

# Food spectrum and trophic position of an Arctic cephalopod, *Rossia palpebrosa* (Sepiolida), inferred by stomach contents and stable isotope ( $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ) analyses

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**ABSTRACT:** *Rossia palpebrosa* (Sepiolida) is the most abundant nekto-benthic cephalopod in the Arctic; however, its feeding and trophic ecology are largely unknown. This work aims to assess the role of this species in Arctic ecosystems based on the contents of its stomach and analyses of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotopes in its beak. The main taxa identified in the food spectrum were Crustacea (frequency of occurrence: 52.1%), followed by Polychaeta (14.6%) and fishes (6.3%). Sipuncula and Echinoidea were occasionally found and were recorded here as *R. palpebrosa* prey for the first time, as well as Polychaeta and Euphausiacea. A significant geographic increase in  $\delta^{13}\text{C}$  values (mean  $\pm$  SE,  $-19.3 \pm 0.2\text{‰}$ ) from the Barents Sea to West Greenland was found, but no significant ontogenetic increase, suggesting no migrations occurred among different water masses. Values of  $\delta^{15}\text{N}$  ( $8.7 \pm 0.2\text{‰}$ ) and trophic level (TL;  $3.6 \pm 0.1$ ) revealed significant ontogenetic increases and an absence of geographic patterns, suggesting the trophic role of this species is similar throughout the studied part of the Arctic. Stable isotope values, TL and food spectrum for *R. palpebrosa* are close to Arctic nekto-benthic predatory fishes and shrimps, especially *Pandalus borealis*. However, sepiolids prey on organisms exceeding their own size and do not scavenge. A gradual ontogenetic decrease in isotopic niche width, while increasing diversity in the food spectrum of larger specimens, was observed in *R. palpebrosa*. However,  $\delta^{13}\text{C}$  values, i.e. variation in primary productivity supporting food sources, were more responsible for these ontogenetic differences in niche size than  $\delta^{15}\text{N}$  values.

**KEY WORDS:** Bobtail squid · Arctic marine food webs · Trophic ecology · Feeding · Ecological niche · Competition · *Pandalus borealis* · Northern shrimp

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## 1. INTRODUCTION

Cephalopods are important components of marine ecosystems worldwide with roles as both prey and

predators (Rodhouse & Nigmatullin 1996, Boyle & Rodhouse 2005), but they remain largely understudied, especially within polar ecosystems (Golikov et al. 2013a, Xavier et al. 2018). Ten species of

cephalopods live permanently in the Arctic (Golikov et al. 2013a, Xavier et al. 2018), but only the pelagic squid species, *Gonatus fabricii*, is relatively well-studied (reviews: Nesis 1987, Roper et al. 2010). Regarding nekto-benthic species, i.e. sepiolids (bobtail squids) of the genus *Rossia*, only scarce information on their distribution, morphology and biology is available (Mercer 1968, Nesis 1987, Golikov et al. 2013b, Golikov 2015). *Rossia palpebrosa* Owen, 1834 is amongst the most abundant bottom-living cephalopod species in the Arctic (Nesis 1987, Golikov et al. 2017), having a vast, probably circumpolar, distribution in the Arctic and reaching South Carolina and the North Sea to the south (Nesis 1987, Golikov et al. 2013b, Golikov 2015). The maximum mantle length (ML) of *Rossia palpebrosa* is up to 58 mm (Golikov et al. 2013b, Golikov 2015). Despite its abundance, nothing is known regarding the trophic position of *R. palpebrosa*, and the stomach contents of this species have been studied only once, in the Canadian Arctic (Mercer 1968) (see Table S1 in the Supplement at [www.int-res.com/articles/suppl/m632p131\\_supp.pdf](http://www.int-res.com/articles/suppl/m632p131_supp.pdf)). Different forms of unknown taxonomic status or proposed subspecies have been distinguished in *R. palpebrosa* by some authors (e.g. Akimushkin 1965, Nesis 1987). It has even been suggested (e.g. Verrill 1881, Grimpe 1933) that 2 very similar species exist: *R. glaucopsis* Loven, 1845 and *R. palpebrosa* Owen, 1834. Nonetheless, our preliminary morphological (Golikov et al. 2013b, Golikov 2015) and molecular (Morov et al. 2011) data, as well as other work regarding species morphology (Mercer 1968), suggest that *R. palpebrosa* exists as one single species.

The stomach contents of cephalopods, in general, have been studied for a long time (reviews: Rodhouse & Nigmatullin 1996, Boyle & Rodhouse 2005). Generally, an ontogenetic shift in the diet of cephalopods towards larger prey means that their trophic level (TL) increases with growth. This is accompanied by an increase in their food spectrum diversity (review: Rodhouse & Nigmatullin 1996). Recently, this has been observed for several cephalopod species using stable isotope analyses (Cherel & Hobson 2005, Cherel et al. 2008, Guerra et al. 2010, Trasviña-Carrillo et al. 2018), including the Arctic squid *G. fabricii*, in which its important role in the Arctic was revealed using stable isotope analysis (Golikov et al. 2018). Stable isotopes of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are the most commonly used for ecological studies in marine environments:  $\delta^{13}\text{C}$  provides information on habitat and the identity of primary carbon sources, and  $\delta^{15}\text{N}$  provides information regarding the role of a species within an ecosystem

and its respective TL (reviews: Post 2002, Boecklen et al. 2011, Layman et al. 2012).

In this study, we analyzed *R. palpebrosa* stomach contents from the Barents Sea and analyzed beak stable isotope signatures from West Greenland and the Barents Sea (1 specimen from East Greenland was analyzed as well). The main goals of this study were to assess the role of *R. palpebrosa* in the Arctic marine ecosystems by classical and novel methods of niche analysis while reviewing the records of previously established prey and predators of *R. palpebrosa* (Table S1). Specifically, we expected: (1) food spectrum to increase with growth; (2) ontogenetic increases of  $\delta^{15}\text{N}$  and TL; (3) overall lower  $\delta^{15}\text{N}$  values and TL than Arctic squid and pelagic predatory fishes, as those groups are positioned higher in the Arctic trophic web; and (4)  $\delta^{13}\text{C}$  values to be higher than in the pelagic Arctic squid, as *R. palpebrosa* is a nekto-benthic species. Depth-related trends in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values have been found in some groups of benthos, either ecological or taxonomical, both within and outside the Arctic (Mintenbeck et al. 2007, Bergmann et al. 2009, Lin et al. 2014, Divine et al. 2015, Bell et al. 2016, Stasko et al. 2018). Thus, depth-related trends were analyzed for *R. palpebrosa* to test these assumptions.

## 2. MATERIALS AND METHODS

### 2.1. Study area and samples

Material was sampled in Greenland waters by the RV 'Paamiut' (Greenland Institute of Natural Resources) and in the Barents Sea by the RV 'Vilnius' and the RV 'Dalnie Zelentsy' (Polar Branch of All-Russian Research Institute of Fisheries and Oceanography and Murmansk Marine Biological Institute, respectively). Samples were collected during July and August between 2011 and 2017 (Table 1, Fig. 1). As *Rossia palpebrosa* is known to grow continuously throughout its life cycle, and its size at maturity is known to be highly variable (Golikov et al. 2013b, Golikov 2015), specimens were categorized into 3 arbitrary ontogenetic size groups: ML < 21 mm (small), ML 21 to 40 mm (medium) and ML > 41 mm (large), corresponding roughly to immature, maturing and mature specimens, respectively. Six specimens from each size group (preferably 3 females and 3 males) were randomly chosen for stable isotope analyses from both West Greenland and the Barents Sea, overall being  $n = 18$  from the Barents Sea and  $n = 18$  from West Greenland, with 12 small, 12 medium

Table 1. Sample sizes (n), sampling year, area and mantle length (ML) of specimens of *Rossia palpebrosa* used for stomach content and stable isotope analyses. In addition, 1 female from East Greenland (ML: 31 mm; sampled in 2016) was included in the stable isotope analysis

	Stomach contents analysis			Stable isotope analysis					
	n	Sampling year	ML (mm)	West Greenland			Barents Sea		
				n	Sampling year	ML (mm)	n	Sampling year	ML (mm)
All	48	2011–2017	11–49 (30.1 ± 1.6)	18	2016	10–56 (31.3 ± 3.5)	18	2011–2017	12–48 (29.6 ± 3.0)
Females	25	2011–2017	16–48 (31.7 ± 2.1)	9	2016	10–56 (33.6 ± 5.3)	10	2011–2017	13–45 (31.6 ± 4.2)
Males	23	2011–2017	11–49 (28.4 ± 2.4)	9	2016	10–46 (29.0 ± 4.8)	8	2011–2017	12–48 (27.1 ± 4.6)

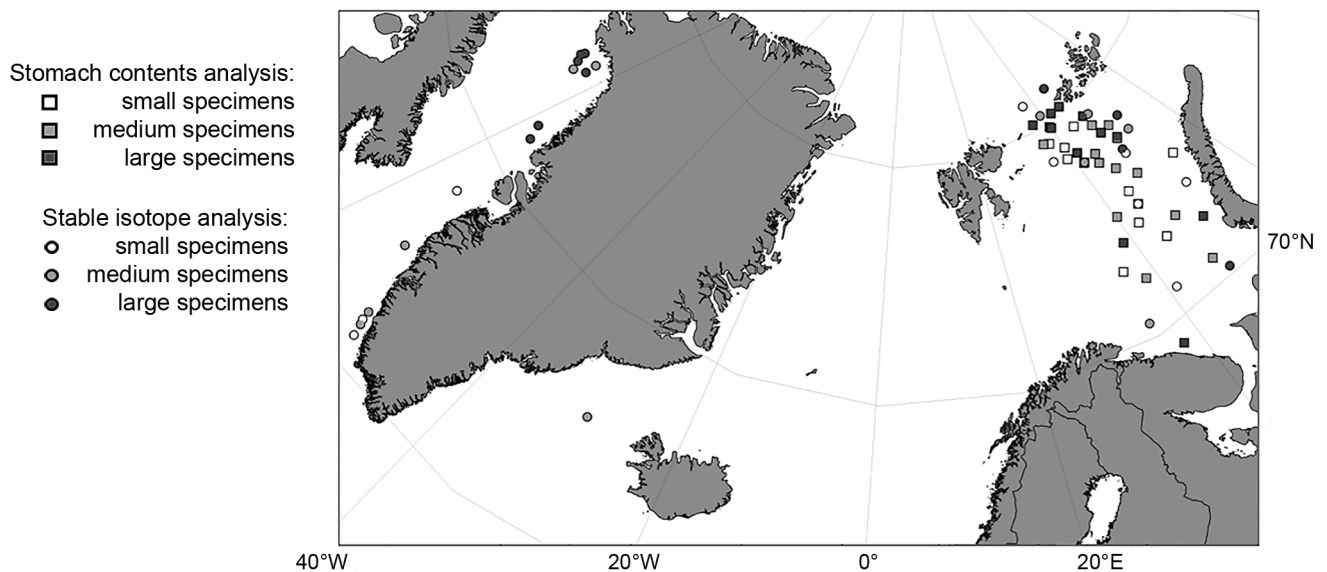


Fig. 1. Sampling locations of *Rossia palpebrosa* in west Greenland, east Greenland and the Barents Sea

and 12 large specimens (Table 1). The sample size is adequate for comparisons of isotopic niches between sexes and areas and among ontogenetic groups, according to Syväranta et al. (2013). Additionally, 1 medium female from East Greenland was available for analysis. The specimens collected in the Barents Sea were used for stomach contents analysis ( $n = 48$ ), and these were randomly selected among all available specimens corresponding to each size group (Table 1).

Specimens were fixed in formalin onboard. Mantle length was measured, and sex and maturity stage were assessed in fixed specimens onshore. Then, the stomachs were taken from the specimens selected for stomach contents analysis ( $n = 48$ ), and lower beaks were taken and their rostrums measured ( $n = 37$ ) from the specimens selected for stable isotope analysis.

## 2.2. Stomach contents analysis

This study aimed to identify the diet composition qualitatively (for comparison with stable isotope data); therefore, the stomach contents of the specimens were not weighed. The caecum, crop and esophagus contents were not used in the study, as they often contain parts eaten in the trawls (Breiby & Jobling 1985, Rodhouse & Nigmatullin 1996), and caecum contents were too digested to be of use (Bidder 1966, A. V. Golikov pers. obs.). All prey fragments found in stomachs were identified, counted and measured when possible. Jelly mass (not prey remains) was found in the majority of stomachs (91.7%; see Section 3.1); thus, stomachs having jelly mass inside and without any prey material were considered empty.

The frequency of occurrence ( $F$ , %) was calculated for all identified prey groups (in relation to the total

number of stomachs examined), using the equation outlined by Breiby & Jobling (1985) and Watanabe et al. (2004) as follows:

$$F_i = \frac{\text{n of stomachs with prey object } i \text{ inside}}{\text{total n of studied stomachs}} \quad (1)$$

Empty stomachs were included in the calculation. The ratio ( $Cn$ , %) was calculated for all identified prey groups (in relation to the total number of prey objects identified), following Breiby & Jobling (1985) and Watanabe et al. (2004):

$$Cn_i = \frac{\text{total n of prey object } i}{\text{total n of prey objects identified in stomachs}} \quad (2)$$

Cumulative prey curve analysis was used to assess the adequacy of the sample size in describing the diet overall, with the order in which stomachs were examined being randomized 5 times and plotted against the cumulative number of new prey items (Ferry & Cailliet 1996).

### 2.3. Stable isotope analysis

The lower beaks were chosen for stable isotope analysis, as they have been used in related studies (Cherel & Hobson 2005, Cherel et al. 2008, Guerreiro et al. 2015, Golikov et al. 2018) and are more often found in the stomach contents of predators (Xavier et al. 2007). The stable isotope analyses were carried out at the Centro de Ciências do Mar e do Ambiente – University of Coimbra (Portugal). Transparent areas of the beaks were cut off before proceeding with stable isotope analyses, as they have different isotopic concentrations (Cherel et al. 2009b). The beaks were dried at 60°C and ground into a fine powder using a mill (Mixed Mill MM 400). Powder sub-samples were weighed (~0.3 mg) with a micro balance scale and sterile-packed in tin containers. Stable isotope values were determined by a Flash EA 1112 series elemental analyzer coupled online via a Finnigan ConFlo II interface to a Delta VS mass spectrometer (Thermo Scientific) and expressed as  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ , where  $R = {}^{13}\text{C}/{}^{12}\text{C}$  and  ${}^{15}\text{N}/{}^{14}\text{N}$ , respectively. The carbon and nitrogen isotope ratios were expressed in delta ( $\delta$ ) notation relative to Vienna-PDB limestone (V-PDB) for  $\delta^{13}\text{C}$  and atmospheric nitrogen (Air) for  $\delta^{15}\text{N}$ . Replicate measurements of internal laboratory standards (acetanilide STD: Thermo Scientific PN 338 36700) in every batch ( $n = 14$ ) indicated precision  $< 0.2\%$  for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values). The mass C:N ratio of all samples was  $3.33 \pm 0.05$  (mean  $\pm$  SE).

### 2.4. Data analysis

Dispersion analysis was used to assess differences among ontogenetic groups, geographical areas and between sexes; groups were compared using the non-parametric Kruskal-Wallis  $H$  and Mann-Whitney  $U$ -tests (Zar 2010). Regression analysis was used to find equations fitting our data. Frequency tables of stomach contents analysis were checked with Fisher's exact test (Zar 2010) and Fisher's angular transformation ( $\phi$ ) (Ly et al. 2017) to assess differences in the food spectrum among size groups. These 2 tests are best suitable for small sample sizes with many empty table cells than the commonly used chi-squared analysis ( $\chi^2$ ) (Zar 2010, Ly et al. 2017). All tests were performed using a significance value of  $\alpha = 0.05$ .

Fixation in either formalin or ethanol does not significantly affect  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in cephalopod beaks (Ruiz-Cooley et al. 2011). Values of  $\delta^{15}\text{N}$  in cephalopod beaks, in contrast to  $\delta^{13}\text{C}$  values, are typically from 3.1‰ to 6.1‰ (mean: 4.8‰) lower than values from muscle tissue (Cherel & Hobson 2005, Hobson & Cherel 2006, Cherel et al. 2008, Ruiz-Cooley et al. 2011). Therefore, muscle  $\delta^{15}\text{N}$  values obtained from the literature for comparison with our data were corrected by subtracting 4.8‰. However, when estimating TL, this value was added to raw beak  $\delta^{15}\text{N}$  values, as proposed by Cherel et al. (2008) and Golikov et al. (2018).

An equation from Hussey et al. (2014a,b) with scaled trophic enrichment factor (TEF), adapted to be used in the Arctic by Linnebjerg et al. (2016), was chosen to estimate TL as the most up-to-date approach (cf. 'classical' TEF of 3.4‰ worldwide in Post [2002] or 'Arctic' TEF of 3.8‰ [Hobson et al. 2002]). Reference values for TL = 2.0, as required for the equation, were  $\delta^{15}\text{N}$  of 7.92‰ in Greenland (i.e. mean value of *Calanus finmarchicus*; Linnebjerg et al. 2016) and  $\delta^{15}\text{N}$  of 7.20‰ in the Barents Sea (i.e. mean value of *C. glacialis*; Søreide et al. 2013). Interpretation of TLs in the Arctic ecosystems followed recent stable isotope studies on the topic (Hobson et al. 2002, Tاملander et al. 2006, Søreide et al. 2013, Linnebjerg et al. 2016, Golikov et al. 2018).

The stable isotopic niche widths of different groups were analyzed using the recent metrics based on a Bayesian framework (Stable Isotope Bayesian Ellipses in R: SIBER; Jackson et al. 2011), which allows robust statistical comparisons. The standard ellipse area corrected for small sample sizes (SEAc, an ellipse that contains 40% of the data regardless of sample size) and the Layman metric of convex hull

area (TA) were estimated using the software package SIBER in R v.3.5.0 (Layman et al. 2007, Jackson et al. 2011, R Core Team 2018). The Bayesian approximation of the standard ellipse area (SEAb) was adopted to compare niche width among groups (i.e. p, the proportion of ellipses in one group that were lower than in another group; Jackson et al. 2011). Trophic levels were used instead of  $\delta^{15}\text{N}$  values (y-axis) in niche estimations. According to classical definition, a niche is a multivariate space with coordinates showing biometric and scenopoetic ecological information (i.e. habitat usage and trophic level) (Layman et al. 2007, Newsome et al. 2007). Thus, this approach improved the ecological meaning of the data: appropriate equations based on obtained and baseline values of  $\delta^{15}\text{N}$  to estimate TL are a tool to avoid biases when comparing specimens from different areas and ecosystems (e.g. Cherel et al. 2008, Linnebjerg et al. 2016).

Statistical analysis, calculations and plots were performed with Statistica 10.0 (Statsoft), PAST 3.15 (Hammer et al. 2001) and MS Excel 2010. Values are presented as mean  $\pm$  SE unless otherwise stated.

### 3. RESULTS

#### 3.1. Analysis of stomach contents

The main taxa in the food spectrum of *Rossia palpebrosa* were Crustacea ( $F = 52.1\%$ ,  $Cn = 65.8\%$ ), followed by Polychaeta ( $F = 14.6\%$ ,  $Cn = 18.4\%$ ) and fishes ( $F = 6.3\%$ ,  $Cn = 7.9\%$ ), while Sipuncula (2 stomachs,  $F = 4.2\%$ ,  $Cn = 5.3\%$ ) and Echinoidea (1 stom-

ach,  $F = 2.1\%$ ,  $Cn = 2.6\%$ ) were occasionally found (Table 2). Among the Crustacea, Decapoda (68.0% of all crustacean remains) was the most representative taxon, with the majority composed of shrimps (88.2% of all remains of Decapoda). Euphausiacea (20.0% of all crustacean remains) were also very common (Table 2). Overall, in 78.7% of stomachs with prey, we found remains of only 1 prey taxa or prey item. Stomachs were empty in 33.3% of studied specimens. In both empty stomachs and stomachs with prey remains, a jelly mass of yellow or orange color was found (in 91.7% of studied specimens). This jelly mass was not cnidarian remains because it was much harder and lacked cnidocytes or any other cells. It was present in larger quantities in specimens having bad fixation conditions (e.g. too little fixation liquid added onboard or being in bad shape prior to fixation already) or in those which had been fixed for a longer time. Thus, this jelly mass was likely to be digestive enzymes affected by fixation. Cumulative prey curve analysis showed the diet of *R. palpebrosa* was well described, as only 1 new prey item over 8.5 stomachs appeared after the 18<sup>th</sup> stomach, and the overall diet reached a stable level at the 35<sup>th</sup> stomach with  $n = 48$  (Fig. 2).

There were significant differences in the food spectrum of different sexes: females had significantly higher percentages of decapods, shrimps and *Pandalus* spp., while for males, the percentage of polychaetes was significantly higher (Table 3). However, Crustacea in general as well as Euphausiacea and other non-decapod crustaceans were of equal importance to both sexes (Table 3).

In terms of ontogenetic differences, small *R. palpebrosa* specimens consumed significantly lower quan-

Table 2. Frequency of occurrence ( $F$ , %) and ratio ( $Cn$ , %) of all identified prey groups, jelly mass and empty stomachs (only  $F$  for the last 2) in *Rossia palpebrosa* from the Barents Sea. -: not applicable. Stomachs with jelly mass and without any prey remains were considered empty (see Section 2.2)

Prey taxa	All (n = 48)		Small (n = 15): ML $\leq$ 20 mm		Medium (n = 19): ML 21–40 mm		Large (n = 14): ML > 40 mm	
	$F$	$Cn$	$F$	$Cn$	$F$	$Cn$	$F$	$Cn$
Crustacea	52.1	65.8	33.3	71.4	57.9	68.8	64.3	60.0
Decapoda	35.4	44.7	6.7	14.3	47.4	56.3	50.0	46.7
Caridea	31.3	39.5	6.7	14.3	36.8	43.8	50.0	46.7
<i>Pandalus</i> spp.	20.8	26.3	6.7	14.3	26.3	31.3	28.6	26.7
Other Crustacea	22.9	29.0	26.7	57.1	21.1	12.5	21.4	13.3
Euphausiacea	10.4	13.2	6.7	14.3	15.8	12.5	7.1	6.7
Polychaeta	14.6	18.4	6.7	14.3	15.8	18.8	21.4	20.0
Osteichthyes	6.3	7.9	0.0	0.0	5.3	6.3	14.3	13.3
Sipuncula	4.2	5.3	0.0	0.0	5.3	6.3	7.1	6.7
Echinoidea	2.1	2.6	6.7	14.3	0.0	0.0	0.0	0.0
Jelly mass	91.7	–	93.3	–	89.5	–	92.9	–
Empty stomachs	33.3	–	53.3	–	31.6	–	14.3	–

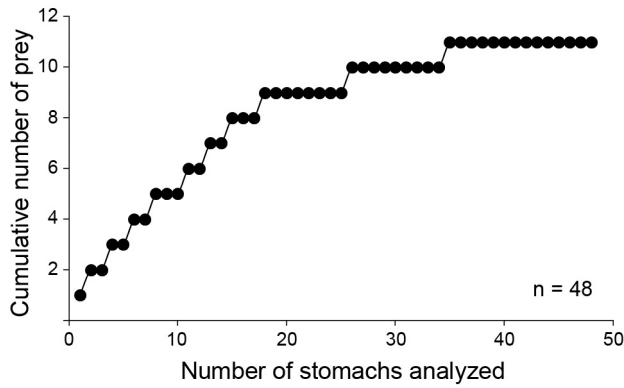


Fig. 2. Cumulative prey curve for total number of analyzed stomachs (order randomized 5 times) of *Rossia palpebrosa*

tities of decapods and shrimps than medium specimens and significantly lower quantities of crustaceans, decapods and shrimps than large specimens (Tables 2 & 3). No differences were found in the food spectra between medium and large specimens (Table 3). Non-decapod crustaceans (other than Euphausiacea) were the most important prey for small specimens. Euphausiacea were consumed mostly by medium specimens. Rare prey items such as fishes and sipunculids were found only in the medium and large size groups, while sea urchins were present in only 1 small specimen (Tables 2 & 3). Additionally, small specimens had a significantly higher percentage of empty stomachs than large specimens but not medium specimens (Tables 2 & 3).

### 3.2. Stable isotope analysis

Values of  $\delta^{13}\text{C}$  ranged from  $-21.6$  to  $-17.0\text{‰}$  ( $-19.3 \pm 0.2\text{‰}$ ), with no differences between sexes ( $U = 145$ ,  $p = 0.46$ ) (Table 4). No ontogenetic differences in  $\delta^{13}\text{C}$  values were found either overall ( $H_{2,37} = 0.27$ ,  $p = 0.87$ ), in West Greenland ( $H_{2,18} = 1.97$ ,  $p = 0.37$ ) or in the Barents Sea ( $H_{2,18} = 0.75$ ,  $p = 0.69$ ) (Table 4). Overall,  $\delta^{13}\text{C}$  values were not significantly different among size groups ( $n = 37$ ,  $R^2 = 0.01$ ,  $p = 0.57$ ). There were significant geographic differences in  $\delta^{13}\text{C}$  values between West Greenland and the Barents Sea ( $U = 46$ ,  $p < 0.001$ ), with West Greenland values being the highest ( $-18.6 \pm 0.2\text{‰}$ ), Barents Sea values the lowest ( $-20.0 \pm 0.3\text{‰}$ ) and the single available value from East Greenland being intermediate ( $-19.4\text{‰}$ ) (Table 4). This pattern showing geographic differences in  $\delta^{13}\text{C}$  values, when ontogenetic groups were analyzed separately, was significant for medium ( $U = 3$ ,  $p = 0.017$ ) and large specimens ( $U = 4$ ,  $p = 0.027$ ) but not significant when considering small specimens ( $U = 6$ ,  $p = 0.07$ ). We did not find a significant linear relationship between  $\delta^{13}\text{C}$  values and depth ( $n = 37$ ,  $R^2 = 0.01$ ,  $p = 0.66$ ).

Values of  $\delta^{15}\text{N}$  varied from  $6.0$  to  $11.4\text{‰}$  ( $8.7 \pm 0.2\text{‰}$ ) with no differences between sexes ( $U = 153$ ,  $p = 0.61$ ) (Table 4). No geographic differences were found between West Greenland and the Barents Sea either overall ( $U = 162$ ,  $p = 0.98$ ) or in small ( $U = 15$ ,  $p = 0.70$ ), medium ( $U = 11$ ,  $p = 0.31$ ) or large specimens ( $U = 10$ ,  $p = 0.23$ ) when analyzed separately

Table 3. Analyses of frequency of stomach contents of *Rossia palpebrosa* from the Barents Sea with Fisher's exact test and Fisher's angular transformation ( $\phi$ ). Significant p-values are in **bold**. –: not applicable. Stomachs with jelly mass and without any prey remains were considered empty (see Section 2)

Prey taxa	Ontogenetic groups						Females vs. Males							
	Small vs. Medium		Small vs. Large		Medium vs. Large		Fisher's exact		Fisher's angular		Fisher's exact		Fisher's angular	
	Fisher's exact test, p	Fisher's angular transformation $\phi$	Fisher's exact test, p	Fisher's angular transformation $\phi$	Fisher's exact test, p	Fisher's angular transformation $\phi$	Fisher's exact test, p	Fisher's angular transformation $\phi$	Fisher's exact test, p	Fisher's angular transformation $\phi$	Fisher's exact test, p	Fisher's angular transformation $\phi$	Fisher's exact test, p	Fisher's angular transformation $\phi$
Crustacea	0.14	1.44	0.08	0.10	1.69	<b>0.045</b>	1.00	0.37	0.36	0.39	0.57	0.29		
Decapoda	<b>0.011</b>	2.88	<b>0.002</b>	<b>0.013</b>	2.82	<b>0.002</b>	1.00	0.15	0.44	0.054	1.94	<b>0.026</b>		
Caridea	<b>0.046</b>	2.27	<b>0.012</b>	<b>0.013</b>	2.82	<b>0.002</b>	0.50	0.76	0.23	<b>0.046</b>	2.04	<b>0.021</b>		
<i>Pandalus</i> spp.	0.15	1.61	0.054	0.14	1.63	0.052	1.00	0.14	0.44	<b>0.050</b>	2.09	<b>0.018</b>		
Other Crustacea	0.51	0.38	0.35	0.54	0.33	0.37	0.65	0.03	0.49	0.44	0.50	0.31		
Euphausiacea	0.40	0.85	0.20	0.74	0.05	0.48	0.43	0.22	0.41	0.20	1.40	0.08		
Polychaeta	0.40	0.85	0.20	0.27	1.19	0.12	0.51	0.41	0.34	<b>0.038</b>	2.32	<b>0.010</b>		
Osteichthyes	0.56	–	–	0.22	–	–	0.38	0.89	0.19	0.53	0.53	0.30		
Sipuncula	0.56	–	–	0.48	–	–	0.67	–	–	0.23	–	–		
Echinoidea	0.44	–	–	0.54	–	–	–	–	–	0.48	–	–		
Jelly mass	0.59	0.40	0.34	0.74	0.05	0.48	0.62	0.340	0.37	0.27	1.16	0.12		
Empty stomachs	0.18	1.29	0.10	<b>0.033</b>	2.32	<b>0.010</b>	0.23	1.19	0.12	0.24	1.03	0.15		

Table 4. Data on stable isotope values ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) in the lower beaks of *Rossia palpebrosa* and its trophic level (TL). Values are minimum to maximum (mean  $\pm$  SE). -: not available; ML: mantle length

Area	Parameter	All specimens	Small: ML $\leq$ 20 mm	Medium: ML 21–40 mm	Large: ML > 40 mm
All specimens (n = 12 in small and large, n = 13 in medium)	$\delta^{13}\text{C}$ , ‰	-21.6 to -17.0 (-19.3 $\pm$ 0.2)	-21.6 to -17.0 (-19.4 $\pm$ 0.4)	-20.9 to -17.0 (-19.2 $\pm$ 0.4)	-21.1 to -18.5 (-19.3 $\pm$ 0.2)
	$\delta^{15}\text{N}$ , ‰	6.0 to 11.4 (8.7 $\pm$ 0.2)	6.4 to 10.2 (8.2 $\pm$ 0.4)	6.0 to 9.7 (8.2 $\pm$ 0.2)	8.3 to 11.4 (9.6 $\pm$ 0.3)
	TL	2.7 to 4.2 (3.6 $\pm$ 0.1)	2.9 to 4.0 (3.4 $\pm$ 0.1)	2.7 to 3.9 (3.4 $\pm$ 0.1)	3.4 to 4.2 (3.8 $\pm$ 0.1)
West Greenland (n = 6 in each group)	$\delta^{13}\text{C}$ , ‰	-19.7 to -17.0 (-18.6 $\pm$ 0.2)	-19.2 to -18.5 (-18.8 $\pm$ 0.1)	-19.7 to -17.0 (-18.3 $\pm$ 0.4)	-19.4 to -18.3 (-18.8 $\pm$ 0.2)
	$\delta^{15}\text{N}$ , ‰	6.0 to 11.4 (8.6 $\pm$ 0.4)	6.4 to 8.4 (8.2 $\pm$ 0.6)	6.0 to 9.3 (7.8 $\pm$ 0.5)	8.5 to 11.4 (10.0 $\pm$ 0.5)
	TL	2.7 to 4.2 (3.5 $\pm$ 0.1)	2.9 to 3.7 (3.3 $\pm$ 0.1)	2.7 to 3.6 (3.2 $\pm$ 0.1)	3.4 to 4.2 (3.8 $\pm$ 0.1)
East Greenland (1 medium female)	$\delta^{13}\text{C}$ , ‰	-19.4	-	-19.4	-
	$\delta^{15}\text{N}$ , ‰	9.7	-	9.7	-
	TL	3.7	-	3.7	-
Barents Sea (n = 6 in each group)	$\delta^{13}\text{C}$ , ‰	-21.6 to -17.0 (-20.0 $\pm$ 0.3)	-21.6 to -17.0 (-20.0 $\pm$ 0.7)	-20.9 to -18.6 (-20.1 $\pm$ 0.4)	-21.1 to -18.7 (-19.8 $\pm$ 0.4)
	$\delta^{15}\text{N}$ , ‰	6.6 to 10.5 (8.6 $\pm$ 0.2)	6.6 to 10.1 (8.3 $\pm$ 0.5)	7.7 to 9.5 (8.4 $\pm$ 0.3)	8.3 to 10.5 (9.2 $\pm$ 0.3)
	TL	3.1 to 4.1 (3.6 $\pm$ 0.1)	3.1 to 4.0 (3.5 $\pm$ 0.1)	3.4 to 3.9 (3.6 $\pm$ 0.1)	3.6 to 4.1 (3.8 $\pm$ 0.1)

(Table 4). Contrary to the observed  $\delta^{13}\text{C}$  values, there was a significant linear ontogenetic increase in  $\delta^{15}\text{N}$  values:  $\delta^{15}\text{N} = 0.04 \text{ ML} + 7.453$  (n = 37,  $R^2 = 0.18$ ,  $p = 0.009$ ) (Fig. 3); thus, significant differences were present among size groups:  $H_{2,37} = 95.08$ ,  $p = 0.009$  (Tables 4 & 5, Fig. 3). However, only large specimens had significantly higher  $\delta^{15}\text{N}$  values (9.6  $\pm$  0.3‰), compared to small (8.2  $\pm$  0.4‰) and medium specimens (8.2  $\pm$  0.2‰) (Tables 4 & 5). Significant ontogenetic differences within West Greenland and the Bar-

ents Sea were not found when analyzed separately (Tables 4 & 5), probably due to the small number of beaks analyzed. The relationship between  $\delta^{15}\text{N}$  values and depth was not significant (n = 37,  $R^2 = 0.08$ ,  $p = 0.99$ ).

### 3.3. TL and isotopic niches

The estimated TL ranged from 2.7 to 4.2 (3.6  $\pm$  0.1), with no differences between sexes ( $U = 156.5$ ,  $p = 0.69$ ) nor between West Greenland and the Barents Sea ( $U = 119.5$ ,  $p = 0.18$ ) (Table 4). Following the patterns established for  $\delta^{15}\text{N}$  values, geographical differences in TL were not found with respect to any of the ontogenetic stages, i.e. small ( $U = 13$ ,  $p = 0.47$ ), medium ( $U = 7$ ,  $p = 0.06$ ) or large specimens ( $U = 17.5$ ,  $p = 0.99$ ) (Table 4). A significant ontogenetic increase was observed (TL = 0.01ML + 3.246, n = 37,  $R^2 = 0.16$ ,  $p = 0.016$ ), also with significant ontogenetic differences:  $H_{2,37} = 10.56$ ,  $p = 0.005$  (Tables 4 & 5, Fig. 3). Similar to  $\delta^{15}\text{N}$  values, only large specimens had significantly higher TL (3.8  $\pm$  0.1) than small (3.4  $\pm$  0.1) and medium specimens (3.4  $\pm$  0.1) (Tables 4 & 5). When the Barents Sea and West Greenland were analyzed separately, significant ontogenetic differences were found only within West Greenland, and these were only between medium and large speci-

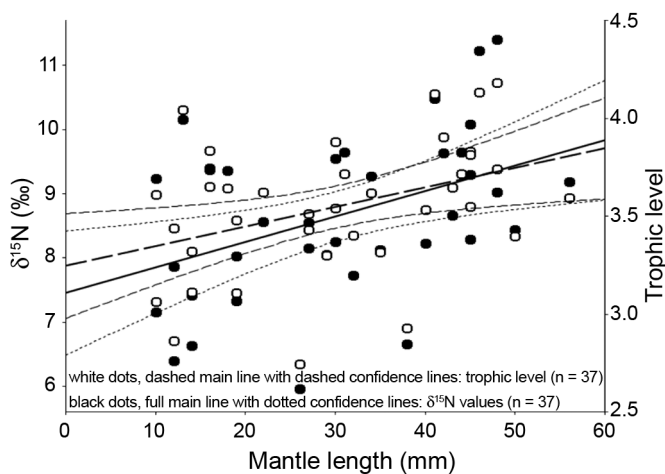


Fig. 3. Increase of  $\delta^{15}\text{N}$  values and trophic levels of *Rossia palpebrosa* during ontogenesis

Table 5. Ontogenetic comparison of  $\delta^{15}\text{N}$  values in the lower beaks of *Rossia palpebrosa* and its trophic levels, using Kruskal-Wallis  $H$ -test and Mann-Whitney  $U$ -test values for between-groups comparisons. Significant  $p$ -values are in **bold**. –: not applicable

Stages	$\delta^{15}\text{N}$ , ‰	Trophic levels
All specimens	$H_{2,37} = 95.08$ , <b><math>p = 0.009</math></b>	$H_{2,37} = 10.36$ , <b><math>p = 0.005</math></b>
Small vs. Medium	$U = 70$ , $p = 0.68$	$U = 76.5$ , $p = 0.95$
Small vs. Large	$U = 32$ , <b><math>p = 0.023</math></b>	$U = 27.5$ , <b><math>p = 0.011</math></b>
Medium vs. Large	$U = 23$ , <b><math>p = 0.003</math></b>	$U = 22.5$ , <b><math>p = 0.003</math></b>
West Greenland	$H_{2,18} = 6.75$ , $p = 0.06$	$H_{2,18} = 7.58$ , <b><math>p = 0.021</math></b>
Small vs. Medium	–	$U = 13.5$ , $p = 0.53$
Small vs. Large	–	$U = 5.5$ , $p = 0.06$
Medium vs. Large	–	$U = 2$ , <b><math>p = 0.013</math></b>
Barents Sea	$H_{2,18} = 4.35$ , $p = 0.11$	$H_{2,18} = 4.32$ , $p = 0.11$

mens (Tables 4 & 5). The relationship between TL and depth was not significant ( $n = 37$ ,  $R^2 = 0.06$ ,  $p = 0.73$ ).

The probability that the stable isotopic niche width is smaller in females than in males was 77.8%, and the probability it is larger was 22.2%. Thus, niches were not significantly different between sexes. This was also the case comparing West Greenland and the Barents Sea, with a 70.0% probability of niche width being smaller in West Greenland than in the Barents Sea (and 30.0% probability of being larger). In terms of ontogenetic changes, large specimens had significantly smaller isotopic niche than small specimens, but not than medium specimens (Table 6, Fig. 4). Overall, isotopic niche width was decreasing gradually through ontogenesis (Table 6, Fig. 4).

## 4. DISCUSSION

### 4.1. Analysis of stomach contents

The only previous study of the food spectrum of *Rossia palpebrosa* showed that fishes and Crustacea were consumed, the former unidentified and the latter represented by Mysidacea, Isopoda (*Idotea* sp.), Amphipoda and Decapoda (with *Lithodes* sp. and *Pandalus* sp. identified and the rest indicated as unidentified Decapoda) (Mercer 1968) (Table S1). Thus, the overall importance of Crustacea, Decapoda, shrimps and *Pandalus* spp. in the diet of *R. palpebrosa* is strongly supported by both this study and Mercer (1968). Isopoda, Amphipoda and Mysidacea were found only by Mercer (1968), and copepods were not found in this study nor by Mercer (1968). However, Isopoda, Amphipoda and Mysidacea are most likely included in the 'other Crus-

tacea' group in this study, which consists of unidentified crustacean remains. Notwithstanding, this study found Echinoidea, Sipuncula, Polychaeta and Euphausiacea in the diet of *R. palpebrosa* for the first time (the latter 2 taxa being relatively common), thus expanding the known food spectrum of *R. palpebrosa*. Two other species of *Rossia* inhabiting the Arctic have an even smaller number of known taxa present in their food spectrums, with Crustacea being the most important group in the diet of *R. megaptera* and Osteichthyes the most important group in the diet of *R. moelleri* (Mercer 1968) (Table S1). In terms of prey size preferences, sepiolids are known to prey on animals up to 4 times their size (Bergström 1985, Yau & Boyle 1996, Hanlon et al. 1997). Being able to prey on animals much larger than themselves is characteristic of most squids and cuttlefishes (reviews: Rodhouse & Nigmatullin 1996, Boyle & Rodhouse 2005).

Table 6. Ontogenetic comparison of stable isotopic niches in *Rossia palpebrosa*. Significant values are in **bold**. SEAc: standard ellipse area corrected for small sample sizes; SEAb: Bayesian estimation of standard ellipse area; TA: Layman metric of convex hull area. –: not applicable

Stage	Small	Medium	Large
n	12	13	12
TA	2.82	2.07	1.23
SEAc	1.45	1.09	0.71
SEAb	$1.45 \pm 0.46$	$1.13 \pm 0.34$	$0.71 \pm 0.22$
Small	–	0.264	<b>0.044</b>
Medium	0.736	–	0.123
Large	<b>0.956</b>	0.877	–

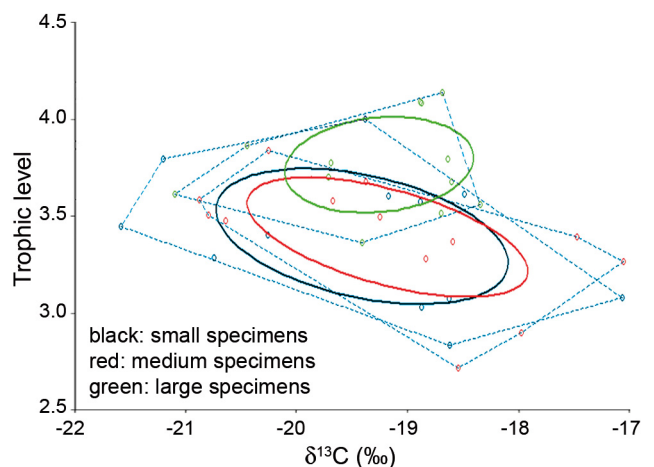


Fig. 4. Isotopic niches of *Rossia palpebrosa*: ontogenetic approach



While other squids, cuttlefishes and incirrate octopods tend to turn their diet from crustaceans to fishes as they increase in size, and, thus, predatory abilities (reviews: Rodhouse & Nigmatullin 1996, Boyle & Rodhouse 2005), the diet of *R. palpebrosa* shifted from smaller crustaceans to larger crustaceans, with fishes remaining of minor importance. There was a significant ontogenetic increase in larger-sized crustacean taxa in the food spectrum of *R. palpebrosa*: small specimens ate mostly crustaceans (unidentified), which were neither Euphausiacea nor Decapoda, while medium specimens were eating more decapods and euphausiids, and large specimens preyed mostly on decapods. The food spectrum of larger groups was more diverse, and fishes were only present in medium and large specimens. However, the largest isotopic niche of the small specimens suggested they consume many different crustacean taxa ('other Crustacea' in our study: those not belonging to copepods, euphausiids and decapods and not exactly identifiable) as well as some unusual groups, such as sea urchins (Table 2). The Arctic squid *Gonatus fabricii* had a much more diverse food spectrum compared to *R. palpebrosa* in this study, with patterns of ontogenetic changes similar to those known of other cephalopods (i.e. change from crustaceans to fishes; cf. Golikov et al. 2018 and reference therein).

Like other sepiolids, *Rossia palpebrosa* is a nekto-benthic predator (Nesis 1985, Jereb & Roper 2005, this study). In the Arctic ecosystems, large active shrimps and fishes are also nekto-benthic predators, but they partly rely on scavenging in their diets (Wienberg 1981, Nesis 1985, Shumway et al. 1985, Hobson et al. 2002, Tamelander et al. 2006, Bergmann et al. 2009, Divine et al. 2015, Bell et al. 2016). Sepiolids are known to eat only live prey (Mercer 1968, Bergström 1985, Yau & Boyle 1996, Hanlon et al. 1997), unless specially trained to prey on pieces of dead fish under aquarium conditions (Nabhitabhata et al. 2005). The food spectrum of *R. palpebrosa* is closer to that of the Northern shrimp *Pandalus borealis* than any other Arctic nekto-benthic shrimp and fish species, with some exceptions: i.e. small specimens of *P. borealis* prey considerably on copepods, and all life stages opportunistically rely on scavenging (Wienberg 1981, Shumway et al. 1985). The similarities were also supported in terms of the stable isotope composition of *R. palpebrosa* (see Section 4.2). The food spectrum of *P. borealis* mostly includes crustaceans: copepods are the most important prey of small specimens, euphausiids are the most important

prey of medium specimens and, together with polychaetes, molluscs and fishes are the most important prey of large specimens (Wienberg 1981, Shumway et al. 1985). Cephalopod remains were found only once in *P. borealis* (Wienberg 1981). *P. borealis* is not an active hunter of fishes, but rather scavenges on their remains (Wienberg 1981, Shumway et al. 1985), so cephalopods were most probably scavenged as well. However, deepwater pelagic predatory pandalid shrimps in the Mediterranean of the same size or smaller than *P. borealis* often prey on cephalopods, including sepiolids (Fanelli & Cartes 2004).

#### 4.2. Stable isotope analysis, TL and isotopic niches

To our knowledge, this is the first ontogenetic study using stable isotope analysis of a nekto-benthic cephalopod. Ontogenetic increase of both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  was less pronounced in *R. palpebrosa* than in pelagic squids (Cherel & Hobson 2005, Cherel et al. 2008, Guerra et al. 2010, Trasviña-Carrillo et al. 2018.), including the Arctic squid *G. fabricii* (Golikov et al. 2018). Values of  $\delta^{13}\text{C}$  in *R. palpebrosa* did not reveal significant ontogenetic increase, suggesting there were no migrations among different water masses. However, there were significant geographical differences in  $\delta^{13}\text{C}$  values. In contrast,  $\delta^{15}\text{N}$  values and TL demonstrated significant ontogenetic increase but not significant geographic differences, suggesting a similar trophic role of the species throughout the studied part of the Arctic (Tables 4 & 5). Similar results were found in the Arctic squid (Golikov et al. 2018).

Values of  $\delta^{13}\text{C}$  in *R. palpebrosa* ( $-21.6$  to  $-17.0\text{‰}$ ) (Table 4) were higher than in pelagic squid from the same ecosystem ( $-23.0$  to  $-18.4\text{‰}$ ) (Golikov et al. 2018). This was expected since *R. palpebrosa* is a nekto-benthic species, in contrast to the pelagic *G. fabricii*:  $\delta^{13}\text{C}$  values are known to be lower in pelagic animals (i.e. relying on pelagic primary source of carbon) than in bottom-associated ones (i.e. relying on bottom primary sources of carbon) (reviews: Post 2002, Boecklen et al. 2011, Layman et al. 2012). However, both species showed similar large ranges of  $\delta^{13}\text{C}$  values (i.e.  $4.6\text{‰}$ ; Golikov et al. 2018, this study). An increase of  $\delta^{13}\text{C}$  values in the westward direction, from the Barents Sea to West Greenland through East Greenland, was observed in *R. palpebrosa*, as it was found in the Arctic squid (Golikov et al. 2018), indicating regional differences in the base-

line  $\delta^{13}\text{C}$  values. The single studied specimen of *R. palpebrosa* from East Greenland fitted well into that pattern.

Beak or corrected muscle  $\delta^{15}\text{N}$  values in about half of studied North Atlantic squid species were higher than in *R. palpebrosa* (Das et al. 2003, Hobson & Cherel 2006, Cherel et al. 2009a, Guerra et al. 2010, Chouvelon et al. 2012). In contrast, beak or corrected muscle  $\delta^{15}\text{N}$  values in studies of benthic and pelagic octopods, and in nekto-benthic cuttlefishes, were similar or lower than those of *R. palpebrosa* (Das et al. 2003, Hobson & Cherel 2006, Cherel et al. 2009a, Chouvelon et al. 2012). Values of  $\delta^{15}\text{N}$  in sepiolids from the Bay of Biscay were also lower, 5.2 to 11.0‰ ( $7.8 \pm 0.4$ ‰) (Chouvelon et al. 2012). In *R. pacifica* from the Japan Sea,  $\delta^{15}\text{N}$  values were 11.4 to 12.7‰ ( $12.0 \pm 0.5$ ‰) (Takai et al. 2000), with baselines near the previous region or slightly higher (Somes et al. 2010). Therefore, sepiolids, due to their smaller sizes in relation to squids, octopods and cuttlefishes (Nesis 1985, Boyle & Rodhouse 2005, Jereb & Roper 2005), predictably occupied lower positions in food webs.

Apart from this study, TLs of cephalopods were estimated by Cherel et al. (2008), Braid & Bolstad (2014), Rosas-Luis et al. (2016) and Golikov et al. (2018). All of the aforementioned studies that occurred outside the Arctic (i.e. except Golikov et al. 2018) used the Antarctic pelagic tunicate as a baseline, which was recently proven to be imprecise even within the Antarctic: tunicates are part of the microbial food web, thus they do not represent TL 2.0 in the classical pelagic trophic web, as previously assumed (Pakhomov et al. 2019). Still, the TL of *R. palpebrosa* (up to 4.2) is much lower compared to the TL of Antarctic (Cherel et al. 2008) or Arctic (Golikov et al. 2018) squid species (up to 6.1 and 5.1, respectively).

We did not find a difference in isotopic niche between sexes in *R. palpebrosa*. This pattern was recently found in the squids *Dosidicus gigas* and *Ommastrephes bartramii* from the Pacific (Kato et al. 2016, Gong et al. 2018) and was concluded based on indirect proofs for some other cephalopods (Arkhipkin & Middleton 2002, Takeshita & Sato 2016, Trasviña-Carrillo et al. 2018). In general, isotopic niches are usually not provided in cephalopod literature; however, the niche of *R. palpebrosa* was smaller than the niche of Arctic squid (Golikov et al. 2018) or the squid *D. gigas* from the Pacific (Trasviña-Carrillo et al. 2018) but was larger than in 3 species of South Atlantic squids (Rosas-Luis et al. 2016). Gradual ontogenetic decrease in isotopic niche width, while

increasing diversity in the food spectrum of larger specimens, was observed in *R. palpebrosa*. The same was observed in *G. fabricii* in the Arctic (Golikov et al. 2018) and in *Doryteuthis gahi* in the South Atlantic (Rosas-Luis et al. 2016). In *D. gigas*, both ontogenetic niche decrease (Trasviña-Carrillo et al. 2018) and increase (Gong et al. 2018) were found in different parts of its range. Large nektonic sharks and predatory bony fishes also demonstrate both ontogenetic increase and decrease of niche sizes, while the food spectrum becomes more diverse, in different species and different parts of their ranges (Grubbs 2010, Carlisle et al. 2015, Sardenne et al. 2016). As an increase in the food spectrum diversity with growth is well known and common in cephalopods (reviews: Rodhouse & Nigmatullin 1996, Boyle & Rodhouse 2005), the ontogenetic niche pattern established here is probably common as well. Overall, values of  $\delta^{13}\text{C}$  are generally more responsible for these ontogenetic differences in niche sizes (i.e. less variation in larger specimens) than values of  $\delta^{15}\text{N}$  (Grubbs 2010, Carlisle et al. 2015, Sardenne et al. 2016, Golikov et al. 2018, this study).

Apart from sepiolids, other Arctic nekto-benthic predators usually show similar and lower (shrimps) or similar and higher (fishes)  $\delta^{13}\text{C}$  values in the Barents Sea, Canadian low Arctic, central high Arctic and Greenland, with their  $\delta^{15}\text{N}$  values and TL (if estimated) showing the same patterns (Hobson et al. 2002, Tamelander et al. 2006, Bergmann et al. 2009, Linnebjerg et al. 2016, Yurkowski et al. 2018). In particular,  $\delta^{13}\text{C}$  values in *P. borealis* ranged from  $-19.6$  to  $-15.8$ ‰,  $\delta^{15}\text{N}$  values ranged from 11.8‰ to 14.4, and TL ranged from 2.1 to 3.8 (Hobson et al. 2002, Tamelander et al. 2006, Linnebjerg et al. 2016, Yurkowski et al. 2018). The lowest TL estimations are probably inaccurate; from what is known regarding the food spectrum of species (Wienberg 1981, Shumway et al. 1985), values should start from 2.5 or 2.7, as we found in *R. palpebrosa*. Both nekto-benthic shrimps and fishes from the Canadian high Arctic had higher  $\delta^{15}\text{N}$  values (and nearly the same TL, as the baseline  $\delta^{15}\text{N}$  values for copepods were higher as well) than their relatives from other parts of the Arctic compared above (Hobson et al. 2002, Divine et al. 2015, Bell et al. 2016, Yurkowski et al. 2018). Thus,  $\delta^{13}\text{C}$  values from most of the predatory nekto-benthic fishes from the Canadian high Arctic were lower and  $\delta^{15}\text{N}$  values, even in shrimps, were higher than those in *R. palpebrosa* from this study. Apart from the Arctic top predators, benthic predatory and nekto-benthic scavenging Arctic

invertebrates and fishes overall had higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and TL than *R. palpebrosa* (cf. Hobson et al. 2002, Tamelander et al. 2006, Bergmann et al. 2009, Divine et al. 2015, Bell et al. 2016, Linnebjerg et al. 2016, Yurkowski et al. 2018). In terms of isotopic niche size, the Northern shrimp *P. borealis* was the only available invertebrate species for comparison, with a slightly larger niche (Linnebjerg et al. 2016) than *R. palpebrosa*. Many nekto-benthic predatory fishes had larger niches as well (Linnebjerg et al. 2016). This can be explained by the presence of an opportunistic scavenging component in the diet of nekto-benthic shrimps and fishes, higher environmental tolerance and increased habitat usage capabilities.

An increase in  $\delta^{15}\text{N}$  values with depth is well known in different ecological groups of benthos from different taxa and can be explained (at least in part) by the biogeochemical degradation of particulate organic matter with depth (Mintenbeck et al. 2007, Bergmann et al. 2009, Lin et al. 2014, Divine et al. 2015, Bell et al. 2016, Stasko et al. 2018). However, TL usually remains constant if baseline values are depth-adjusted (Divine et al. 2015, Bell et al. 2016). Additionally, nekto-benthic predators usually do not show depth-related increase in  $\delta^{15}\text{N}$  values, due to their high mobility and reliance on prey of both pelagic and benthic origin (Bergmann et al. 2009, Lin et al. 2014, Divine et al. 2015, Bell et al. 2016, Stasko et al. 2018). *R. palpebrosa* is not an exception. Decreases in  $\delta^{13}\text{C}$  values with depth are less common in benthic taxa than the aforementioned increase in  $\delta^{15}\text{N}$  values (Lin et al. 2014). This decrease in  $\delta^{13}\text{C}$  values was not found in nekto-benthic predators, possibly due to the same reasons outlined previously for  $\delta^{15}\text{N}$  values (Lin et al. 2014). However, no benthic or nekto-benthic cephalopods were studied in terms of depth-related trends with respect to either  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values or TL, apart from *R. palpebrosa*.

### 4.3. Conclusions

The ontogenetic increase in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and of TL in *R. palpebrosa* was less pronounced than in pelagic squids, as size increases, and consequently, changes in prey consumption are far less pronounced in *R. palpebrosa*. No other nekto-benthic or benthic cephalopods have been studied to date, and comparisons are currently not possible within the same life form.

A significant geographic increase in  $\delta^{13}\text{C}$  values was found in a westward direction from the Barents

Sea to West Greenland. We found no significant ontogenetic increase in  $\delta^{13}\text{C}$  values, suggesting an absence of migrations between different water masses. In contrast,  $\delta^{15}\text{N}$  values and TL showed significant ontogenetic increase but without a geographic pattern, suggesting the same trophic role of the species throughout the studied part of the Arctic. The TL of *R. palpebrosa* was lower than that of Arctic and Antarctic squids and of other Arctic nekto-benthic predators, including shrimps and fishes. In particular, the food spectrum, stable isotope values and TL of *R. palpebrosa* were close to those of the Northern shrimp *P. borealis*. Accordingly, *R. palpebrosa* is placed within the nekto-benthic predator guild in Arctic ecosystems, sharing their common features (food spectrum,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and their depth-related patterns, isotopic niche size and TL). Still, the sepiolids and other nekton-benthic predators show some important differences. Sepioids are much more active predators, they eat prey exceeding their own size, they choose their prey carefully, and they avoid scavenging, while shrimps and fishes of the same life form are more opportunistic and often rely on scavenging.

The observed gradual ontogenetic decrease in isotopic niche width, while increasing diversity in the food spectrum of larger specimens, is unexpected. However, this pattern is known for some squids (e.g. *G. fabricii*, *Dosidicus gigas* and *Doryteuthis gahi*), while other squid species demonstrate ontogenetic niche increase (e.g. *D. gigas*). Moreover, both predatory bony and cartilaginous fishes demonstrate both increase and decrease of niche sizes in ontogeny along with increasing diversity in the food spectrum of larger specimens (e.g. tunas, white and salmon sharks). Thus, as an increase in the food spectrum diversity with growth is well known and common in cephalopods, the ontogenetic niche decrease established here is probably common as well due to more variation in primary productivity supporting food sources during earlier stages, although more variation in the taxonomic food spectrum existed in larger individuals. It means values of  $\delta^{13}\text{C}$  are generally more responsible for these ontogenetic differences in niche size than values of  $\delta^{15}\text{N}$ .

The observed small food spectrum and very short list of known predators of *Rossia* in the Arctic (Table S1) only reflect the current state of knowledge, rather than reality. There are many known predators of nekto-benthic shrimps and fishes, and it is likely similar for *Rossia*. Thus, future studies may show that *R. palpebrosa* is more important to Arctic ecosystems than the present knowledge indicates.

**Acknowledgements.** We are grateful to 'PINRO-IMR Ecosystem Survey in the Barents Sea' project and 'Initiating North Atlantic Benthos Monitoring (INAMon)' project for providing parts of the samples, to the scientific groups and crews of the mentioned RVs, especially Pavel A. Lubin and Igor E. Manushin, for help onboard, to Arina V. Yunusova for help with stomach contents analysis and to Alexandra Baeta, Zara F. Teixeira and Cláudia S. Moreira for help with stable isotope analysis. INAMon was financially supported by the Greenland Institute of Natural Resources, North Atlantic Cooperation (nora.fo; J. nr. 510-151), Sustainable Fisheries Greenland, the Ministry for Research in Greenland (IKIIN) and the Environmental Protection Agency (Dancea) of the Ministry of Environment and Food of Denmark (J. nr. mst-112-00272). The work is also part of the Danish Presidency project in Nordic Council of Ministers, mapping seabed biodiversity and vulnerability in the Arctic and North Atlantic. This research was co-sponsored by the Foundation for Science and Technology (FCT – Portugal) and the European Social Fund (POPH, EU) through a post-doc grant to F.R.C. (SFRH/BPD/95372/2013) and by strategic program of MARE (MARE – UID/MAR/04292/2019). We thank Dr. Mike Vecchione, 2 anonymous reviewers of the manuscript and the editor Dr. James McClintock for their valuable comments to improve the quality of the paper.

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*Editorial responsibility: James McClintock,  
Birmingham, Alabama, USA*

*Submitted: July 1, 2019; Accepted: October 7, 2019  
Proofs received from author(s): November 27, 2019*