

Functional traits explain trophic allometries of cephalopods

Kieran J. Murphy^{1*}, Gretta T. Pecl¹, Shane A. Richards², Jayson M. Semmens¹,
Andrew T. Revill³, Iain M. Suthers^{4,5}, Jason D. Everett^{4,6}, Rowan Trebilco^{1, 3}, and
Julia L. Blanchard¹

¹Institute for Marine and Antarctic Studies, University of Tasmania, Hobart,
Tasmania, Australia

²School of Natural Sciences, University of Tasmania, Hobart, Australia

³CSIRO Oceans and Atmosphere, Hobart, Australia

⁴School of Biological, Earth, and Environmental Sciences, University of New South
Wales, Sydney, Australia

⁵Sydney Institute of Marine Science, Mosman, Australia

⁶Centre for Applications in Natural Resource Mathematics, The University of
Queensland, St Lucia, 4067 Queensland, Australia

* Corresponding author: Kieran.Murphy@utas.edu.au

Abstract

1. Individual body size strongly influences the trophic role of marine organisms and the structure and function of marine ecosystems. Quantifying trophic position-individual body size relationships (trophic allometries) underpins the development of size-structured ecosystem models to predict abundance and the transfer of energy through ecosystems. Trophic allometries are well studied for fishes but remain relatively unexplored for cephalopods.
2. Cephalopods are important components of coastal, oceanic, and deep-sea ecosystems, and they play a key role in the transfer of biomass from low trophic positions to higher predators. It is therefore important to resolve cephalopod trophic allometries to accurately represent them within size-structured ecosystem models.
3. We assessed the trophic positions of cephalopods in an oceanic pelagic (0 - 500 m) community (sampled by trawling in a cold-core eddy in the western Tasman Sea), comprising 22 species from 12 families, using bulk tissue stable isotope analysis and amino acid compound-specific stable isotope analysis. We assessed whether ontogenetic trophic position shifts were evident at the species-level and tested for the best predictor of community-level trophic allometry among body size, taxonomy, and functional grouping (informed by fin and mantle morphology).
4. Individuals in this cephalopod community spanned 2 trophic positions and fell into 3 functional groups on an activity level gradient: low, medium, and high. The relationship between trophic position and ontogeny varied among species, with the most marked differences evident between species from

different functional groups. Activity level-based functional group and individual body size best explained cephalopod trophic positions (marginal $R^2 = 0.43$).

5. Our results suggest that the morphological traits used to infer activity level, such as fin-to-mantle length ratio, fin musculature, and mantle musculature are strong predictors of cephalopod trophic allometries. Contrary to established theory, not all cephalopods are voracious predators. Low activity level cephalopods have a distinct feeding mode, with low trophic positions and little-to-no ontogenetic increases. Given the important role of cephalopods in marine ecosystems, distinct feeding modes could have important consequences for energy pathways and ecosystem structure and function. These findings will facilitate trait-based and other model estimates of cephalopod abundance in the changing global ocean.

Keywords

Food web, mesopelagic, octopus, ontogenetic diet shift, predator-prey interaction, size spectrum, squid, trophodynamics

INTRODUCTION

Cephalopods are a major component of marine ecosystems globally. They are abundant in coastal, oceanic and deep-sea ecosystems, are considered as voracious predators with rapid growth, short life spans, high production rates, and are an important prey source for many large marine predators (Clarke, 1983; Hunsicker, Essington, Watson, & Sumaila, 2010; Rodhouse & Nigmatullin, 1996). The abundance of cephalopods, from a diverse range of life-history types, is increasing across all oceanic regions, most likely due to climate change and the removal of their predators by fishing (Doubleday et al., 2016). This means their role

in marine ecosystems could be expanding. Cephalopods can be difficult to sample (Rodhouse & Nigmatullin, 1996), especially in oceanic ecosystems for species with limited fisheries interest, so using ecosystem models to understand their role in the structure and function of marine ecosystems is desirable. However, there is a paucity of critical data to facilitate quantifying the role of cephalopods in ecosystem models, which is exacerbated by the difficulty in representing them in ecosystem models appropriately due to their unique life-history and feeding characteristics (de la Chesnais, Fulton, Tracey, & Pecl, 2019). This creates a barrier to understanding the functional role of cephalopods in marine ecosystems and how it may respond in a changing climate.

Energy flow through food webs of marine ecosystems is underpinned by body size-dependent predator-prey relationships (Blanchard, Heneghan, Everett, Trebilco, & Richardson, 2017; Jennings, Pinnegar, Polunin, & Boon, 2001; O’Gorman, Jacob, Jonsson, & Emmerson, 2010). Quantifying the relationships between trophic position and individual body size (trophic allometries) provides a simple, yet powerful, means to explain and predict ecosystem structure and function (Blanchard et al., 2014, 2012, 2009; Hunt et al., 2015; McCormack et al., 2019). Size-structured models can now resolve important functional groups with distinctive traits (Blanchard et al., 2017; Heneghan, Everett, Blanchard, & Richardson, 2016). However, cephalopods are largely neglected within size-structured models and are poorly resolved in ecosystem models in general (de la Chesnais et al., 2019).

To include species or functional groups in size-structured models, trophic allometries are needed, but these relationships are variable and uncertain for cephalopods.

Cephalopod species often exhibit ontogenetic increases in trophic position (Cherel, Fontaine, Jackson, Jackson, & Richard, 2009; Chouvelon et al., 2011; Parry, 2008;

Ruiz-Cooley, Villa, & Gould, 2010). An extreme example is the Arctic species *Gonatus fabricii*, that undergoes an estimated 2.6 trophic position increase from the juvenile epipelagic phase to the adult bathypelagic phase (Golikov et al., 2018). On the other hand, two squid species in the north Atlantic, *Alloteuthis subulata* (mean weight of ~ 8 g and trophic position ~ 4.5) and *Loligo forbesii* (mean weight of ~ 375 g and trophic position ~ 4.7) displayed no ontogenetic changes in trophic position, nor much deviation in overall trophic position despite their size differences (Jennings & van der Molen, 2015). However, there may be other factors that contribute to variability across studies, as *L. forbesii* has been found to exhibit ontogenetic trophic shifts in a separate study (Chouvelon et al., 2011). Another species that has been shown to undergo no ontogenetic increase in trophic position is the vampire squid, *Vampyroteuthis infernalis*, in a global assessment of the species' trophic ecology (Golikov et al., 2019). When assessed at a community-level, cephalopods can exhibit ontogenetic shifts in trophic position, but it is variable and not all species undergo shifts (Staudinger et al., 2019). Additionally, cephalopods have been found to have similar or higher trophic positions than many much larger fish species in multiple ecosystem assessments (Jennings & van der Molen, 2015; Madigan et al., 2012), suggesting that different trophic allometries may apply to them.

Knowledge gaps are particularly evident for species with smaller maximum adult sizes, due to sampling difficulties and a general lack of commercial fisheries interest (Reid, 2016; Ruiz-Cooley et al., 2010; Villanueva, Perricone, & Fiorito, 2017). In the absence of species' specific knowledge of trophic allometries species' functional traits may provide enough explanatory power to understand broad-scale differences in variation across taxonomically similar or functional groups (Brose et al., 2019), but

the relationship between species' trophic allometries and functional traits have not previously been examined for cephalopods.

Morphological traits, such as fin shape in fishes, indicate swimming and feeding modes, which are key to the transfer of energy in ecosystems, and therefore ecosystem functioning (Bellwood, Streit, Brandl, & Tebbett, 2019; Mihalitsis & Bellwood, 2019; Myers, Anderson, Eme, Liggins, & Roberts, 2020). Cephalopods have a diverse range of morphology and behaviours that could influence their predation and functional role within food webs (Rodhouse & Nigmatullin, 1996; Villanueva et al., 2017). We hypothesise that the morphological traits of cephalopod fins and mantle musculature convey their activity level and feeding mode, enabling the construction of cephalopod functional groups.

Here we examined trophic allometries in an oceanic cephalopod community sampled via midwater trawls in the western Tasman Sea. In this region, cephalopods comprise a major portion of the diets of many top oceanic predators, with species such as blue shark and swordfish appearing to have ontogenetic preference for cephalopods during early and later life stages respectively (Young et al., 2010). We used bulk tissue carbon and nitrogen stable isotope analysis, in conjunction with amino acid compound specific nitrogen isotope analysis, to estimate the trophic allometries of cephalopods within the community, spanning a range of body sizes and morphologies. We asked two key questions: 1) Are there ontogenetic trophic position shifts for individual cephalopod species? and 2) Can trophic allometries be explained by functional traits?

MATERIALS AND METHODS

Sample collection and study location

We sampled cephalopods from a cyclonic cold-core eddy in the western Tasman Sea (Fig. 1), using midwater trawls from 10th – 12th September during a research voyage on Australia's Marine National Facility *RV Investigator*, in the Austral spring of 2017 (Henschke et al., 2019). We collected cephalopods, among other pelagic marine specimens, using two different types of trawl equipment. The first trawl type sampled the upper 100 m of the eddy using a Danish pelagic trawl with 300 μ m mesh, towed at approximately 1 m s⁻¹ (2 knots) for 60 minutes per trawl (n = 9). The second trawl type was an International Young Gadoid Pelagic Trawl (IYGPT) fitted with a MIDwater Open and Closing net system (Marouchos, Underwood, Malan, Sherlock, & Kloser, 2017) with six distinct codends to enable depth stratified sampling. The IYGPT trawl has a mesh size of 200 mm reducing to 10 mm, and the codend mesh size was 500 μ m. The IYGPT trawl was lowered to 500 m, with the first codend sampling obliquely from the surface to 500 m, and each subsequent codend sampled 100 m depth intervals for approximately 20 minutes each at 1 m s⁻¹, as the trawl returned to the surface (n=3). We sorted specimens from codends into broad taxonomic and morphological groups, enumerated them manually or using photography for image analysis and they were stored at -20°C until further analysis.

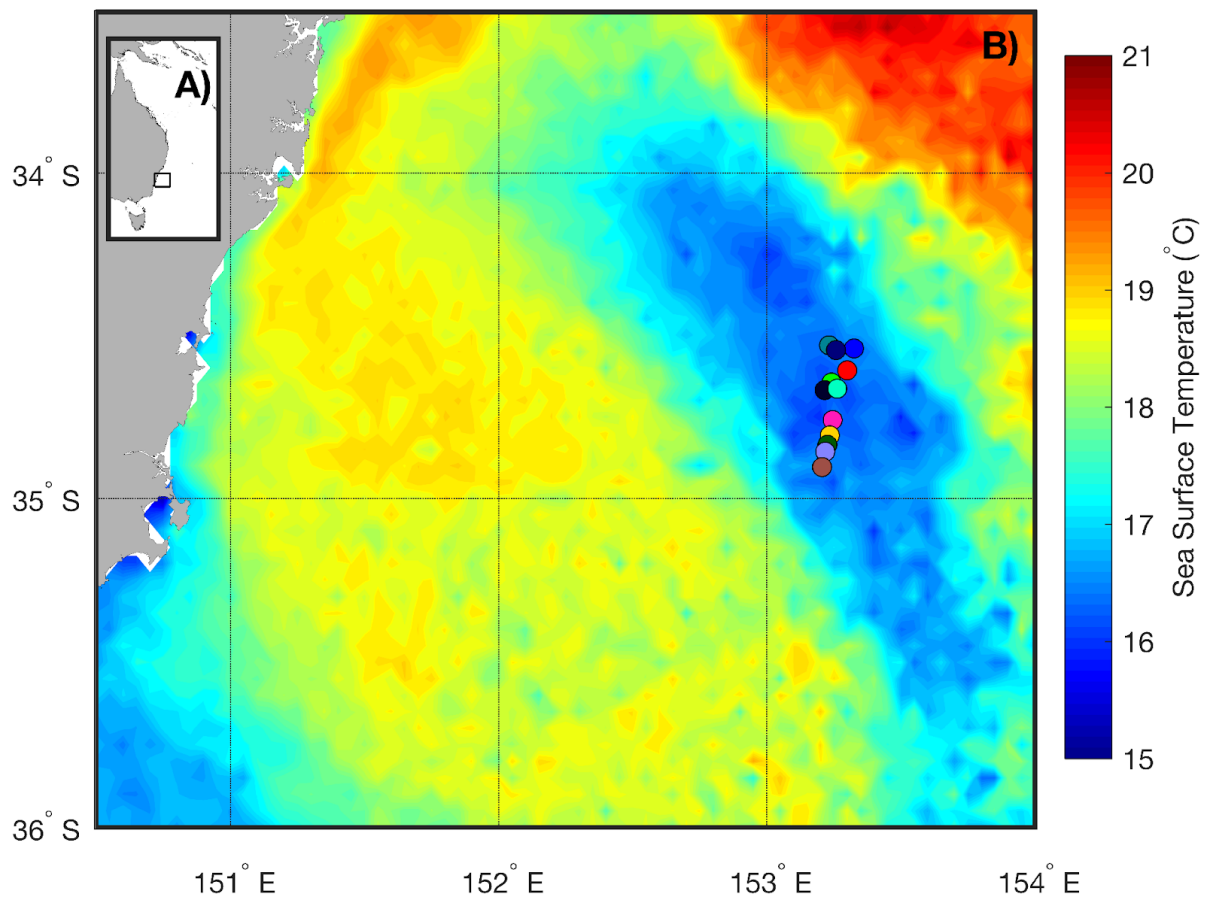


Figure 1: The twelve midwater trawls (filled circles in panel B) used to sample oceanic cephalopods in a cyclonic cold-core eddy from the western Tasman Sea off the coast of southeast Australia (panel A). The background colour is Sea Surface Temperature for September 2017 from MODIS-Aqua extracted from the Integrated Marine Observing System (IMOS) Data Portal (<http://imos.aodn.org.au/imos/>).

Cephalopod identification and preparation for stable isotope analysis

We identified cephalopods using taxonomic keys in Reid (2016). We cleaned and dissected mantle tissue samples from each individual, rinsed them with Milli-Q filtered water, and re-froze them at -20°C, before freeze-drying for 72 – 96 h. We ground freeze-dried samples into a homogeneous powder using an agate mortar and pestle and stored them in centrifuge tubes inside a desiccator, ready to be weighed for isotope analyses.

Bulk stable isotope analysis

We used stable isotope analysis as it provides integrated temporal representation of predators' assimilated diets (Revill, Young, & Lansdell, 2009). Many studies assessing size-based community trophic structure in marine ecosystems, including cephalopod focused research, have used stable isotope analysis (Cherel, Fontaine, et al., 2009; Chouvelon et al., 2011; Hunt et al., 2015; Hussey et al., 2014; Jennings et al., 2001; Jennings & van der Molen, 2015; Reum, Jennings, & Hunsicker, 2015).

Carbon and nitrogen stable isotope signatures originate at the base of all food webs at the stage of primary production (Fry, 2006). Carbon signatures ($\delta^{13}\text{C}$) change relatively little with increasing trophic position in food webs, while nitrogen ($\delta^{15}\text{N}$) increases from one trophic position to the next by the trophic enrichment factor ($\Delta^{15}\text{N}$), which is widely, if not always correctly, assumed to be between 2 - 4‰ (Peterson & Fry, 1987; Pethybridge, Choy, Polovina, & Fulton, 2018; Post, 2002; Revill et al., 2009). In addition to nitrogen stable isotope analysis, we also carried out carbon stable isotope analysis as a control measure. Carbon SIA allowed us to identify any groups or species that may have large spatial or temporal shifts in their diet, that may result in differing nitrogen baseline values, which could influence our interpretation of the nitrogen SIA.

We conducted bulk tissue stable isotope analysis (bulk SIA) on 207 cephalopod specimens, with species sample sizes ranging from 1 to 38 (Table S1). These specimens comprised 34% of the total number and 71% of the total weight of cephalopods sampled from the eddy. We weighed dried mantle tissue (mean sample weight = 0.38 mg \pm 0.08 SD) in tin capsules and analyzed carbon and nitrogen stable isotopes using flash combustion isotope ratio mass spectrometry (varioPYRO

cube coupled to Isoprime100 mass spectrometer) at the Central Science Laboratory,
University of Tasmania (Australia).

Stable isotope signatures are reported in delta (δ) values as the enrichment of the
heavier isotope relative to international reference standards (Pee Dee Belemnite for
C and atmospheric air for N) in parts per mil (‰) from the following equation:

$$\delta X (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000] \quad (\text{Equation 1})$$

where $X = {}^{13}\text{C}$ or ${}^{15}\text{N}$ and R = the ratio ${}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. Seven certified reference
materials with known isotopic composition (NBS-21, USGS-24, USGS-25, USGS-40,
USGS-41, IAEA-N1 and IAEA-N2) were used to correct for instrumental drift and
quality assurance purposes. Sulfanilamide was used to correct for elemental
percentages. One of each standard was measured after every 6th sample, with a
precision of 0.1‰ for both isotopes, while precision for elemental analysis
percentages were 0.02% for carbon and 0.09% for nitrogen.

To convert $\delta^{15}\text{N}$ values of cephalopod tissues to relative trophic positions, we used
 $\delta^{15}\text{N}$ signatures from particulate organic matter (POM) samples as isotopic
baselines. We obtained POM samples for each trawl station from water samples
collected from a CTD rosette with remotely fired Niskin bottles from the surface (4 m)
and the deep chlorophyll maximum (75 – 100 m) prior to and after the trawls. Four
litres of water from each POM sample ($n = 4$) was filtered through 47 μm pre-
weighed, pre-combusted, and acidified glass fibre filters using a low-pressure
vacuum. We froze the filter papers at -20°C until preparation for stable isotope
analysis. To prepare the filter papers, we incubated them at 50°C for 48 h, and then
halved and folded them to fit within tin foil capsules. We prepared and analysed
POM samples at the Australian Nuclear Science and Technology Organisation.

216 Relative trophic position (RTP) was calculated according to Post (2002),

217
$$\text{RTP}_{\text{consumer}} = [(\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{base}}) / \Delta^{15}\text{N}] + \lambda \quad (\text{Equation 2})$$

218 where $\delta^{15}\text{N}_{\text{consumer}}$ is the $\delta^{15}\text{N}$ of each individual, $\delta^{15}\text{N}_{\text{base}}$ is the mean $\delta^{15}\text{N}$ value of
219 the POM samples ($3.6\text{‰} \pm 0.55$), and $\Delta^{15}\text{N}$ is the trophic enrichment factor (3.4‰).

220 *Data analysis*

221 We used linear regression to evaluate ontogenetic shifts in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for the six
222 most abundant species. We considered three groupings to test whether taxonomic
223 identity, or functional grouping, best explained cephalopod community-level trophic
224 allometries. The first grouping was taxonomic and based on family, whereas the
225 other two groupings were functional and based on morphology. Cephalopod trophic
226 position was estimated using linear mixed-effects models (LMM) with grouping and
227 body size as predictors. The relative performance of our three groupings was
228 assessed by model selection using Akaike's Information Criteria (AIC). We
229 considered families that were represented by more than one individual. We treated
230 trawl as a random effect to account for unmeasured local environmental variation
231 influencing trophic position, and to incorporate the effects of variable maximum depth
232 of each trawl and mesh size differences between the two trawl types. We conducted
233 all analyses in R, version 3.5.1 (R Core Team, 2018) and fit LMMs using the function
234 lme4 (Bates, Mächler, Bolker, & Walker, 2015). We also carried out standard ellipse
235 analysis in the R package SIBER (Jackson, Inger, Parnell, & Bearhop, 2011) to
236 determine the combined $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ niche space for the functional groups.

237 For both functional groupings we used morphological traits as proxies for activity
238 level feeding mode, such as fin and mantle morphology. First, we considered a 3-
239 level functional group (FG₃):

1. High activity: thick, muscular mantle with larger, firmer fins and where fin length is approximately 50% or greater than the dorsal mantle length (DML), based on species-level descriptions (Reid, 2016).

2. Medium activity: thick, muscular mantle with softer fins and fins are less than 50% of the DML, based on species-level descriptions (Reid, 2016), or absent entirely (muscular pelagic octopus).

3. Low activity: Thin, muscular mantle tissue, and small fin length relative to DML. All members of the family Cranchiidae we sampled fell within this community. They have large coelomic cavities containing ammonia that enables them to achieve neutral buoyancy (Voight, Pörtner, & O'Dor, 1995), and most likely use jet propulsion infrequently (Clarke, 1962). They also tend to be transparent, using counter illumination to reduce the risk of predation while less active, as well possessing the ability to deter predation by filling their body cavity with ink, hence being unpalatable.

We also considered a simpler 2-level functional grouping (FG₂) that compared the Cranchiidae (low activity) with all other cephalopods (medium-to-high activity). Thus, the medium-to-high activity group in FG₂ combined all individuals from the medium and high activity levels described for FG₃.

Amino acid compound specific nitrogen isotope analysis

In addition to bulk SIA, we used amino acid compound specific nitrogen isotope analysis (AA-CSIA) for several individuals from two species that represented the broadest activity level groupings (low and high, *Leachia pacificus* and *Abraliopsis gilchristi* respectively). Amino acid values of $\delta^{15}\text{N}$ are analysed in AA-CSIA to assess trophic position in a similar fashion to traditional stable isotope analysis, except that the basal resource $\delta^{15}\text{N}$ is also distinguished, meaning that it provides direct

estimates of trophic position without the requirement of separate baseline samples (Bradley et al., 2015; Pethybridge et al., 2018). AA-CSIA is increasingly recognised as a powerful tool in trophic ecology. It is particularly useful in situations where baselines are uncertain or difficult to quantify otherwise, as may occur with ontogenetic changes in habitat (Pethybridge et al., 2018), as well as overcoming the challenges of using chitinous tissue from cephalopod beaks (Cherel, Bustamante, & Richard, 2019)

We prepared and analysed samples for AA-CSIA as per the methods outlined in Dale et al. (2011). We calculated relative trophic position for AA-CSIA according to Bradley et al. (2015),

$$RTP = \frac{\delta^{15}N_{Tr} - \delta^{15}N_{Src} - \beta}{TEF} + 1 \quad (\text{Equation 3})$$

where $\delta^{15}N_{Tr}$ and $\delta^{15}N_{Src}$ are the nitrogen isotopic values for trophic and source amino acids, β is the isotopic offset between the trophic and source amino acid pair in primary producers and TEF is the trophic enrichment factor. The trophic amino acids measured were glutamic acid/glutamine (Glx) and threonine (Thr), while the source amino acids measured were lysine (Lys), phenylalanine (Phe) and serine (Ser). Cherel et al. (2019) provide detailed descriptions of these amino acids and the methods to estimate trophic position using AA-CSIA.

RESULTS

The cephalopod community contained 12 families and 22 species in the sub-sample we selected for bulk SIA. The size range of individuals sampled spanned 0.05 – 720.5 g, while the taxonomic diversity was matched by a wide range of $\delta^{15}N$ values, equivalent to almost two trophic positions based on $\delta^{15}N$ (Equation 2). The five most

abundant families were Cranchiidae, Enoploteuthidae, Pyroteuthidae, Ocythoidae
 and Brachioteuthidae (Table S1). All other families were represented in small
 numbers. Among the six most abundant species, increases in $\delta^{15}\text{N}$ with increasing
 body size were rare, with only one species, of the high activity level *Abraliopsis*
gilchristi, having a significant increase in $\delta^{15}\text{N}$ with body size (F-test, $F_{1,26} = 19.62$, p
 < 0.001 , Fig. 2). Significant changes in $\delta^{13}\text{C}$ with increasing body size were more
 common, with significant negative slopes for both Cranchiidae species, *Liguriella*
podophthalma and *Leachia pacificus*, and a positive slope for *A. gilchristi* ($p < 0.01$
 for all three, Fig. 2). The 2 species from each level of the FG₃ functional group (*L.*
podophthalma and *L. pacificus* = low, *Ocythoe tuberculata* and *Pterygioteuthis*
giardia = medium, and *Brachioteuthis picta* and *A. gilchristi* = high) appear to have
 similar within-group patterns of $\delta^{15}\text{N}$. Low activity level species have low initial $\delta^{15}\text{N}$
 values at small individual body size and do not increase through ontogeny. The
 medium activity group had a higher intercept for the $\delta^{15}\text{N}$ versus body weight
 relationships compared to the low activity group. However, both had a slope not
 significantly different to zero, representing no change through ontogeny, at least
 across the size range we sampled. Standard ellipse analysis showed that there is
 overlap between the 3 groups, but there are distinct characteristics between them.
 Medium and high activity level functional groups had a generally higher trophic
 position and narrower carbon niche space and the low activity functional group had
 lower trophic positions and a wider niche breadth in terms of carbon (Fig. 3).

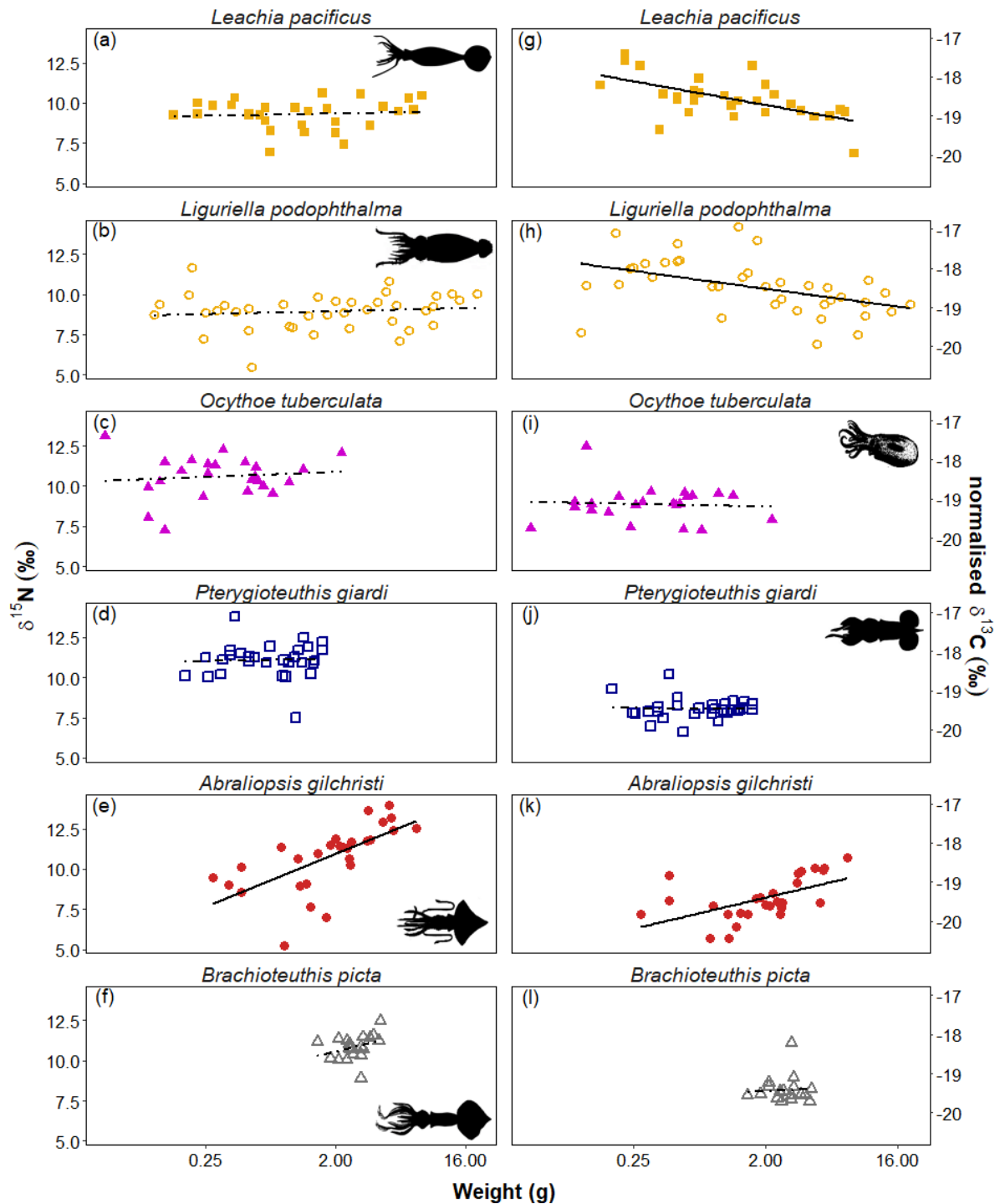
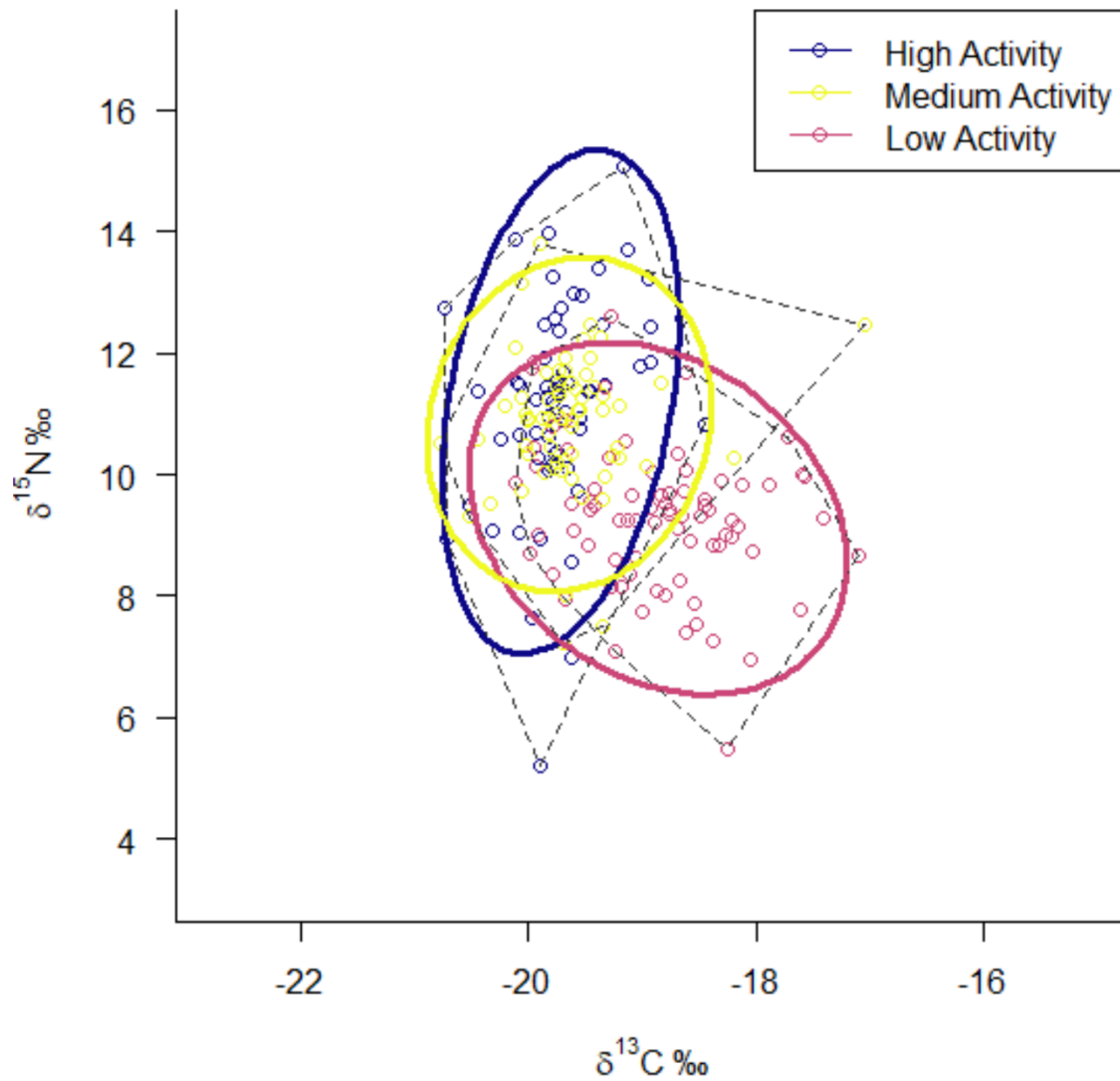


Figure 2: Species-specific trends in $\delta^{15}\text{N}$ (a-f) and normalised $\delta^{13}\text{C}$ (g-l) with increasing body weight, arranged by increasing activity level (low to high from top to bottom while within-group ordering is alphabetical). Significant linear relationships ($p < 0.05$) are presented as solid black lines, while non-significant are dot-dash lines (full linear regression equations and statistics in Table S2).



315

316 Figure 3: Stable isotope ellipses of $\delta^{15}\text{N}$ (‰) and $\delta^{13}\text{C}$ (‰) values for the 3-activity
 317 level-based functional groups. Standard ellipse areas corrected for small sample
 318 sizes (SEAc) (solid lines) represent the core isotopic niche of each functional group,
 319 while the convex hulls of overall niche diversity (dotted lines) encompass all data
 320 points.

321 Trophic position of individuals within the cephalopod community from the western
 322 Tasman Sea eddy was best explained using a model that included the functional
 323 grouping with 3 activity levels (FG_3), body weight and an interaction between
 324 grouping and body weight (marginal $R^2 = 0.43$, conditional $R^2 = 0.45$, Table 1, Fig.

4). The second-best model, which included a 2- rather than 3-level functional grouping, was significantly less informative based on the difference in the Akaike Information Criterion ($\Delta AIC > 8$; Table 1). These results suggest that quantifying activity, in addition to body weight, is informative when estimating trophic position and predicting dynamics of cephalopod communities. The need for an interaction implies that the mechanistic feedbacks between body size and activity on trophic position are likely complex.

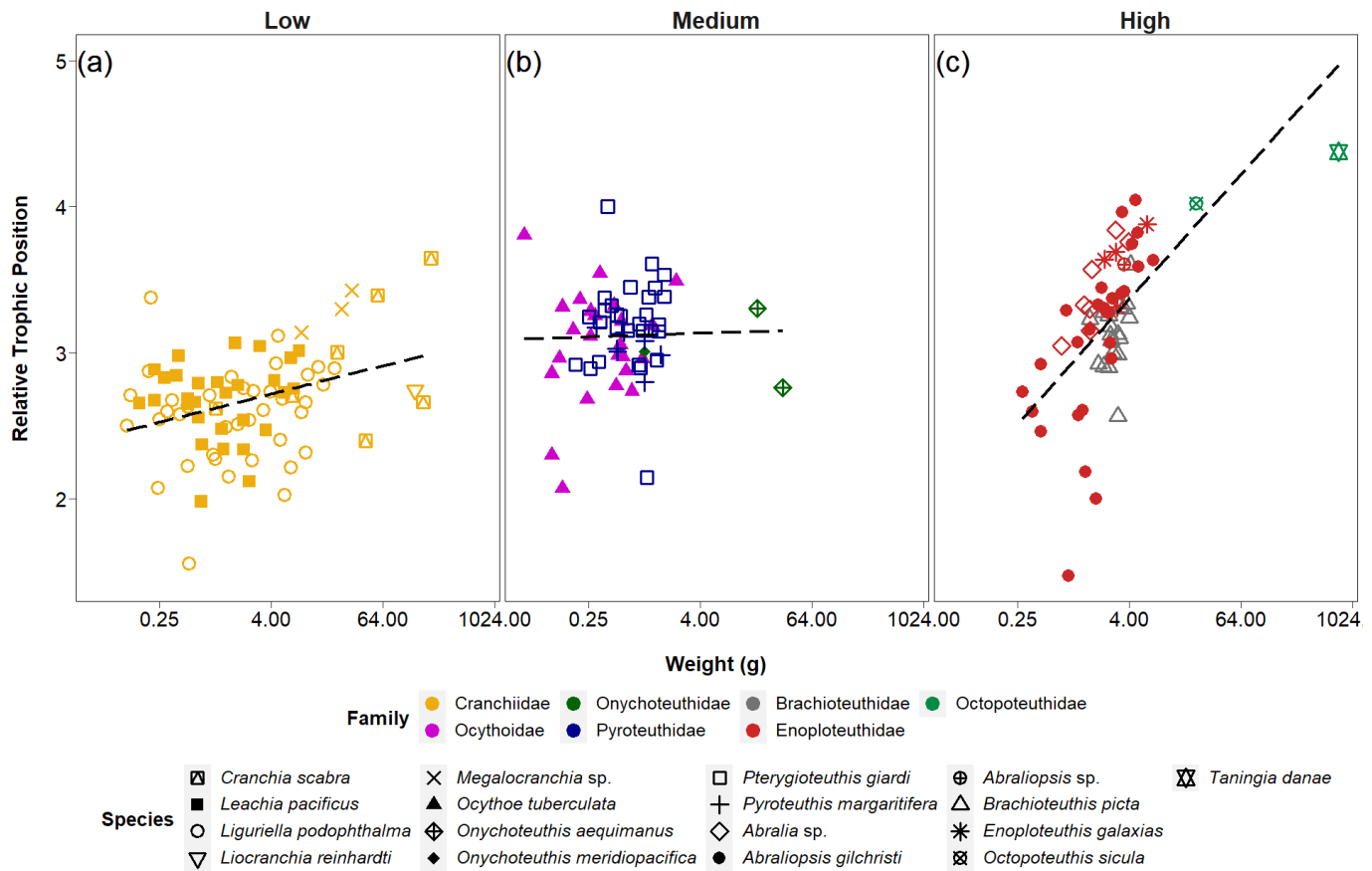


Figure 4: Changes in relative trophic position with increasing body weight for the functional groups within the 3-activity level grouping (FG_3): (a) low; (b) medium; and (c) high. Note that the lower resolution functional group (FG_2) merges the medium and high activity level groups, (b) and (c), into one. Species are represented by

337 shapes and families by colour (see figure legend). Regression lines are fitted from
 338 the best model, $FG_3 * Weight$ (Table 1).

339 Table 1: Ranking of candidate models considered for predicting cephalopod relative
 340 trophic position (RTP). The model predictor variables considered (i.e. fixed effects)
 341 were taxonomic group (Family), a functional grouping with 3 activity levels (FG_3), a
 342 functional grouping with 2 activity levels (FG_2), and log transformed individual body
 343 weight (Weight). K is the number of estimated parameters. * Note that all models
 344 included a random effect of trawl (i.e., + (1|Trawl) has been removed from the model
 345 formulation for clarity).

Model Formulation*	K	ΔAIC
RTP ~ $FG_3 + Weight + FG_3:Weight$	8	0
RTP ~ $FG_2 + Weight$	5	9.55
RTP ~ $FG_2 + Weight + FG_2:Weight$	6	13.20
RTP ~ Family + Weight + Family:Weight	16	13.85
RTP ~ $FG_3 + Weight$	6	14.68
RTP ~ Family + Weight	10	20.21
RTP ~ FG_2	4	27.55
RTP ~ FG_3	5	30.90
RTP ~ Family	9	31.09
RTP ~ 1	3	77.20
RTP ~ Weight	4	77.45

346

347 The source amino acids, Lys and Phe, both had consistent values of $\delta^{15}\text{N}$ across the
348 body weight range of *L. pacificus* (F-test, $F_{1,9} = 0.16$, $p = 0.696$), whereas they both
349 displayed increasing trends across the body weight range of *A. gilchristi* individuals
350 (F-test, $F_{1,4} = 9.33$, $p = 0.046$) (Fig. 5). The consistent source amino acid values
351 indicate that the prey of *L. pacificus* individuals, regardless of body weight, come
352 from the same location. *Abraliopsis gilchristi* individuals, however, have a diet that
353 changes as they get bigger, supported by the increasing source amino acid values
354 with size, although the slope is dependent upon the nitrogen stable isotope type
355 used to calculate relative trophic position (F-test, $F_{1,5} = 46.85$, $p < 0.001$). These
356 ontogenetic changes in relative trophic position are probably due to changes in prey
357 size, geographic region, or depth. AA-CSIA indicated that *A. gilchristi* individuals
358 (which are in the high activity group) had higher trophic positions than *L. pacificus*
359 individuals (which are in the low activity level functional group), regardless of body
360 weight (Fig. 5). This differs to the findings from the bulk SIA, which suggest that the
361 smallest *A. gilchristi* individuals had a comparable relative trophic position to *L.*
362 *pacificus* individuals (Fig. 2). For *L. pacificus*, the AA-CSIA and bulk SIA provide very
363 similar relative trophic position values and suggest there is no ontogenetic change in
364 trophic position (F-test, $F_{1,15} = 0.75$, $p = 0.398$). In contrast, relative trophic position
365 derived from AA-CSIA are consistently higher than bulk SIA results for *A. gilchristi*,
366 while the rate of increase with increasing body size is not as high from the AA-CSIA,
367 primarily due to a higher initial relative trophic position.

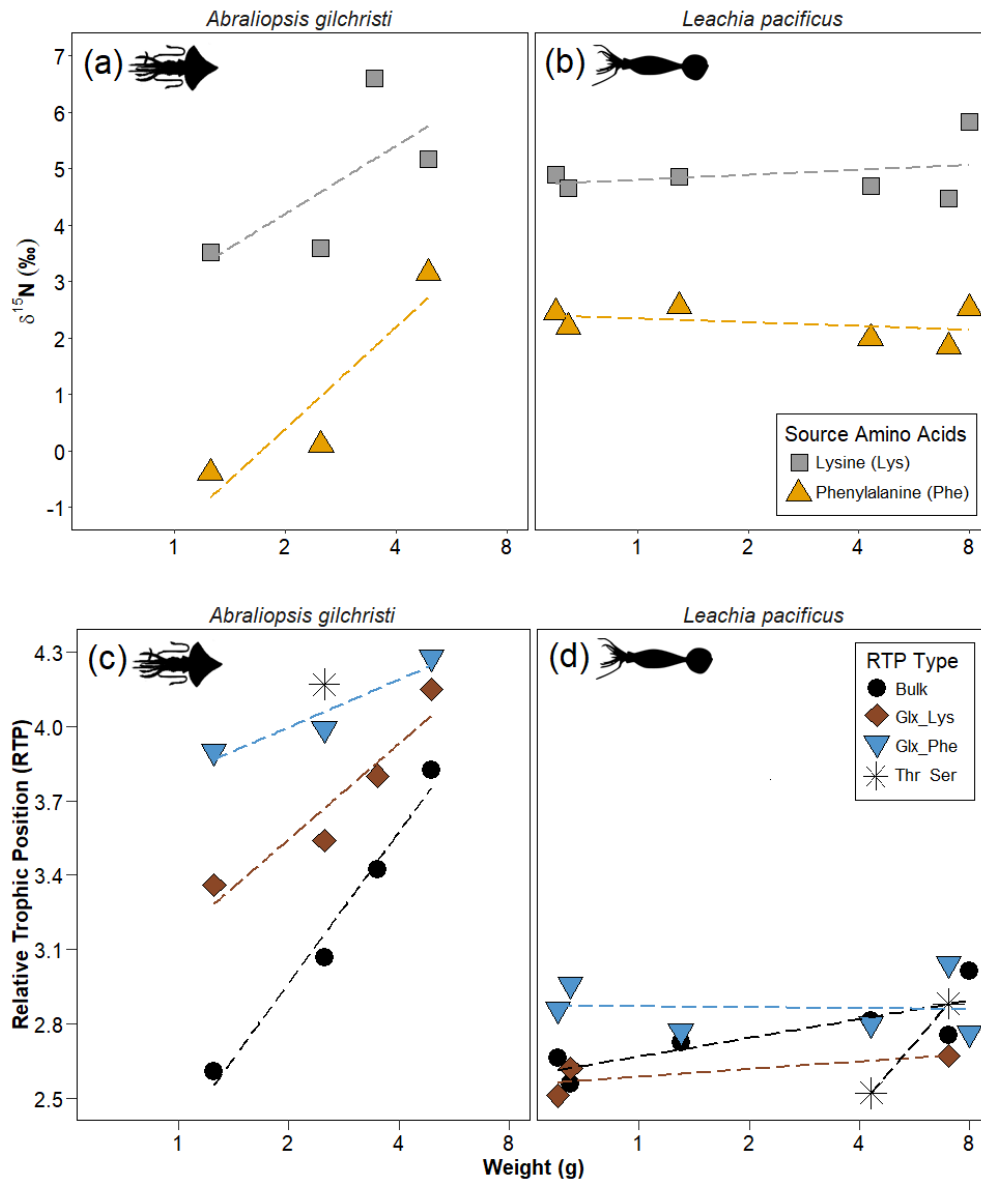


Figure 5: Source amino acid $\delta^{15}\text{N}$ values (a - b) and relative trophic position results from AA-CSIA alongside bulk SIA (c - d) presented against wet weight (g on log₂ scale) for the subset of *Abraliopsis gilchristi* (n = 4) and *Leachia pacificus* (n = 6) individuals, respectively.

DISCUSSION

This is the first study to present the trophic allometries of a spatially and temporally quantifiable oceanic cephalopod community using muscle tissue samples.

Previously, studies have used beaks sampled from predator stomach contents to

378 assess trophic structure of communities using species' mean body sizes (e.g.
379 Cherel, Ridoux, Spitz, & Richard, 2009). Here, variability in the relationship between
380 trophic position versus individual body size for cephalopod species was evident, with
381 some species and families exhibiting strong ontogenetic trophic position shifts, while
382 others did not shift significantly across the size ranges observed (0.05 – 720.5 g).
383 The examples of weak-to-no trophic position shift with increasing body size was
384 particularly unexpected (Jennings et al., 2001).

385 Variation in trophic allometries across species was best explained by aggregating
386 species into 3 functional groups based on a single functional trait: activity level. This
387 finding is slightly unexpected, as previous work has suggested that diverse
388 morphological traits related to feeding and the ability to feed on large prey compared
389 to a predator's own body size can lead to variable feeding modes that may require
390 substantial group- or taxonomic-specific information (Nakazawa, Ohba, & Ushio,
391 2013; Villanueva et al., 2017). Interestingly, 2 of the 3 activity level groups did not
392 conform with the traditional assumption that trophic position increases with body size
393 (Jennings et al., 2001). This finding not only deviates from the standard assumptions
394 applied within size-structured ecosystem models (Andersen et al., 2016; Blanchard
395 et al., 2009), it also differs from the general description of cephalopods as voracious
396 predators.

397 Cranchiidae have been similarly found to exhibit no ontogenetic trophic shift in other
398 locations too, across a similar size range, suggesting that this could be consistent
399 generally (Staudinger et al., 2019). Low activity level cephalopods such as
400 Cranchiidae (which are among the most globally abundant cephalopods), have low
401 trophic positions. They likely feed predominantly on zooplankton, and as an ambush
402 predator, they have low metabolic demands (Seibel, 2007). This distinct functional

403 role is likely to have differing effects on the energy distribution and flow in marine
404 food webs compared to the more stereotypical 'voracious' active cephalopods. How
405 these patterns extend into the much larger size classes is still somewhat unclear.
406 There are assessments of the trophic positions of very large cephalopods, such as
407 the colossal and giant squid, suggesting they feed primarily on zooplanktivores like
408 myctophids (Cherel et al., 2019). Given the colossal squid is a member of the
409 Cranchiidae, the same family that makes up the low activity level functional group
410 here, it perhaps indicates that even though they can reach large sizes (hundreds of
411 kilograms), they do not exhibit large ontogenetic trophic shifts, but instead
412 undergoing a more moderate shift from zooplankton to small fish in comparison to
413 cephalopod species that undergo larger ontogenetic trophic shifts. This is perhaps
414 highlighted by a squid species we designated as having a high activity level in our
415 study, *Tanigia danae*, which had a higher trophic position than the colossal squid in
416 Cherel et al. (2019), despite being less than half the size on average as the colossal
417 squid. This suggests that the steeper trophic allometry found for the high activity
418 group here broadly holds when extended into larger size classes.

419 Large ontogenetic increases in trophic position, such as those seen in the high
420 activity cephalopods, could be partially accounted for by unique predation behaviour
421 observed in some squid that can prey upon large individuals bigger than themselves.
422 Gape limitation is not as constricting for most cephalopods compared to fish and the
423 use of tentacles and arms particularly enhances cephalopods' ability to feed closer to
424 their own body size (Rodhouse & Nigmatullin, 1996). For example, *Gonatus onyx*
425 have been recorded regularly feeding on prey the same size as themselves,
426 resulting in low predator-prey mass ratio values, as well as exhibiting high levels of
427 cannibalism (Hoving & Robison, 2016). It is also possible that juvenile and small

cephalopods can feed closer to their adult stage preferred predator-prey mass ratio, due to enhanced predation capability and reduced gape limitation at smaller sizes when compared to strictly gape-limited species (Villanueva et al., 2017). This could explain why low activity level cephalopods do not increase in trophic position as they grow, as they are able to feed close to their adult preferred prey size even when they are juveniles. It is possible that low activity level cephalopods may eat larger individuals as they grow while maintaining a similar trophic position throughout growth, if they prey largely upon herbivorous zooplankton. If this were the case, the shift in prey size would not be matched with a nitrogen stable isotope enrichment, as most zooplankton of this type would be approximately the same trophic position (Henschke et al., 2015). Even if this is the case, it still suggests that this group have a diet mostly composed of zooplankton and they do not shift to higher levels of piscivory, as is commonly reported for squid species, at least until they are much larger (Cherel, Fontaine, et al., 2009; Hunsicker & Essington, 2006; Rodhouse & Nigmatullin, 1996).

Variation in cephalopod trophic position not explained by functional traits here could partially be accounted for by variable prey availability, for example due to seasonality and changes across the wide range of depths sampled in this study. The trophic allometries presented here do not account for differences that may have occurred due to changes in the prey basal source with depth. Deep sea fish communities have been shown to vary in both their trait composition and trophic allometries with depth (Mindel, Neat, Trueman, Webb, & Blanchard, 2016; Trueman, Johnston, O’Hea, & MacKenzie, 2014). Allometric scaling of predator-prey mass ratios can also be influenced by prey availability and can become more size dependent as prey availability decreases, at least in terrestrial ecosystems (Costa-Pereira, Araújo,

Olivier, Souza, & Rudolf, 2018). This could be the case for deeper oceanic communities where energy availability, particularly from smaller prey, is more limited and would require further study (Mindel, Webb, Neat, & Blanchard, 2016). Despite 3 species having significant ontogenetic changes in bulk carbon isotope values, the AA-CSIA results for two of these species confirmed that the significant trophic shift, or lack thereof for the low activity level functional group, are robust to these changes in carbon isotope values with size. It is possible that the low activity level species that had a change in carbon isotope values with size are undergoing ontogenetic descent which may result in this change. For the high activity level species, *A. gilchristi*, an ontogenetic change in prey type could explain the changing carbon isotope values with size. There are still questions about the broad applicability of this approach to cephalopod trophic allometries. For example, more complete coverage of families within each functional group would help to assess the generality of these traits in explaining trophic allometries. In our case, the low activity level functional group only contains organisms of family Cranchiidae (capturing 4 spp.), but other families are known to exhibit similar trophic allometries (such as *Vampyroteuthis infernalis*, Golikov et al. 2019). An alternative taxonomy-based approach to explain trophic allometries could have been used, but we found a model that used “family” as a predictor was substantially weaker (Table 1).

The dominant members of this cephalopod community are abundant in the temperate and tropical global oceans (Bower et al., 1999; Brodeur & Yamamura, 2005; Judkins, Vecchione, Cook, & Sutton, 2017; Lischka, Piatkowski, & Hanel, 2017; Reid, 2016; Ruvalcaba-Aroche et al., 2018; Voss, 1980) and broadly represent the diversity of activity levels exhibited by cephalopods in oceanic ecosystems. To further validate and expand the applicability of this functional approach of community

478 trophic structure will require testing in additional ecosystem types. Oceanic
479 ecosystems, such as the one we studied, may require different functional groupings
480 to coastal ecosystems, where benthic dominated groups such as octopuses and
481 cuttlefish are more prominent. The coupling of benthic and pelagic environments in
482 oceanic and coastal ecosystems also needs consideration, as functional roles could
483 vary depending on factors such as ontogenetic descent, spatial and temporal shifts
484 in diet, as well as ontogenetic changes in morphology (Fernández-Álvarez,
485 Machordom, García-Jiménez, Salinas-Zavala, & Villanueva, 2018; Golikov et al.,
486 2019). Exploring the consequences and importance of different functional grouping
487 resolution will be key moving forward.

488 There is growing appreciation of the important role cephalopods have in the transfer
489 of energy from low-mid trophic positions to large fish, marine mammals, and
490 seabirds (Cherel, Hobson, Guinet, & Vanpe, 2007; Hunsicker et al., 2010;
491 McCormack, Melbourne-Thomas, Trebilco, Blanchard, & Constable, 2020),
492 particularly with the trend of increasing abundance of cephalopods (Doubleday et al.,
493 2016). This work provides a starting point to explore how trait-based cephalopod
494 functional groups influence ecosystem processes and enables us to ask key
495 questions about their impacts on trophic transfer efficiency, size structure, and
496 biomass residency time of marine ecosystems. The high growth efficiency of
497 cephalopods (Boyle & Rodhouse, 2008), combined with their increasing abundance,
498 could increase the trophic transfer efficiency of ecosystems where cephalopods are
499 abundant. This will be particularly true in areas where cephalopods are able to 'fill
500 the void' left by fishing, where their fish predators and competitors are reduced.

501 **CONCLUSIONS**

We show that trophic positions of oceanic cephalopods are variable but can be described by a combination of activity level and body size. It is a misconception that all cephalopods are 'voracious predators'. Oceanic cephalopods with lower activity levels maintain low trophic positions throughout their body size range whereas other more active cephalopod species exhibit steep ontogenetic shifts with higher trophic positions at larger body sizes. This means that not all cephalopods occupy the same role in the ecosystem and thus should not be characterised in the same way within ecosystem models. The most abundant species in this cephalopod community are broadly representative of the traits commonly observed within their respective families, which in turn are represented in circumglobal subtropical and temperate ecosystems. Future work needs to be carried out to assess the generality of these different trophic allometries for high versus low activity level cephalopod groups. Representing cephalopods via their traits within size-structured models and accurately representing their distinct trophic roles will further improve the understanding of marine ecosystem structure and function. This will facilitate model estimates of cephalopod abundance in the changing global ocean, as well as improving assessment of anthropogenic implications for marine ecosystems.

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AUTHORS' CONTRIBUTIONS

KJM, GTP, JMS, and JLB conceived the study. KJM led the writing of the manuscript. KJM and IMS collected the data. KJM performed cephalopod identification and sample preparation for bulk SIA. ATR performed AA-CSIA. KJM and SAR analysed the data. All authors contributed to aspects of the methodological design and substantially contributed to the critical assessment of drafts and gave final approval for publication.

CONFLICT OF INTERESTS

The authors report no conflict of interests.

DATA AVAILABILITY STATEMENT

800 Data supporting this study are available from a GitHub repository archived using
801 Zenodo: <https://doi.org/10.5281/zenodo.3980411> (Murphy et al., 2020).

802 **ORCID**

803 *Kieran J. Murphy*  <https://orcid.org/0000-0002-9697-2458>

804 *Gretta T. Pecl*  <https://orcid.org/0000-0003-0192-4339>

805 *Shane A. Richards*  <https://orcid.org/0000-0002-9638-5827>

806 *Jayson M. Semmens*  <https://orcid.org/0000-0003-1742-6692>

807 *Andrew T. Reville*  <https://orcid.org/0000-0003-2486-5976>

808 *Iain M. Suthers*  <https://orcid.org/0000-0002-9340-7461>

809 *Jason D. Everett*  <https://orcid.org/0000-0002-6681-8054>

810 *Rowan Trebilco*  <https://orcid.org/0000-0001-9712-8016>

811 *Julia L. Blanchard*  <https://orcid.org/0000-0003-0532-4824>

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