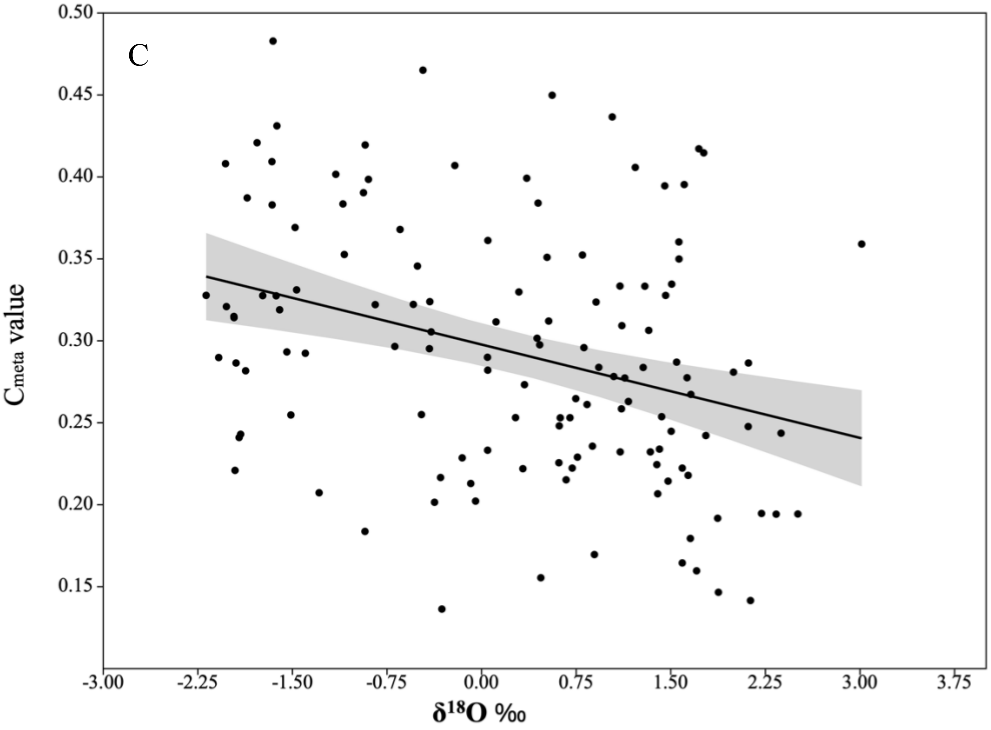
1.0
1.5
2.0
δ18O ‰


C
Cmeta value
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0.50
0.45
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0.25
0.20
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-1.50
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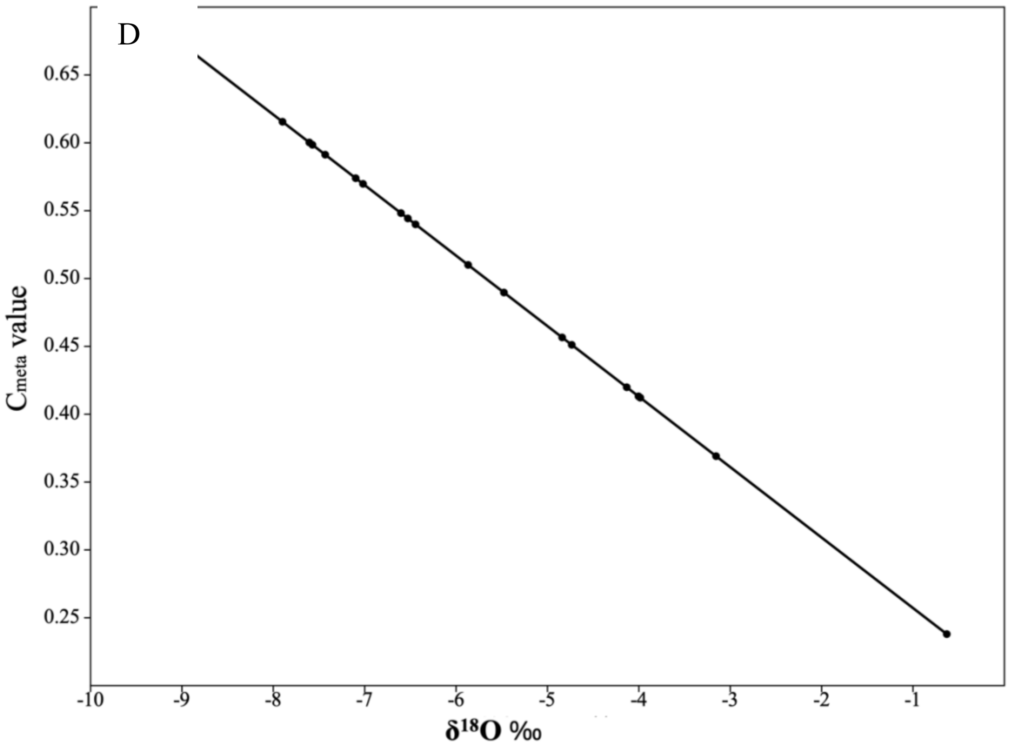
0.75
1.50
2.25
3.00
3.75


D
Cmeta value
δ18O ‰
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-10
-9
-8
-7
-6
-5
-4
-3
-2
-1

Figure 4. Relationship between $\delta^{18}O$ and C_{meta} across four groups of phragmocone-bearing cephalopods. (A) Fossil nautiloids show a strong negative correlation between $\delta^{18}O$ and C_{meta}, suggesting increased incorporation of metabolic carbon at lower $\delta^{18}O$ values, likely linked to warmer or more metabolically active conditions. (B) Extant nautiloids display a much weaker trend, reflecting a narrower $\delta^{18}O$ range and greater physiological buffering in modern taxa inhabiting relatively stable mesopelagic environments. (C) Sepiids also exhibit a weak but discernible negative trend, consistent with more active metabolisms and dynamic coastal habitats, though with considerable scatter. (D) Ammonoids show a near-perfect inverse relationship, with lower $\delta^{18}O$ values strongly associated with higher C_{meta} values, reflecting elevated metabolic activity and limited physiological control of carbonate chemistry. Shaded bands represent 95% confidence intervals around the regression lines. Each point represents a septal sample analyzed for oxygen isotopic composition and carbon metabolic contribution.

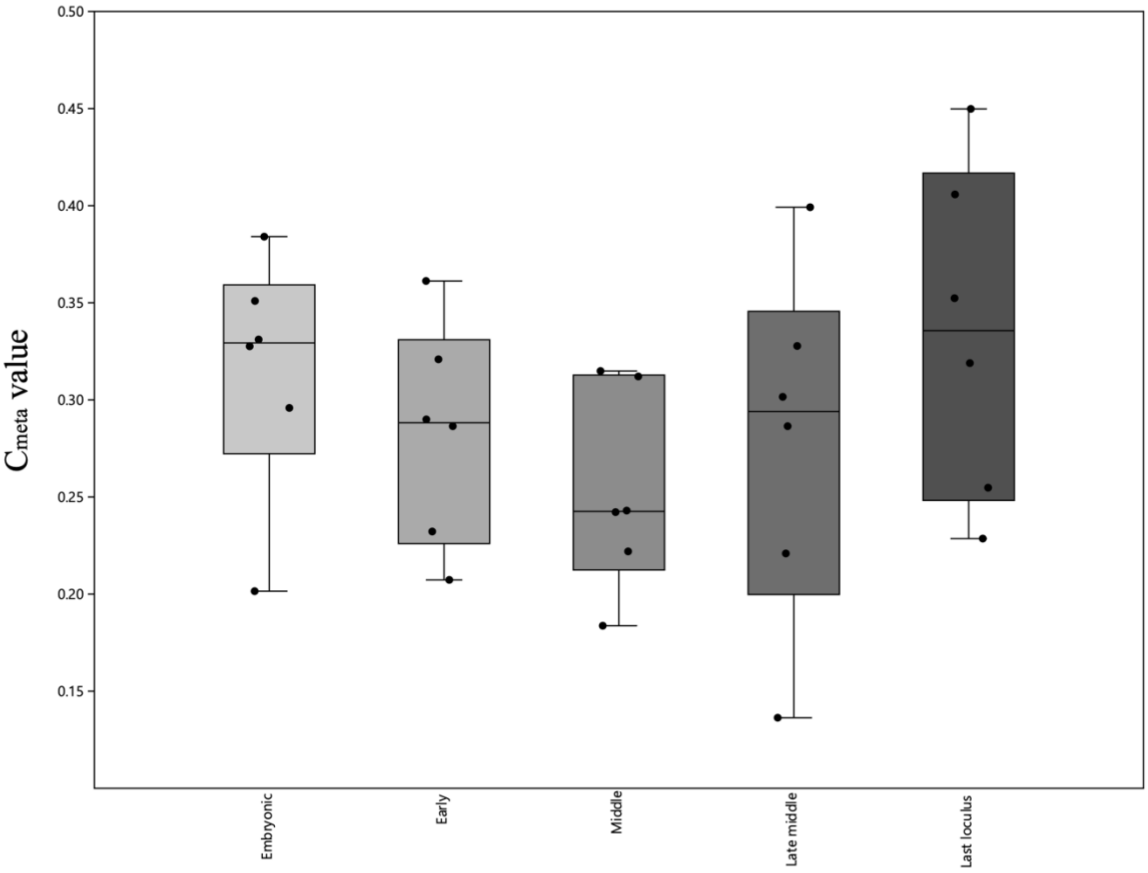

Figure 5. Boxplots of C_{meta} values across ontogenetic stages in Sepia spp., from embryonic to final septa (last loculus). C_{meta} represents the proportion of metabolically derived carbon incorporated into shell carbonate, serving as a proxy for metabolic activity. While early developmental stages (embryonic and early) show moderately high C_{meta} values, a decline is observed during middle ontogeny, followed by an increase in later stages. This pattern may reflect shifts in metabolic demand related to growth, maturation, and reproductive investment. Individual data points are overlaid; boxes represent interquartile ranges, with medians indicated by horizontal lines.

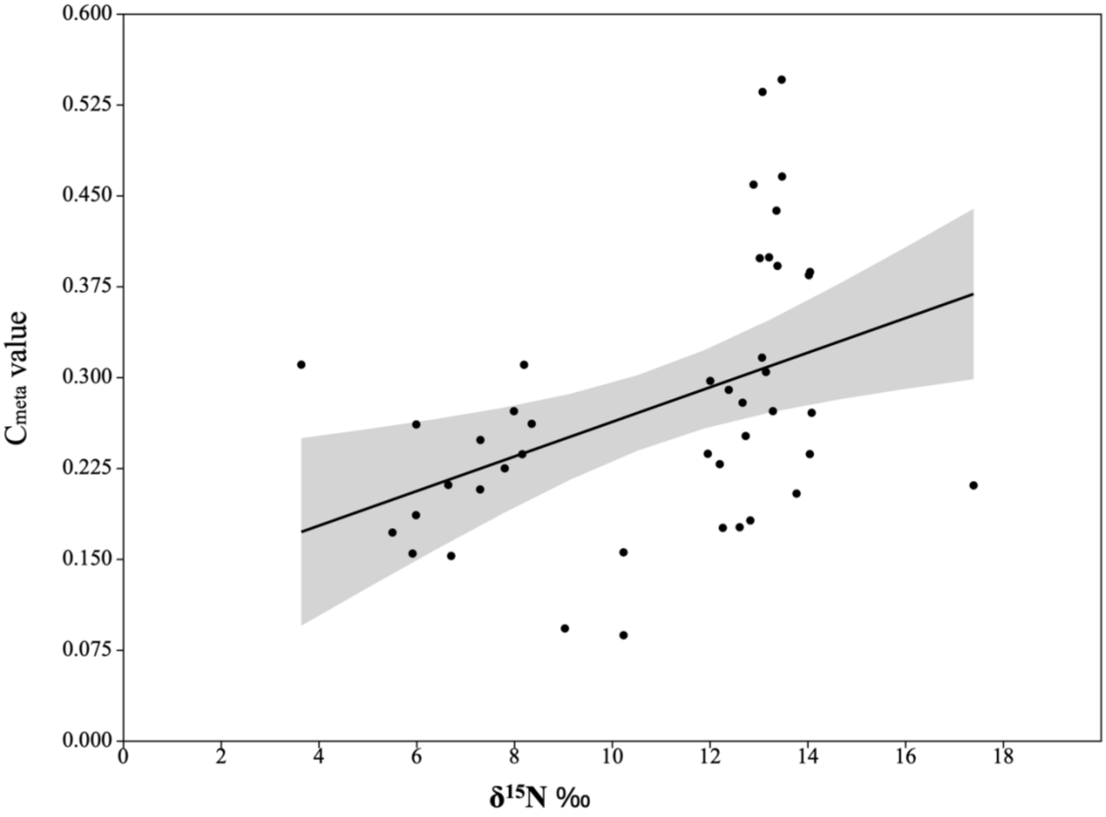

Figure 6. Relationship between $\delta^{15}N$ values and C_{meta} in extant nautiloid shell carbonate. C_{meta} represents the proportion of metabolically derived carbon incorporated into shell material and is used here as a proxy for metabolic activity. $\delta^{15}N$ values, indicative of trophic position, show a positive correlation with C_{meta}, suggesting that individuals with higher $\delta^{15}N$—likely occupying higher trophic levels—also exhibit elevated metabolic carbon incorporation. Shaded area represents the 95% confidence interval of the linear regression. Each point corresponds to an individual septal sample

General Conclusions

This dissertation examined the ecological dynamics of nautiloids and other marine organisms across multiple spatial, temporal, and functional contexts. By integrating population-level isotopic analyses, trophic level estimation, and comparative metabolic assessments, these studies provide a holistic view of how ancient and modern phragmocone-bearing cephalopods interact with, and respond to, their environments.

In the first chapter, stable isotope analyses revealed distinct isotopic niches among nautiloid populations across the Pacific. These patterns suggest that geographic isolation, local environmental conditions, and potential differences in prey availability drive niche differentiation, offering insight into the population structure and connectivity of species once thought to have homogeneous ecologies.

The second chapter extended this isotopic approach to the mesophotic zone, focusing on scavenger communities. By estimating trophic levels of nautiloids and associated species, this work demonstrated their role as mid- to high-level consumers within deep reef ecosystems. It highlighted the functional significance of nautiloids in recycling organic matter at depth and underscored the vulnerability of mesophotic scavenger networks to habitat degradation and fishing pressures.

The third chapter applied comparative metabolic analysis (Cmeta) to phragmocone-bearing cephalopods, integrating data from both modern and extinct taxa. Results showed clear metabolic contrasts between nautiloids and co-occurring squid and cuttlefish, reflecting evolutionary trade-offs in growth rate, mobility, and ecological strategy. These findings provide a physiological framework for interpreting the persistence of nautiloids in certain environments and the extinction of others.

Together, these chapters show that nautiloids are not passive relics of a bygone era but active, ecologically significant components of marine food webs. Their isotopic niches, trophic roles, and metabolic strategies reveal a complex interplay between evolutionary history, physiology, and environmental change. This integrated approach not only advances our understanding of cephalopod ecology but also informs conservation strategies for species facing increasing anthropogenic and climatic pressures.