



Realized dietary specialization in an Antarctic keystone species, the sea urchin *Sterechinus neumayeri*

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Abstract

Dietary specialization is a key trait influencing species resilience in changing environments, yet it is often inferred as the fundamental niche—an inherent species feature—rather than the realized niche, which reflects actual resource use under specific ecological conditions and can vary across spatial, temporal and biological organizational scales (individuals to populations). In Antarctic ecosystems undergoing rapid changes, understanding trophic plasticity in keystone species is essential. Here, the dietary composition and specialization of the Antarctic sea urchin *Sterechinus neumayeri* were assessed across spatial, temporal and organizational scales along the Western Antarctic Peninsula (WAP, 64–67°S; 61–66°W). Samples were collected at thirteen sites spread over two expeditions conducted in February–March 2023 (southern WAP) and 2024 (northern WAP), spanning a north–south gradient along the WAP and providing spatial coverage across the region. Temporal variability was inferred from complementary dietary tracers applied to the same individuals: gut content metabarcoding (COI gene tag), providing short-term, high-resolution taxonomic information, and stable isotope analysis ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$), offering a longer-term view of assimilated diet at lower resolution. Results revealed significant spatial variation in dietary composition and specialization. Although diatoms dominated diet across sites, contributions of macroalgae and invertebrates varied. Alpha and beta prey diversity and isotopic niche volumes differed significantly among sites, and correspondence between short-term and longer-term dietary patterns was weak. Finally, population-level averages masked substantial individual dietary specialization within and between sites. These findings indicate that *Sterechinus neumayeri* exhibits context-dependent dietary specialization, emphasizing the importance of multi-scale approaches in marine ecosystems.

Keywords Stable isotope analysis · Metabarcoding · Trophic plasticity · Trophic markers · Realized dietary specialization · Western Antarctic Peninsula (WAP)

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Introduction

Determining the degree of dietary specialization in a species is intrinsically complex and often far less straightforward than it may initially appear. Many studies treat dietary specialization as a fixed, species-level trait, independent of local conditions (Ferry-Graham 2002; Devictor et al. 2010; Cusa et al. 2019). This perspective typically refers to the fundamental specialization, a degree of specialization that is an inherent species trait independent of localised biotic or abiotic influences (Ferry-Graham 2002; Devictor et al. 2010; Cusa et al. 2019). A complete assessment of the dietary specialization, however, also requires consideration of the realized specialization, a degree of specialization which is shaped by local ecological conditions and reflects the actual

set of resources used at a given time and place. The realized specialization is inherently flexible, constrained by both biotic and abiotic factors that may vary spatially, temporally, and across levels of biological organization (individual vs. population-level variation) (Devictor et al. 2010; Bolnick et al. 2011; Costa et al. 2015; DeSantis et al. 2022). As such, measurements of dietary specialization at a given place and time may represent only a subset of the ecological conditions that a species can experience elsewhere or over longer timescales. Similarly, dietary specialization assessed solely at the population level may obscure individual-level variation, despite the important role such variation plays in shaping population responses to environmental change and evolutionary dynamics (Bolnick et al. 2003). Indeed, even species generally considered ecological generalists may exhibit considerable individual-level variation. Populations can either consist of generalist individuals with high within-individual variation in diet, or of specialist individuals that differ from each other, resulting in high between-individual variation (Bolnick et al. 2003; Devictor et al. 2010; Costa et al. 2015; DeSantis et al. 2022).

Given the magnitude and pace of current environmental shifts in many regions of the world, assessing the degree of dietary specialization through a framework that explicitly integrates spatial, temporal and organisational scales is crucial for evaluating species' acclimative and adaptive potential. In such context, generalist individuals are often considered the "winners" compared to specialist feeders (Clavel et al. 2011; Haaland et al. 2020; Griffiths et al. 2024). Generalists are typically characterized by a broader fundamental niche and a wider dietary breadth, consuming a diverse array of prey taxa. In contrast, specialists tend to be more selective, with narrower dietary niches focused on a limited number of prey species (Devictor et al. 2010; Liang et al. 2020). Owing to their greater plasticity, generalists are therefore expected to perform better and become more dominant under changing environmental conditions, a pattern reported across multiple ecosystems (Clavel et al. 2011; Gerking 2014; Michel et al. 2016; Griffiths et al. 2017; Haaland et al. 2020; Liang et al. 2020; Stuart-Smith et al. 2021; Meijenfildt et al. 2022). Yet, this "generalist-specialist" classification is often reported in a context-free manner, implicitly treated as a fixed species-level trait and generalized across space, time and organizational scales (individual to population level). Such an approach overlooks the fact that dietary specialization is inherently dynamic, varying with local environmental conditions, temporal context, and inter-individual differences. As a consequence, substantial variability in dietary niche width and composition may be masked, oversimplified, or entirely overlooked, despite its central importance for understanding trophic strategies and predicting species' responses to environmental change.

Sterechinus neumayeri is an abundant, endemic sea urchin species with a circum-Antarctic distribution, and is considered a key component of Antarctic benthic ecosystems (McClintock 1994; Ortiz et al. 2017; Caputi et al. 2020). It is commonly described as a trophic generalist, omnivorous and sometimes even opportunistic, feeding on a wide range of food items including diatoms, macroalgae, detritus, and invertebrates such as bryozoans, hydrozoans, amphipods, sponges, and polychaetes, as supported by gut content and stable isotopes analyses (Calizza et al. 2018; Caputi et al. 2020; Galloway et al. 2024; McClintock 1994; Michel et al. 2016, 2019; Norkko et al. 2007; Rossi et al. 2019). This large array of prey and food items documented for *S. neumayeri* has cemented its reputation as a generalist, often without explicit consideration of the spatial, temporal and ecological contexts in which these diets were initially assessed. Understanding how *Sterechinus neumayeri*' dietary specialization varies across these scales is essential to better characterize a facet of its realized niche, and to refine our understanding of its trophic ecology. In this regard, the Western Antarctic Peninsula (WAP) offers an ideal natural laboratory. The WAP is characterized by strong environmental gradients, including spatial variation in sea-ice cover, benthic habitat structure and community composition, as well as a marked and predictable seasonality (Constable et al. 2014; Lee et al. 2017; Cardona et al. 2021; Amsler et al. 2023; Griffiths et al. 2024)—all of which may influence feeding patterns. Importantly, the Antarctic Peninsula is also one of the region's most strongly impacted by global change, with rapid warming, shifts in sea-ice dynamics, and subsequent effects on marine ecosystems (Meredith and King 2005; Constable et al. 2014; Siegert et al. 2019; Griffiths et al. 2024).

The objectives of this study were therefore two-fold. First, we aimed to assess the dietary composition and realized dietary specialization of *Sterechinus neumayeri* by accounting for variation across three ecological scales—spatial, temporal, and organizational (individual to population-level; population defined here as conspecific individuals sampled at the same site and sharing the same habitat and environmental conditions at the time of sampling). Based on the premise that realized specialization is a plastic trait shapes by ecological conditions, we specifically hypothesized that i) dietary specialization varies spatially, in response to differences in habitat characteristics and benthic community composition along the WAP; ii) dietary specialization varies temporally, due to the strong ecological dynamics that structure Antarctic ecosystems; and iii) dietary specialization is an individual-level trait, often hidden by population-level averages, and must be assessed at the appropriate scale to be fully understood. Second, we sought to critically evaluate

the species' long-assumed generalist status considering these multiscale patterns.

To address these hypotheses, two complementary approaches were employed: gut content metabarcoding and stable isotope analysis. These methods differ in both their temporal scope and taxonomic resolution, offering a synergistic framework for investigating trophic ecology. Metabarcoding provides high-resolution taxonomic information on ingested material, but represents only short-term snapshots of recent dietary intake and does not distinguish between ingested and effectively assimilated material. In contrast, stable isotopes offer temporally integrated information of assimilated resources, though with lower taxonomic resolution (Whitaker et al. 2019; Cordone et al. 2022; Kuhrt et al. 2024). When combined, these approaches gather fine-scale taxonomic detail with integrated measures of resource use and trophic position, yielding a more comprehensive characterization of dietary patterns and niche structure (Kuhrt et al. 2024). Considering their temporal complementarity, they can also be used on the same individuals to explore trophic variation at different temporal scales (Cordone et al. 2022; Hartman et al. 2024). Even though combining these methods is increasingly recognized as essential for a comprehensive understanding of trophic ecology (Kuhrt et al. 2024), such integrative approaches remain scarce and, to date, have never been applied jointly in Antarctic marine invertebrates.

By integrating both approaches across multiple ecological scales, this study provides the first multiscale, dual-method investigation of dietary specialization in *Sterechinus neumayeri*. It broadly aims to emphasize the importance of considering realized rather than solely fundamental dietary specialization in ecological research, and advocates for a multiscale approach to evaluate the degree of dietary specialization in marine species.

Materials and methods

Sample collection and processing

Specimens of *Sterechinus neumayeri* were collected along the Western Antarctic Peninsula (WAP) during two research expeditions: TANGO1, conducted in the southern WAP in February–March 2023 (Danis et al. 2023), and TANGO2, conducted in the northern WAP in February–March 2024 (Danis et al. 2024). As habitat conditions vary strongly along the WAP (Michel et al. 2016; Siegert et al. 2019; Griffiths et al. 2024; Katz et al. 2025), the sampling design aimed to cover a wide range of shallow habitats and environmental variability across this north–south gradient, thereby capturing the full potential dietary niche of the species in the Peninsula (Fig. 1). In this context, thirteen sites were selected

across six regions, spanning distances from a few hundred meters to approximately 700 km (Danis et al. 2023, 2024). Sampling was conducted by scuba divers operating from the research vessel *Australis*, a fully equipped motorsailer (Danis et al. 2023, 2024). At each site, 20 to 30 individuals were collected at depths ranging from 5 to 30 m (see Online Resource 1), yielding a total of 333 *Sterechinus neumayeri* specimens across the TANGO1 and TANGO2 expeditions. Of these, 252 individuals were retained for the present study.

Onboard, *Sterechinus neumayeri* specimens were directly measured to get the ambitus diameter and height, then dissected. Muscles associated with the Aristotle's lantern were removed using sterile dissection tools, along with stomach and intestinal contents collected as completely as possible throughout the digestive tract, except for the posterior (anal) section of the gut. The stomach and gut content are hereafter collectively referred to as “gut content”. Gut content was collected along the digestive tract to capture diet composition over hours to days via metabarcoding (Klinger et al. 1994; Lawrence and Klinger 2001), while muscles associated to the Aristotle's lantern and chosen for stable isotope analyses cover a time window of a few weeks to months in polar regions (Carter et al. 2019; Kuhrt et al. 2024; Thomas & Crowther 2015). The Aristotle's lanterns were directly dried up at 60 °C for 24 h then kept at -20 °C in glass tubes, while gut content was preserved in sterile Eppendorf tubes containing RNAlater for 24 h at 4 °C, then at -20 °C. All dissecting tools were sterilized between individuals and tissues, following a three-step process by rinsing in absolute ethanol, sterile water and keeping them for a few seconds in a sterilizer containing glass micro-beads (Micro Bead Sterilizer, Sigma-Aldrich, St. Louis, MO, USA; cat. No. Z378569).

In order to characterize the sex of each individual, histological sections of the 252 specimens were prepared by adapting the procedure described by Nunes & Jangoux 2004. For each sample, a small piece of gonad was dehydrated in butanol, embedded in paraffin wax, cut into 5-mm thick sections, stained with Masson's Trichrome (Gabe, 1968) and observed under a light microscope.

Dietary niche width assessment approaches

Gut content characterization via DNA metabarcoding

DNA extraction The 252 gut contents were extracted using the DNeasy PowerSoil DNA extraction kit (QIAGEN). The manufacturer's protocol was followed with specific modifications: samples were heated on PHMT Thermoshaker (Grant) for 10 min then shaken for 20 min using the horizontal Vortex adapter to ensure a better lysis of the tissues, and final DNA elution was repeated twice with 100 µl of the

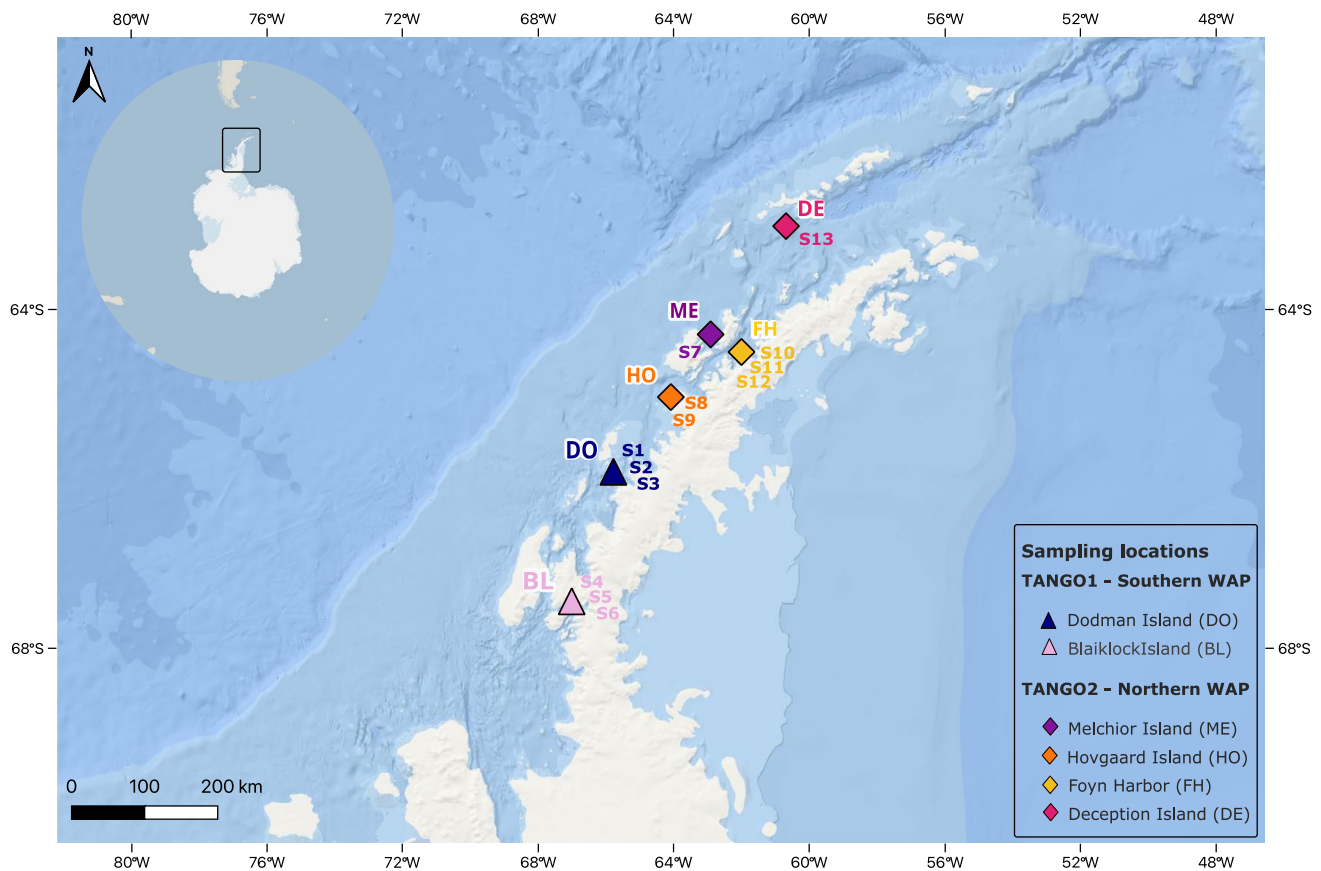


Fig. 1 Map presenting the thirteen sites sampled through the six regions situated along the Western Antarctic Peninsula, during TANGO1 (2023) and TANGO2 (2024) expeditions. Map created using QGIS, with data provided by Quantarctica (Matsuoka et al. 2021)

elution agent provided by the kit and an incubation period of 10 min at room temperature between the two elution steps. A purification step was added prior to PCR and sequencing steps using the DNeasy PowerClean Pro Cleanup kit (QIAGEN) following the manufacturer's protocol with the following modifications: the final DNA elution was repeated twice with 70 µl of the elution agent provided by the kit and an incubation time of 10 min at room temperature between the two elution steps. Negative controls were included in each extraction round to verify that no contamination occurred during DNA extraction. DNA concentration was determined using a Qubit High Sensitivity dsDNA Assay kit with a Qubit 4.0 fluorometer (Thermo Fisher Scientific) according to the manufacturer's protocol.

DNA metabarcoding and sequencing Library preparation and sequencing were carried out by AllGenetics & Biology SL (www.allgenetics.eu) as follows. The forward and reverse primers used to perform the polymerase chain reaction (PCR) amplifying a 313-bp segment of the mitochondrial cytochrome c oxidase subunit I (COI) gene were: mICOIntF-XT (5' GGWACWRGWTGRACWITITAYC-

CYCC 3'; Leray et al. 2013; Wangenstein et al. 2018a, b) and jgHCO2198 (5' TAIACYTCIGGRTGICCRAARA-AYCA 3'; (Geller et al. 2013). These primers allow the detection of a wide range of metazoan prey items, and have already been reported as efficient on marine invertebrates (Casey et al. 2019; Geller et al. 2013; Leray et al. 2013; van der Loos & Nijland 2021; Wangenstein et al. 2018a, b). In the first amplification step, PCRs were carried out in a final volume of 12.5 µL, containing 1.25 µL of template DNA, 0.5 µM of the primers, 3.13 µL of Supreme NZYTaq 2 × Green Master Mix (NZYTech), and ultrapure water up to 12.5 µL. The reaction mixture was incubated as follows: an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 54.7 °C for 45 s, 72 °C for 45 s, and a final extension step at 72 °C for 7 min. PCRs of samples that failed to produce a sharp band in the agarose gels were repeated by doubling the amount of template DNA and the amount of Supreme NZYTaq 2 × Green Master Mix (NZYTech). The oligonucleotide indices that are required for multiplexing different libraries in the same sequencing pool were attached in a second amplification step, carried out in a final volume of 25 µL, containing 2.5 µL of PCR product from the first amplification step, 1 µM of the dual-indexed

primers, 6.5 μ L of Supreme NZYTaQ 2 $\times$ Green Master Mix (NZYTech), and ultrapure water up to 25 μ L. The reaction mixture was incubated as follows: an initial denaturation at 95 °C for 5 min, followed by 5 cycles of 95 °C for 30 s, 60 °C for 45 s, 72 °C for 45 s, and a final extension step at 72 °C for 7 min. A negative control that contained no DNA was included in every PCR round to check for contamination during library preparation. The library size was verified by running the libraries on 3% agarose gels stained with GreenSafe (NZYTech) and imaging them under UV light. Then, the libraries were purified using the Mag-Bind RXNPure Plus magnetic beads (Omega Bio-tek), following the instructions provided by the manufacturer. Finished libraries were pooled in equimolar amounts according to the results of a Qubit dsDNA HS Assay (Thermo Fisher Scientific) quantification. The pool was sequenced in a fraction of a NovaSeq PE250 flow cell (Illumina) aiming for a total output of 7 gigabases.

Bioinformatic pipeline A custom pipeline was used to process COI reads, inspired by Brandt et al. (2021). Pairs of Illumina reads were first corrected with Cutadapt (Martin 2011) to remove any potential trace of adapter dimers, then with DADA2 (Callahan et al. 2016) following the online tutorial for paired-end data (<https://benjjneb.github.io/dada2/tutorial.html>). Trimming lengths were adjusted based on the quality profiles of the samples and primers were removed (truncLen at 250 and 220, maxEE at 2, truncQ at 2, maxN at 0). Error model calculation, read correction, read merging and chimera removal were performed at default settings implemented in DADA2. Prior to taxonomy assignment, ASVs were clustered into OTUs using swarm v3.1.5 (Mahé et al. 2015) with an iterative local threshold of $d=13$, based on settings previously used in the literature for COI (Rodríguez-Barreras et al. 2020; Brandt et al. 2021; Angulo-Preckler et al. 2023). Two taxonomy assignment methods were used: BOLDigger (Buchner and Leese 2020) and BLAST+ (Basic Local Alignment Search Tool v 2.16.0) based on minimum similarity and minimum coverage (-perc_identity 80 and -qcov_hsp 80). These two methods were used parallelly to counteract for the still poorly characterized Antarctic communities, i.e. the large proportion of taxa not identified in DNA reference databases. More stringent parameters were indeed not implemented in this study for the same reason. Both datasets were manually checked and refined to combine them in a final dataset used for the analyses, and each taxonomic annotation was corrected using World Register of Marine Species (WoRMS; <https://marinespecies.org>). Assignments with less than 80% identity similarity or unassigned at the class level were excluded. A >97% threshold was used to define species level, >95%

to genus, >90% to family, >85% to order and >80% to class and phylum level (Wangensteen and Turon 2016; Ruiz et al. 2024). Final refinement of the dataset was based on the filtering process of Ruiz et al. 2024: i) removal of every OTU that consisted of non-target taxa (bacteria, fungi, terrestrial taxa), potential contaminants (predator and human DNA) or parasites, ii) removal of every OTU with an abundance in the negative controls higher than 10% of the total reads and iii) removal of OTUs whose relative abundance was smaller than 0.002% (Wangensteen et al. 2018a). After all these refinements steps, a final step consisting in the removal of samples with less than 50 reads, and of OTUs with less than 5 reads in total was applied (Rodríguez-Barreras et al. 2020).

Stable isotope analysis ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$, $\delta^{34}\text{S}$)

A total of 252 Aristotle's lantern samples with associated muscle tissue were used, corresponding to the same individuals analyzed for their diet by metabarcoding. Upon return from the expeditions, samples were dried again at 60 °C for 72 h. Muscle tissue was then carefully scraped from the Aristotle's lantern, homogenized, and ground into a fine powder using a mortar and pestle. For each individual, approximately 2.5 mg of homogenized muscle tissue were weighed into tin capsules. Stable isotope analyses were performed using carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$), and sulfur ($\delta^{34}\text{S}$). Carbon and nitrogen isotopes are widely used in food-web studies, with $\delta^{13}\text{C}$ indicating the origin of basal carbon sources and $\delta^{15}\text{N}$ reflecting trophic position. Sulfur isotopes ($\delta^{34}\text{S}$), although less commonly applied, provide complementary information by distinguishing benthic and pelagic pathways that cannot always be resolved using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ alone (Connolly et al. 2004; Pinzone et al. 2019). The combined use of these three isotopes therefore allows a more detailed characterization of trophic ecology and isotopic niche than a dual-isotope approach. These ratios were measured by continuous-flow elemental analysis–isotope ratio mass spectrometry (CF-EA-IRMS) at the University of Liège, using a vario MICRO Cube elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany) coupled to a precisiON mass spectrometer (Elementar UK, Cheadle, UK). Isotopic ratios were expressed using the widespread δ notation (Coplen 2011), in ‰ and relative to the international references Vienna Pee Dee Belemnite (for carbon), atmospheric air (for nitrogen) and Vienna Canyon Diablo Troilite (for sulphur). IAEA (International Atomic Energy Agency, Vienna, Austria) certified reference materials caffeine (IAEA-600; $\delta^{13}\text{C} = -27.771 \pm 0.043\text{‰}$; $\delta^{15}\text{N} = 1.0 \pm 0.2\text{‰}$; means $\pm$ SD), and barium sulphate (IAEA-SO-5; $\delta^{34}\text{S} = 0.5 \pm 0.2\text{‰}$) were

used as primary analytical standards. Sulfanilic acid (Sigma-Aldrich; $\delta^{13}\text{C} = -25.6 \pm 0.4\text{‰}$; $\delta^{15}\text{N} = -0.13 \pm 0.4\text{‰}$; $\delta^{34}\text{S} = 5.9 \pm 0.5\text{‰}$; means $\pm$ SD) was used as secondary analytical standard. Standard deviations on multi-batch replicate measurements of secondary and internal lab standards (amphipod crustacean muscle) analysed interspersed with samples (one replicate of each standard every 15 analyses) were 0.2‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and 0.4‰ for $\delta^{34}\text{S}$.

Data analysis workflows

The analyses detailed in Sects. "Metabarcoding dietary data analysis" and "Isotopic niche metrics analysis" were initially processed separately for the two expeditions (TANGO1 and TANGO2) to evaluate a potential sampling year-effect (see Online Resource 1). As trends were identical in both years, and given the study design—aimed at encompassing the widest range of environmental conditions and habitats to maximise the captured dietary variability of *Sterechinus neumayeri* along the WAP, the thirteen sites were then combined into a single dataset and analyzed as follows.

Metabarcoding dietary data analysis

Metabarcoding data were used to characterize dietary composition and degree of dietary specialization at a short temporal scale. The diet composition and diversity of *Sterechinus neumayeri* along the Western Antarctic Peninsula were evaluated through a semi-quantitative framework inspired by Wangenstein et al. (2018a, b). Because metabarcoding data are affected by various biases—such as primer specificity, variability in gene copy number, and stochastic amplification errors—a rank-based scoring system was employed to capture abundance patterns while reducing the influence of quantitative biases (Wangenstein et al. 2018a, b). For each sample, OTUs were sorted according to their read abundance and classified into ranks from 0 to 10: 0 denoting absence, and ranks 1 to 10 reflecting increasing relative abundance intervals within that sample (Rank 1: $\leq 10\%$; Rank 2: $> 10\text{--}20\%$; Rank 3: $> 20\text{--}30\%$; ... Rank 10: $> 90\text{--}100\%$). These processed data were then used exclusively for comparative analyses among individuals and sites, assuming that residual amplification biases would remain consistent across all samples (Wangenstein et al. 2018a, b).

All analyses were performed in R (version 4.3.3; R Core Team 2013), using vegan, mgcv, WdStar and RInSp packages (Zaccarelli et al. 2013; Bates et al. 2015; Hamidi et al. 2019; Oksanen et al. 2022), based on the class-level metabarcoding data. Dietary alpha diversity was assessed for each individual using richness, Shannon, and Simpson diversity indices, calculated from the semiquantitative

OTU abundance data. Differences in dietary alpha diversity between sites were tested using Kruskal–Wallis tests, while differences between sexes and sizes were processed using linear mixed-effects models (lmer). A Principal Coordinates Analysis (PCoA) based on Bray–Curtis dissimilarity matrices was performed for visual exploration of dietary composition across individuals and sites. Differences in the variability of diet composition between sites were assessed by a distance-based Welch MANOVA using the WdS.test() function. This analyse was preceded by a test to calculate the multivariate homogeneity of group dispersions using betadisper(), and the significance of dispersion differences between sites was tested using permutation tests (permutest()). To specifically evaluate the effects of sex and size (ambitus diameter) on individual dispersion values, we fitted linear mixed-effects models (lmer). Analyses were conducted with sex and size as explanatory variables, while constraining permutations within site.

Individual degree of specialization was assessed by calculating the Proportional Similarity index (PSi) (Bolnick et al. 2002), which compared an individual's diet to that of the population (Bolnick et al. 2002; Andrews et al. 2020). PSi values range from 1 (complete overlap between individual and population resource use) to 0 (complete individual specialization). Statistical significance of individual specialization was assessed via Monte Carlo permutation tests (9999 iterations), generating null distributions of PSi values for each population. Individuals with observed PSi values exceeding 95% of the null distribution were considered significantly specialized. Differences in PSi values between sites and between sizes and sexes were also assessed using, respectively, Kruskal–Wallis test and the linear mixed-effects model. The IS value (i.e. the mean of all PSi values of a site) was also calculated for each site.

Isotopic niche metrics analysis

Stable isotopes data were analyzed to provide insights into the degree of dietary specialization at longer temporal scales. All analyses and visualizations of stable isotope data were conducted in R (version 4.3.3; R Core Team 2013), using vegan, SIBER, nicheROVER, ggplot2, WdStar and plotly packages (Jackson et al. 2011; Swanson et al. 2015; Wickham 2016; Hamidi et al. 2019; Sievert 2020; Oksanen et al. 2022). Inter-site differences in each isotope ratio ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$) were first tested individually using Kruskal–Wallis tests. For each isotope, lmer were fitted to assess the effects of sex and size, while constraining permutations within site. Multivariate isotopic beta diversity was further assessed based on the combined isotope data ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$). As detailed previously, multivariate dispersion was first analyzed using betadisper() and permutest() to evaluate

differences in dispersion among sites and verify homogeneity of variances prior to multivariate analyses. Distance-based Welch MANOVA using the `WdS.test()` function was used to test for differences in multivariate isotopic composition between sites. Additionally, a modified script from Skinner et al. (2019) was used to perform multidimensional isotopic niche analyses. To assess the isotopic niche volume at each site and niche overlap across the thirteen sites, three-dimensional ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$) ellipsoids were built using a Bayesian approach. Standard ellipsoid volumes (SEVb) were estimated with 10^5 iterations.

Multimethod integration: combining metabarcoding and stable isotope data to capture different temporal scales

Dietary specialization temporal variability was compared between short-term (metabarcoding) and longer-term (stable isotope) data, as well as investigated at the within- and between-sites levels. By this way, complementary metrics were used and allowed the integration of the different temporal and organizational levels (*i.e.* individual and populations) considered in this project.

For visual exploration, Principal Coordinates Analysis (PCoA) were performed by the `betadisper()` function on metabarcoding and stable isotopes data, and the coordinates obtained were used to visualize the spatial distribution of individuals for each method. PCoA was also used as input for Procrustes analysis (Oksanen et al. 2022) which tested for the similarity in the multivariate structure between metabarcoding and stable isotope data. Significance of the Procrustes fit was assessed via permutation tests (9999 permutations).

Within-site variability was assessed using site and individual-level dispersions (distances to centroid) as main metric. Distances to centroid reflect the dispersion of individuals within each site and can be interpreted as a proxy for population dietary niche breadth, as it provides a measure of the average degree of dietary diversity (Layman et al. 2007). Multivariate dispersion was calculated for each individual as its distance to the site centroid using the `betadisper()` function from the `vegan` R package, based on Bray–Curtis dissimilarities (metabarcoding data) and Euclidean distances (stable isotope data). These individual dispersions were also averaged by site to assess the within-site variability at the site-level. Comparisons of this metric between metabarcoding and stable isotope data were performed at the site level and individual level using Pearson's correlations.

Then, between-site variability was assessed by first calculating mean OTU abundances and mean stable isotope values for each site. Dissimilarity matrices between sites were then computed using Bray–Curtis distances (metabarcoding) and Euclidean distances (stable isotopes). The

resulting site-level dissimilarity matrices were compared across methods using Pearson's correlation to evaluate the correspondence between the two methods to reflect between-site dietary differences.

Results

From the 252 individuals initially processed, 233 were included in the final dataset. Despite successful amplifications and sequencing, 17 individuals did not pass the metabarcoding filtering criteria. An additional 2 specimens could not be analysed for stable isotope composition due to analytical issues.

Dietary niche breadth across spatial scale

Metabarcoding-based dietary diversity

A total of 16,876,583 COI raw reads were generated through sequencing of the initial 252 gut content samples, with a mean of 61,369 reads per sample (sequencing depth ranged from 42,492 to 260,757 reads). After quality filtering, trimming, removal of singletons and chimeras, 15,226,902 reads were obtained. From these 15mi reads, 2663 OTUs were produced. After the filtering steps, including the removal of 12,565,230 reads assigned to the host *Stereochinus neumayeri* (~82.5%), 486 OTUs were finally kept for further analyses. From the 486 OTUs retained, only 8% were assigned to the species level, 11% to the genus, 21% to the family and 54% to the order (see Online Resource 2).

Shannon diversity indices varied among sites, as detected with Kruskal–Wallis test ($\chi^2=87.48$, $df=12$, $p<0.001$; Fig. 2), with the lowest mean observed at site 13 (1.6 ± 1.1) and the highest at site 8 (3.7 ± 0.3).

The Principal Coordinate Analysis (PCoA) based on Bray–Curtis dissimilarities showed some overlaps between diet composition in sea urchins from different sites (Fig. 3). Gut content data revealed that most sites shared a broadly similar prey pool, whose relative contribution of key dietary components (*e.g.*, diatoms, macroalgae, and invertebrates) differed however across sites (see Online Resource 1). Diatoms dominated the diet across all sites except sites 11 and 13, but the proportions of other taxa varied, which explained the partial dietary overlap. As the permutation test of multivariate homogeneity of groups dispersions showed significant differences in dispersion (*i.e.* differences in how variable individual diets are within each site) across sites ($p<0.001$), a distance-based multivariate Welch MANOVA was performed to evaluate site-level differences in diet composition. This analysis revealed a highly significant site

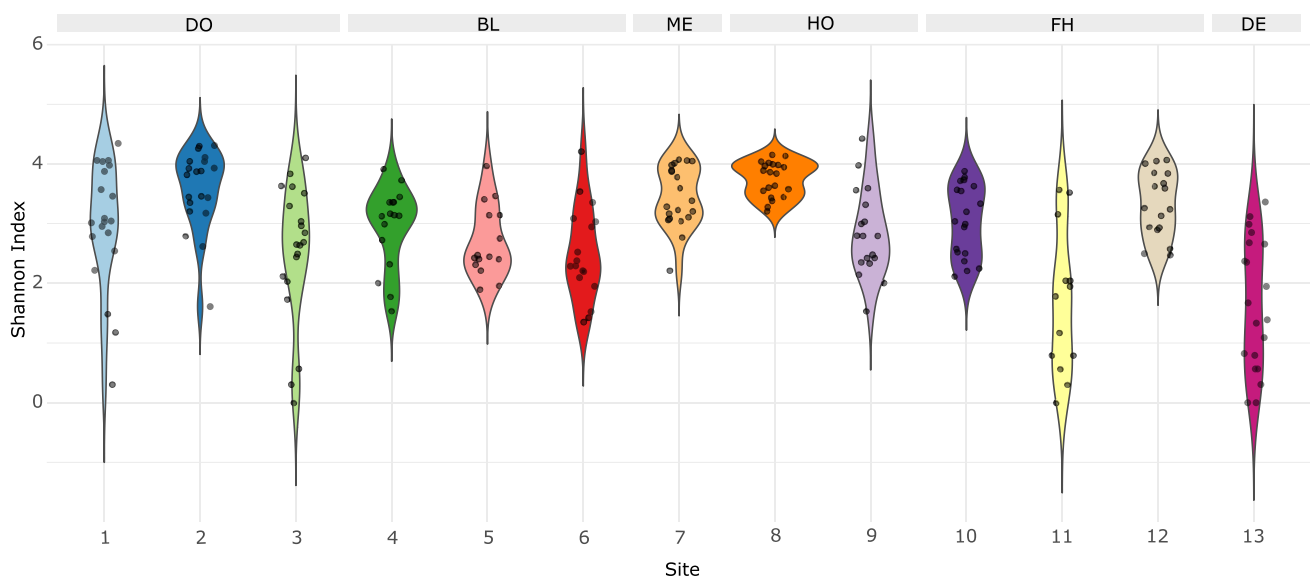


Fig. 2 Distribution of individual Shannon diversity indices of *Sterechnus neumayeri* diets across sampling sites, inferred from gut content metabarcoding analyses. Sites are grouped by region, with regional

affiliations indicated above the x-axis by abbreviations (DO: Dodman Island; BL: Blaiklock Island; ME: Melchior Island; HO: Hovgaard Island; FH: Foyen Harbor; DE: Deception Island)

effect (WdS=9.73, df=12, $p<0.001$), indicating that overall dietary composition differed among sites.

Isotopic niche width

The 3D ellipsoid representations of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values showed differences in ellipsoid size and positioning between sites (Fig. 4). Some of the ellipsoids gathered and partially overlapped in the central part of the isotopic space, like sites 1 and 12, while some other ellipsoids were located at the outer space reducing the overlap with other sites, like sites 8 and 9. Both the 3D representation (Fig. 4) and the violin plot of ellipsoid volume distribution (Fig. 5) suggested variation in isotopic niche width across sites. Some sites such as site 8, site 9, and site 10 exhibited larger ellipsoids, while sites such as site 2, site 11, and site 13 displayed smaller ellipsoids. Kruskal–Wallis tests revealed significant differences between sites for each isotope: $\delta^{13}\text{C}$ ($\chi^2=203.88$, df=12, $p<0.001$), $\delta^{15}\text{N}$ ($\chi^2=170.99$, df=12, $p<0.001$), and $\delta^{34}\text{S}$ ($\chi^2=176.20$, df=12, $p<0.001$). Permutation test of multivariate homogeneity of groups dispersions showed significant differences across sites ($p=0.013$). A distance-based multivariate Welch MANOVA was therefore processed to test for site-level differences in multi-isotopic composition, and revealed significant differences in isotopic composition between sites (WdS=80.84, df=12, $p<0.001$).

Dietary niche width: accounting for temporal scale

The PCoA biplots presented different dispersion structures between metabarcoding (short-term) and stable

isotope (longer-term) data (Fig. 6). Structural differences were reflected in the Procrustes analysis, which indicated a significant yet low concordance and a high mismatch in multivariate structure between methods (Procrustes correlation=0.36, $m^2=0.870$, $p<0.001$; 9999 permutations).

At the within-site level, a weak correspondence between short-term and longer-term dietary data was observed, both at the site- (mean distance to centroid) and individual-level (Fig. 7). At the site-level, no significant correlation was detected between metabarcoding and stable isotopes data when comparing the mean distances to centroid among individuals (Pearson's $r=0.109$, $p=0.721$; Fig. 7a). When comparing individual-level distances to centroid obtained from metabarcoding and stable isotope data, a weak but significant positive correlation was observed (Pearson's $r=0.129$, $p=0.049$; Fig. 7b).

At the between-site level, a moderate but significant positive correlation was found between the dissimilarity matrices calculated from mean OTU abundances and from mean stable isotope values of each site (Pearson's $r=0.331$, $p=0.003$).

Dietary specialization: organizational scale matters

Kruskal–Wallis test indicated significant differences of Proportional Similarity Index (PSi) values between sites ($\chi^2=82.47$, df=12, $p<0.001$), ranging from 0.17 (indicating a significant specialization) to 0.71 (indicating a broad generalism) across all sites (Fig. 8).

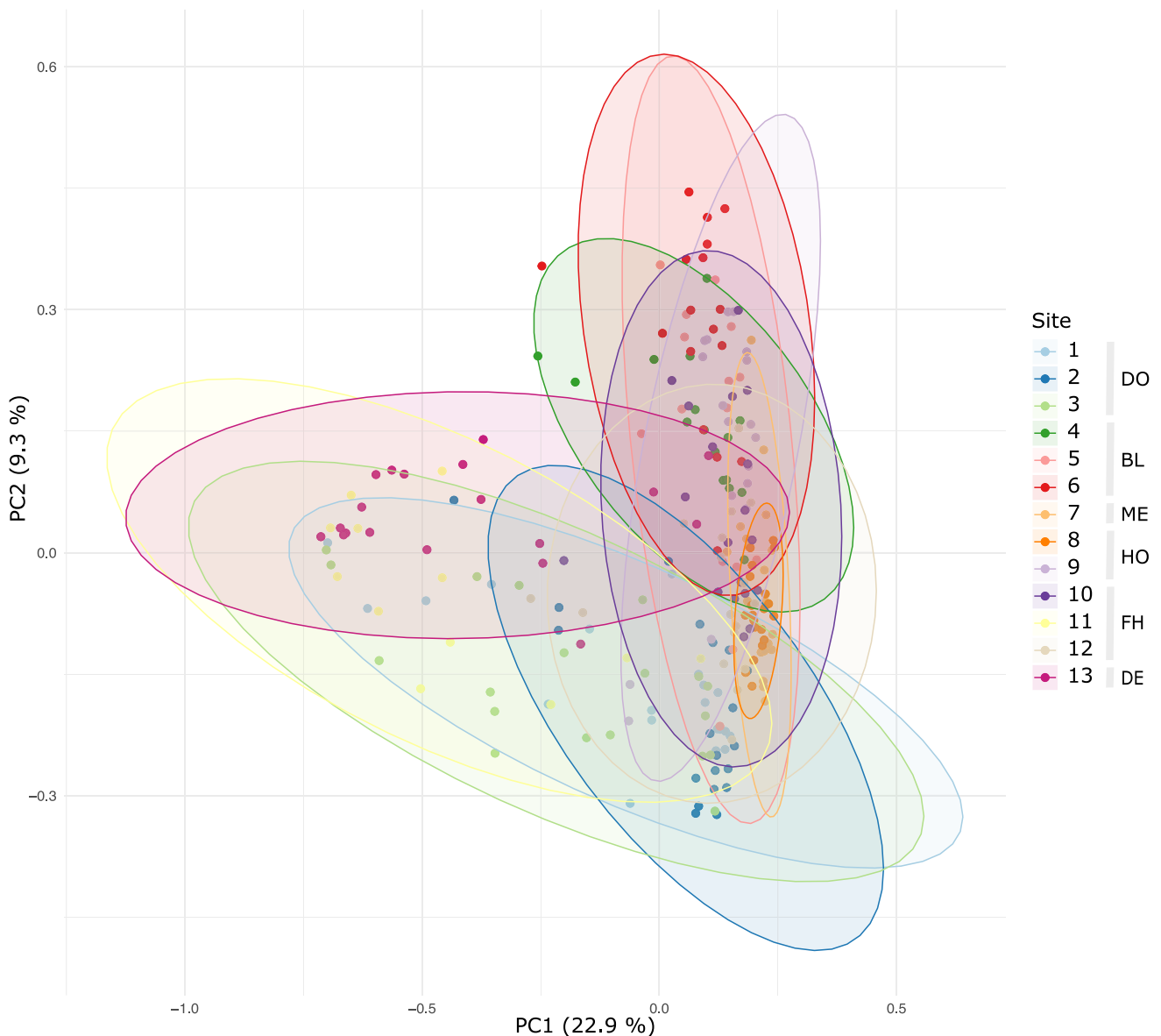


Fig. 3 Principal Coordinate Analysis (PCoA) based on Bray-Curtis dissimilarity matrix of dietary composition, inferred from gut content metabarcoding in *Stereochinus neumayeri* individuals across sites. Each point represents an individual, and ellipses denote the 95% confidence

interval for site-level grouping. The regions to which each site belongs are indicated in the legend by their abbreviations (DO: Dodman Island; BL: Blaiklock Island; ME: Melchior Island; HO: Hovgaard Island; FH: Foyr Harbor; DE: Deception Island)

Influence of biological traits on dietary specialization

The linear mixed-effects model revealed that Shannon diversity was significantly influenced by sex, with males showing a higher diversity than females ($t=2.66$, $df=212$, $p=0.008$), and by size, with diversity increasing with the size ($t=2.73$, $df=122$, $p=0.007$), while accounting for site-level variation. The linear mixed-effects model investigating dietary beta diversity (measured as distance to centroid) showed no significant effect of sex ($p=0.853$) or size ($p=0.719$) on within-site dietary variability.

Linear mixed-effects models revealed significant effects of sex and body size on both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. $\delta^{13}\text{C}$ increased with individual size ($t=4.64$, $df=247$, $p<0.001$) and differed significantly between sexes, with females exhibiting higher values than males ($t=-2.82$, $df=235$, $p=0.005$). Similarly, $\delta^{15}\text{N}$ values increased with size ($t=4.20$, $df=227$, $p<0.001$) and were higher in females than in males ($t=-3.08$, $df=236$, $p=0.002$). In contrast, no significant effects of sex or size were detected for $\delta^{34}\text{S}$ values ($t=-0.54$, $df=236$, $p=0.591$; $t=0.80$, $df=225$, $p=0.422$, respectively).

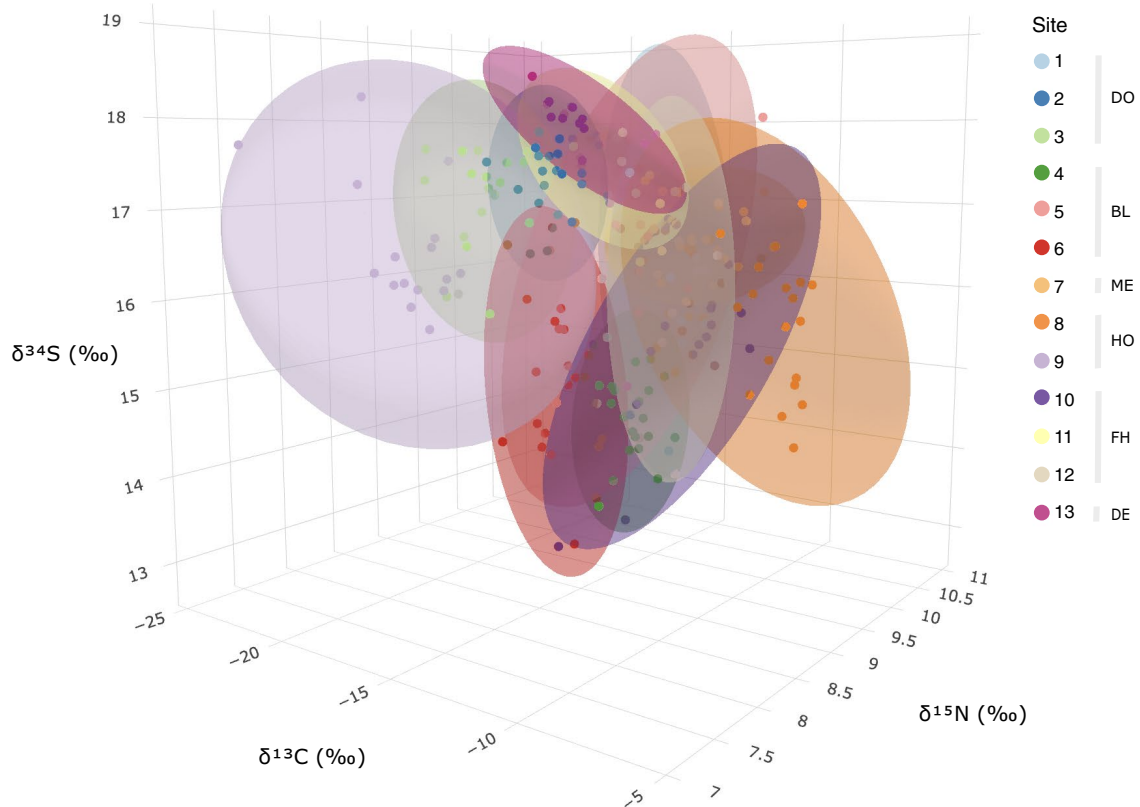


Fig. 4 Three-dimensional isotopic niche space of *Sterechinus neumayeri* individuals across the thirteen sampling sites, based on $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ values. Each point represents an individual, and ellipsoids corresponds to the 95% Bayesian isotopic niche estimated for each site (i.e., the multivariate dispersion of isotope values at the site

level). Regional affiliations of sites are shown in the legend using abbreviated regional names (DO: Dodman Island; BL: Blaiklock Island; ME: Melchior Island; HO: Hovgaard Island; FH: Foyn Harbor; DE: Deception Island). An interactive 3D version is available in Online Resource 3

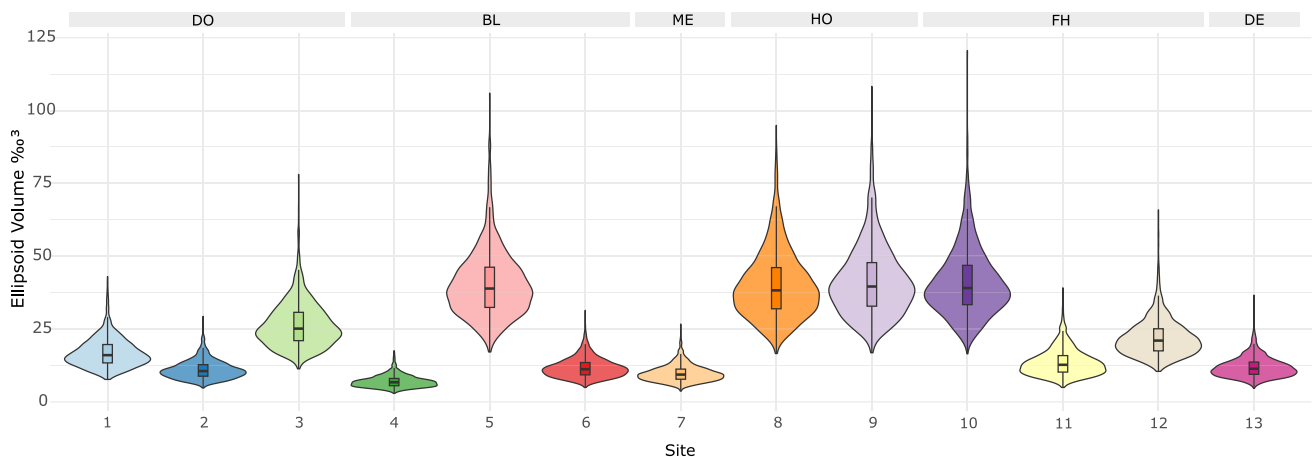


Fig. 5 Violin plots of isotopic niche volumes across sampling sites, based on the three-dimensional ellipsoid volumes defined by $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ values for each site. The volume represents the isotopic niche width estimated from the three stable isotope data. Sites

are grouped by geographic region, indicated above the graph by their abbreviations (DO: Dodman Island; BL: Blaiklock Island; ME: Melchior Island; HO: Hovgaard Island; FH: Foyn Harbor; DE: Deception Island)

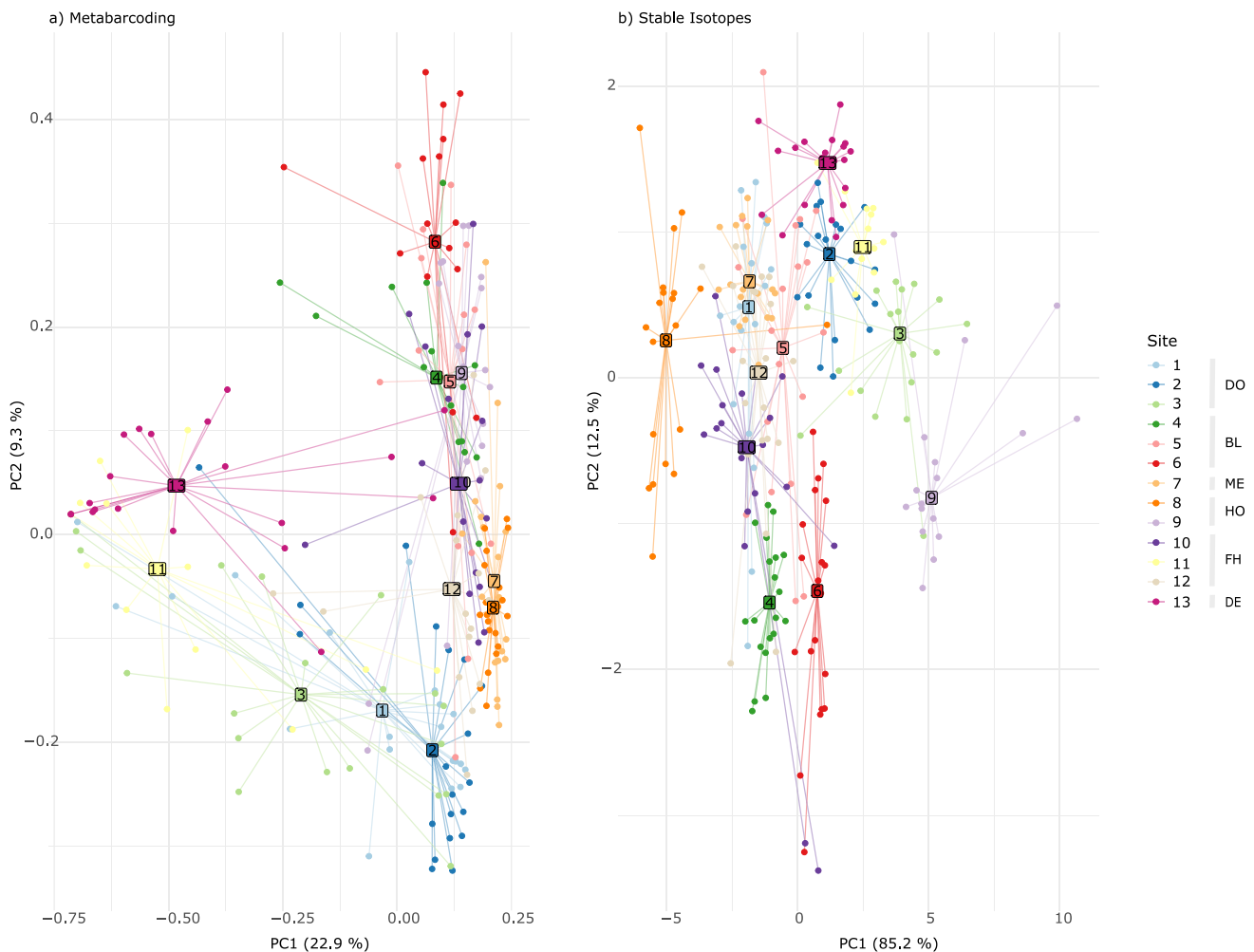


Fig. 6 Principal Coordinate Analyses (PCoA) showing individual-level dispersion across the thirteen sampled sites based on two complementary approaches. **a)** PCoA based on Bray-Curtis dissimilarities of diet composition inferred from gut content metabarcoding. **b)** PCoA based on Euclidean distances calculated from $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ stable isotope values. Each point represents an individual *Sterechnus*

neumayeri. Lines connect individuals to their respective site centroids, illustrating within-site dispersion and variability in dietary composition (**a**) and isotopic niche space (**b**). Regional affiliations of sites are shown in the legend (DO: Dodman Island; BL: Blaklock Island; ME: Melchior Island; HO: Hovgaard Island; FH: Foyen Harbor; DE: Deception Island)

Linear mixed effects model showed a significant effect of sex, with males having higher PSi values than females (Estimate=0.0226, $p=0.044$), and a significant positive effect of body size on PSi (Estimate=0.0026, $p=0.0019$).

Discussion

Dietary specialization varies across different scales

In this study, our aim was to estimate the degree of dietary specialization of *Sterechnus neumayeri* in the Western Antarctic Peninsula, by accounting for variation across three ecological scales—spatial (between sites), temporal (short—metabarcoding—vs longer-terms—stable isotopes), and organizational (individual vs. population levels).

Our hypotheses were that diet composition and degree of dietary specialization vary at those three scales. Additionally, biological traits such as sex and size influence on dietary specialization were assessed. With this framework, we now discuss how our findings support or challenge these expectations and what they reveal about the trophic ecology of *S. neumayeri*.

Overall, both metabarcoding and stable isotopes analyses revealed spatial variation in the dietary composition and degree of dietary specialization of *S. neumayeri* along the Western Antarctic Peninsula. While gut content data indicated a broadly shared prey pool dominated by diatoms and macroalgae, differences in the relative contribution of other taxa generated a significant differentiation in dietary composition and specialization among individuals and sites. The stable isotope analyses revealed strong spatial structuring,

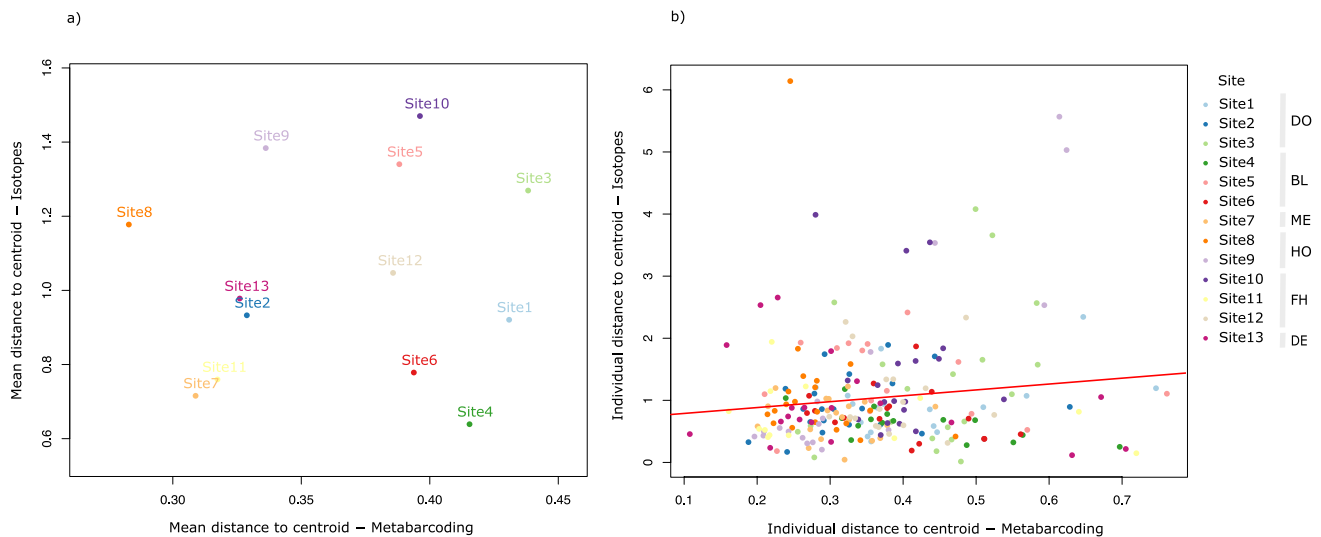


Fig. 7 Pearson correlation analysis comparing within-site dispersion estimated from short-term dietary data (metabarcoding) and longer-term isotopic signals (stable isotopes). **a)** Site-level comparison of mean distances to site centroids derived from gut content metabarcoding (Bray-Curtis dissimilarity) and from stable isotopes data (Euclidean distances based on $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$). Each point represents a sampling site. **b)** Individual-level comparison of distances to site

centroids estimated from the two approaches. Each point represents an individual *Sterechinus neumayeri*, with colors indicating site affiliation. Red lines show linear regression fits used to assess Pearson correlations. The regions to which each site belongs are indicated in the legend by their abbreviations (DO: Dodman Island; BL: Blaklock Island; ME: Melchior Island; HO: Hovgaard Island; FH: Foyen Harbor; DE: Deception Island)

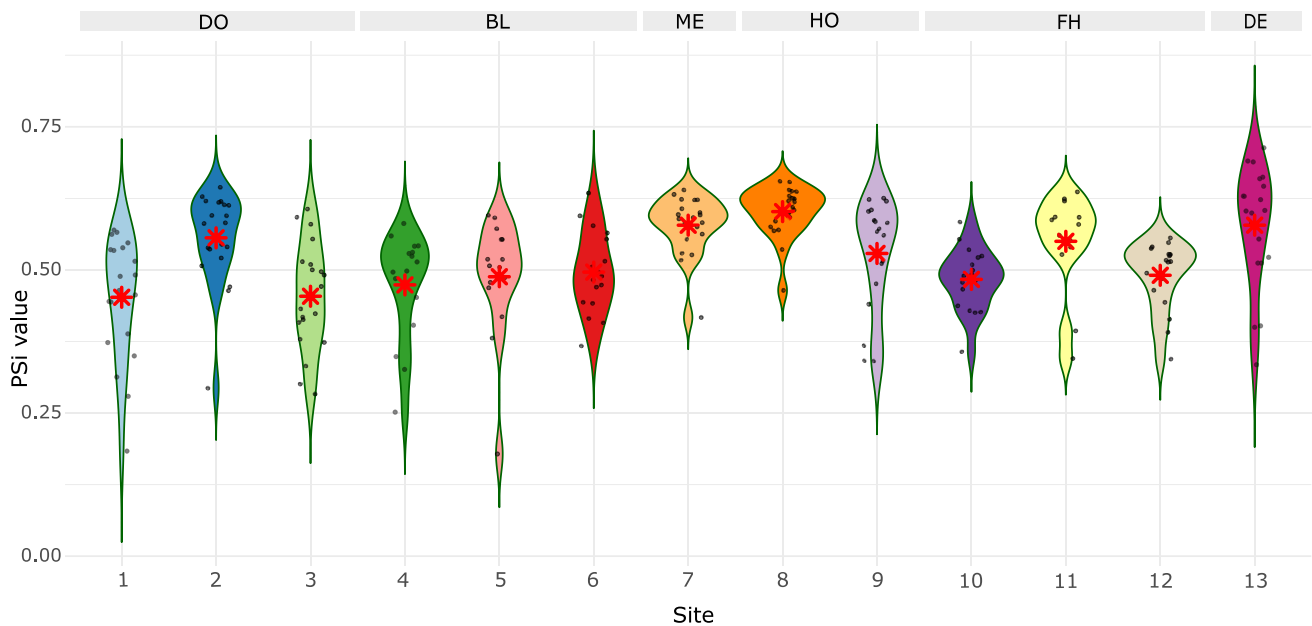


Fig. 8 Distribution of individual PSi (Proportional Similarity index) values across sites, inferred from gut content metabarcoding, representing the degree of dietary specialization for each *Sterechinus neumayeri* individual. Red asterisks indicate population-level IS values, corresponding to the average degree of diet specialization for each site.

Geographic regions corresponding to each site are indicated above the graph by abbreviations (DO: Dodman Island; BL: Blaklock Island; ME: Melchior Island; HO: Hovgaard Island; FH: Foyen Harbor; DE: Deception Island)

with sites not only occupying distinct positions in isotopic space (i.e. displaying significant differences in isotopic composition), but also having different niche widths. These patterns indicate that urchins from different sites assimilate resources that differ in both origin and trophic pathway.

Differences in $\delta^{13}\text{C}$ among sites suggest shifts in the primary carbon sources supporting individuals, particularly in Antarctic ecosystems where primary producers display marked isotopic variability. For instance, water column-associated production typically exhibits very low $\delta^{13}\text{C}$

values (Seyboth et al. 2018) a strong $\delta^{13}\text{C}$ also characteristic of many macroalgae (Gillies et al. 2012; Michel et al. 2019). In contrast, other benthic producers, such as micro-phytobenthos, display less negative $\delta^{13}\text{C}$ signatures (Corbisier et al. 2004; Pasotti et al. 2015), while organic matter associated with Antarctic sea ice is even more enriched in $\delta^{13}\text{C}$ (Michel et al. 2016; Calizza et al. 2018). Consequently, the spatial variation in $\delta^{13}\text{C}$ among sites likely reflects differences in the relative contribution of macroalgal or water column-associated production at sites with more negative values, versus a stronger influence of sea-ice-derived or benthic carbon sources at sites exhibiting higher $\delta^{13}\text{C}$ signatures. Variation in $\delta^{15}\text{N}$, in turn, reflects differences in trophic position among sites. Because nitrogen isotopes exhibit predictable and stepwise enrichment with increasing trophic level (DeNiro and Epstein 1981), higher $\delta^{15}\text{N}$ values indicate greater reliance on animal-derived organic matter through predation or scavenging (Mincks et al. 2008). This suggests spatial differences in trophic strategy among sites, ranging from stronger reliance on animal-derived resources to greater dependence on primary producers or low-trophic-level inputs. Finally, site-specific differences in $\delta^{34}\text{S}$ indicate contrasting reliance on benthic versus pelagic production pathways. Pelagic primary producers assimilate seawater sulphates, resulting in relatively high $\delta^{34}\text{S}$ values, whereas sedimentary recycling processes promote incorporation of lighter sulphur isotopes and lower $\delta^{34}\text{S}$ values (Fry et al. 1988; Connolly et al. 2004). Thus, spatial variation in $\delta^{34}\text{S}$ suggests differences in the relative influence of pelagic versus sediment-linked pathways across sites. Taken together, the combined variability in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ indicates that sea urchins along the WAP do not exploit a single, homogeneous resource pool. Instead, populations appear to rely on distinct combinations of primary carbon sources, trophic levels, and production pathways depending on local conditions. Moreover, differences in isotopic niche width suggest that some populations exploit a broader spectrum of resources, whereas others appear to display less trophic diversity. Since stable isotopes represent a proxy for the realized ecological niche, they inherently capture both dietary inputs and habitat-related influences. The differences observed could therefore partly be explained by inter-site variation in isotopic composition of food items. However, previous studies showed relatively consistent isotopic composition of baseline producers over part of our sampling area (Voisin et al. 2025). Overall, isotopic differences between sites are therefore unlikely to be caused only by indirect baseline effects. Altogether, our findings based on metabarcoding and stable isotopes analyses suggest that *Sterechinus neumayeri* might rely on different food sources and display variable trophic diversity along the Western Antarctic Peninsula. This aligns with previous studies: that of

Michel et al. (2016) which suggested a strong trophic plasticity of *S. neumayeri* in Weddell Sea and Bransfield Strait; findings from Rossi et al. (2019) that supported a variation in resource use between individuals within local populations in the Ross Sea; and findings from Galloway et al. (2024) that indicated a relatively high fatty acids dispersion across their sampled sites in the WAP. A likely explanation for this recurrent trophic variability lies in spatial differences in benthic community composition. Marked heterogeneity in benthic assemblages and resource availability has been documented across the region (Katz et al. 2025), and such spatial differences in benthic communities are likely to shape the dietary composition and degree of specialization in *S. neumayeri*, providing an ecological basis for the trophic differentiation observed. This benthic heterogeneity is itself expected to arise from variability in environmental and habitat conditions along the WAP (Michel et al. 2016; Post et al. 2017; Puccinelli et al. 2018; Vause et al. 2019; Grimes et al. 2025), among which sea-ice cover emerges as a central structuring factor. The WAP is characterized by a pronounced north–south gradient in sea-ice cover, which plays a major role in shaping benthic ecosystems through multiple, interacting pathways (Cardona et al. 2021; Michel et al. 2019). Sea-ice strongly constrains fleshy macroalgal development, with macroalgal cover declining from more than 70% in the northern WAP to less than 1% in the southern WAP (Amsler et al. 2023; Galloway et al. 2024). It also influences phytoplankton production and the relative abundance of benthic invertebrates (Amsler et al. 2023; Clark et al. 2015; Michel et al. 2019; Norkko et al. 2007; Rossi et al. 2019), and affects food availability through the export of sympagic primary production and oceanographic processed regulating pelagic-benthic coupling (Michel et al. 2019). Through this pervasive influence on benthic community structure and resource availability, sea-ice cover is therefore likely to shape benthic compositional heterogeneity along the WAP, and, in turn, contribute substantially to the spatial variation in dietary composition and specialization observed in *Sterechinus neumayeri*.

To assess potential temporal variability in the degree of dietary specialization of *S. neumayeri*, short-term (metabarcoding) and longer-term (stable isotopes) dietary data were compared across the same individuals and sites. The results revealed a partial decoupling between short- and longer-term dietary patterns, with short-term diets not fully reflecting longer-term dietary patterns, suggesting a temporal variability in individual feeding habits. This mismatch between short- and longer-term dietary signals may be linked with strong temporal fluctuations in resource availability that characterize Antarctic ecosystems (Clarke 1988; Calizza et al. 2018; Caputi et al. 2020; Clarke and Peat 2022; Conroy et al. 2023). Along the Western Antarctic

Peninsula, these temporal cycles in resource availability are strongly regulated by variability in sea-ice cover (Norkko et al. 2007; Clark et al. 2015; Michel et al. 2019; Rossi et al. 2019; Amsler et al. 2023; Galloway et al. 2024). Changes in sea ice conditions control the timing and magnitude of pelagic and sympagic primary production, thereby shaping the quantity and quality of food available to benthic consumers (Michel et al. 2019). Periods of reduced sea-ice cover are associated with enhanced production of primary producers such as diatoms and macroalgae, whereas more extensive or persistent ice cover limits primary production and favors reliance on detrital and animal-derived material (Cardona et al. 2021; Michel et al. 2019; Rossi et al. 2019). This dynamic might promote greater individual specialization during times of high resource availability, and more generalized feeding strategies during periods of scarcity (Araújo et al. 2011). Such a pattern aligns with predictions from the Optimal Foraging Theory (Pyke et al. 1977): when resources are abundant and predictable, individuals would tend to specialize, while in periods of scarcity, they would tend to show greater dietary generalism by coupling distinct energy channels. Together, these dynamics might suggest that sea ice-driven variability in food availability may underpin temporal shifts in feeding strategies, leading to contrasting short- and longer-term dietary specialization degree in *S. neumayeri*.

Dietary specialization is often assessed at the species or population level, yet individual-level variation can be equally important. Individuals within a population are not necessarily ecologically equivalent—some may specialize on a subset of available resources, even within a same sex or age class (Bolnick et al. 2003, 2011; Wiley et al. 2019). Individual specialization, a taxonomically widespread phenomenon, can shape both ecological and evolutionary dynamics by influencing how populations respond to environmental change (Bolnick et al. 2003, 2011; Araújo et al. 2011). Accordingly, distinguishing the degree of individual specialization within populations has become an important focus in trophic ecology. A key question when studying generalist species or populations is whether they are composed of generalist individuals—each using a wide variety of food types—or of individuals that are themselves specialized on different subsets of resources (Van Valen 1965; Bolnick et al. 2003; Bearhop et al. 2004). Patterns of individual degree of specialization, quantified using the Proportional Similarity Index (PSi), revealed a strong variability within and between-sites. In contrast, the overall population-level index of specialization (IS) remained stable across sites, suggesting that populations are consistently composed of a mix of more specialized or more generalized individuals. The Optimal Foraging Theory (OFT) might be one of the possible theories explaining the observed variability in dietary

specialization among co-occurring individuals. According to OFT (Pyke et al. 1977), two alternatives hypotheses may be at play: i) when all individuals preferentially target the same high-ranked resource, intense intraspecific competition can reduce its availability, driving some individuals to diversify their diet by exploiting alternative resources; ii) alternatively, individuals may initially prefer different resources but, as competition increases, they may converge on more abundant or accessible secondary resources (Bolnick et al. 2011; Araújo et al. 2011). These contrasting scenarios can both lead to variation in the degree of individual specialization within populations. The patterns observed in our data (e.g., the variability in PSi values among individuals within the same site) might be consistent with these theoretical predictions. Together, these results highlight the importance of taking individual-level dietary variation into account, even within populations traditionally classified as generalist ones.

Finally, beyond the spatial, temporal, and individual-scale patterns, biological traits such as sex and size also contributed to dietary variation in *Sterechinus neumayeri*. Significant differences between sexes and size classes were detected in within-individual dietary diversity (e.g. Shannon index), carbon and nitrogen isotopic compositions and PSi values. Specifically, males exhibited higher dietary diversity than females, reflected by higher PSi values, whereas females showed less negative $\delta^{13}\text{C}$ and higher $\delta^{15}\text{N}$ values than males. This pattern points to sex-specific differences in resource use, with a potential shift in sources for females, alongside greater dietary breadth in males. To our knowledge, sex-based differences in the dietary niche of *Sterechinus neumayeri* have never been directly demonstrated, although they have been suggested in earlier studies (Guilou and Lumingas 1999; Martínez-Pita et al. 2010; Gravem and Adams 2012), potentially arising from differences in energetic requirements or reproductive investment. Body size also significantly influenced trophic patterns. Larger individuals displayed broader dietary diversity, as indicated by higher PSi values, and less negative $\delta^{13}\text{C}$ and higher $\delta^{15}\text{N}$ values, consistent with shifts in food sources with size. These patterns align with previous findings by Calizza et al. (2018) who reported that larger *S. neumayeri* individuals consumed a greater diversity of resources compared to smaller ones. That study further showed that smaller individuals tended to rely more on epiphytic diatoms over both the short- and longer-term, whereas larger individuals exploited a broader range of food items, depending on the time scale considered (Calizza et al. 2018). Altogether, these results indicate that morpho-physiological and/or reproductive differences among individuals play an important role in shaping trophic strategies in *Sterechinus neumayeri*. They further suggest that ontogenetic dietary shifts and size-related differences in

foraging behavior may underlie part of the individual-level and scale-dependent variation observed in this study. More broadly, these findings reinforce the idea that dietary specialization in *S. neumayeri* is not only scale-dependent but also shaped by individual biological characteristics, adding another layer of complexity to the interpretation of its trophic ecology.

Generalist species? the importance of considering realized dietary specialization

The assessment of dietary specialization in *Sterechinus neumayeri* across multiple scales—spatial, temporal, and organizational—was important to *in fine* re-discuss its long-assumed generalist status. As mentioned, dietary specialization is often treated as a fixed species-level trait, assumed to be independent of scales. This approach implicitly emphasizes fundamental specialization—an intrinsic species attribute—rather than realized specialization, which reflects the actual degree of specialization expressed under specific environmental and ecological conditions (Ferry-Graham 2002; Devictor et al. 2010; DeSantis et al. 2022). *Sterechinus neumayeri* is frequently described in the literature as a generalist species, feeding on a wide array of diet items – a foundation typically generalised across space, time and individuals rather than evaluated within specific ecological contexts. Our data highlight that the issue of scale is essential: realized dietary specialization can vary significantly depending on spatial heterogeneity, temporal dynamics, organizational-level variation, and biological traits such as sex and size.

- i) Spatial scale: dietary composition and specialization differed significantly among the thirteen sites sampled along the Western Antarctic Peninsula. While diatoms dominated the diet at most sites, the relative contributions of other food sources (e.g., macroalgae and invertebrates) and the overall dietary breadth varied, suggesting site-specific dietary patterns.
- ii) Temporal scale: dietary specialization varied over time, with a low correlation between short-term diets (reflected in gut content metabarcoding) and longer-term trophic integration (revealed by stable isotope analysis) at the individual and population levels. This temporal mismatch indicated that individual diets were not consistent across the different temporal scales considered.
- iii) Organizational scale: dietary specialization assessed solely at population-level would mask individual-level variability. Indeed, the degree of dietary specialization significantly varied at the individual level, both within and between sites. Some individuals exhibited highly

specialized diets, while others were more generalist, highlighting the existence of intraspecific trophic diversity.

By integrating these scales, a more refined—and above all, more nuanced—picture of *Sterechinus neumayeri*'s trophic ecology emerged. While the species may broadly fit the definition of a generalist based on its fundamental dietary niche, it cannot be consistently considered a strict generalist in terms of realized dietary specialization. Rather, it can express more generalist or more specialist traits depending on the local environmental context, moment in time, and individual-level characteristics. The spatial, temporal and inter-individual variability documented here suggests that *S. neumayeri* possesses a degree of trophic plasticity that may modulate acclimation to ongoing environmental change in the WAP.

In this region, the environmental context within which this plasticity is expressed is structured by several interacting ecological drivers, among which sea-ice dynamics likely hold an important place. Sea-ice strongly shapes benthic community composition and temporal patterns of resource availability, thereby influencing primary production, benthic assemblages, and the composition of trophic resource pools. The present results suggest that variability in dietary composition and specialization in *S. neumayeri* is likely influenced—directly and indirectly—by these spatial gradients and temporal fluctuations in environmental conditions, including sea-ice. Such environmentally mediated variability provides a coherent framework for understanding the context-dependent expression of trophic plasticity observed in this species. This plasticity may allow *S. neumayeri* to exploit a range of dietary sources, potentially conferring an advantage in environments where resource availability fluctuates across space and time. However, this potential benefit must be considered in light of the fact that Antarctic benthic organisms have historically experienced strong but relatively predictable sea-ice-driven cycles of resource availability. Trophic plasticity may thus reflect long-term acclimation to seasonal and interannual variability, rather than unlimited flexibility. Ongoing and projected shifts in sea-ice regimes along the WAP, together with other environmental changes, are therefore likely to have complex consequences for benthic food webs, potentially reshaping competitive interactions, resource coupling, and trophic niche structure. In this context, the trophic plasticity exhibited by *S. neumayeri* may represent an important, but not necessarily sufficient, trait for coping with future environmental change.

More broadly, these findings reinforce the idea that determining the degree of dietary specialization of a species is not straightforward. The variability observed across these scales

makes it difficult to assign a single feeding strategy. Classifying species as “generalists” or “specialists” by extrapolating results from a single place or time risks misrepresenting ecological plasticity, while generalizing population-level patterns without considering individual variability may oversimplify trophic dynamics and obscure meaningful ecological responses. As ecosystems are increasingly altered by climate-driven shifts in productivity, habitat structure, and resource availability, the capacity of species to adjust their trophic niche is likely to play a key role in their persistence and ecological role. Recognizing and explicitly incorporating this multi-scale variability is therefore essential for improving predictions of species’ acclimative and adaptive potential, as well as for refining our understanding of food-web structure and ecosystem functioning in rapidly changing environments.

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Authors contributions MB, LM and BD contributed to the study conception and design. Material preparation and data collection were performed by MB, LM, BD, MD, LT, CM. Data analyses were performed by MB. The first draft of the manuscript was written by MB, and all authors commented on previous versions of the manuscript. All authors approved the final manuscript.

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Data availability According to guidelines from both the Antarctic Treaty and the study’s main funding source (BELSPO), all data underlying this article are openly accessible. The COI gene sequence data generated and analyzed in this study have been deposited in the NCBI Sequences Read Archive (SRA) and are publicly available under the BioProject number PRJNA1345069. Stable isotope composition data are freely available through the SCAR Antarctic Biodiversity Portal (biodiversity.aq) at <https://doi.org/10.15468/hpbwya>.

Declarations

Conflict of interests The authors have no relevant financial or non-financial interests to disclose.

Ethics approval This study did not require ethics approval.

Compliance with ethical standards All applicable international, national, and/or institutional guidelines for the care and use of animals were followed and all necessary approvals have been obtained. The authors declare that they have no conflict of interests.

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