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Ontogenetic trends in resource partitioning and trophic geography of sympatric skates (Rajidae) inferred from stable isotope composition across eye lenses

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ABSTRACT: Resource partitioning is expected in sympatric assemblages of predators as a mechanism that reduces competition between individuals of different species or age classes, which in turn can affect population and community interactions as well as resource distribution and availability. However, for species such as benthic skates (Rajidae), the juveniles of which are cryptic and not easily sampled by traditional survey methods, there is a knowledge gap concerning the spatial and trophic ecology during early life stages. The eye lenses of vertebrates grow over their lifetime providing a chronological biochemical record that can be used to infer differences in diet and/or foraging location (trophic geography) throughout the ontogeny of the animal. For the first time, eye lenses of 4 sympatric Rajidae species from the northeast Atlantic were successfully used to recover stable isotope life histories for individual skates. Isotopic separation among species and across life stages within species suggests that habitat partitioning and differences in trophic ecology are present throughout ontogeny. Isotopic data imply that adults are separated from juveniles both spatially and in terms of their diet and the 4 species appear to partition resources more than expected based on previous studies.

KEY WORDS: Sclerochronology · Carbon · Nitrogen · *Raja* spp. · Ray · Skate · Stable isotopes

1. INTRODUCTION

Species living in sympatric assemblages may partition their environment through the differential use of resources such as habitat and prey type (Schoener 1983, Ross 1986, Platell et al. 1998). Such resource partitioning can reduce competition between individuals of different species or age classes, potentially influencing population and community interactions and resource availability (Ross 1986). Habitat partitioning, for example, will affect the spatial distribution of a species, while differences in prey preferences can allow predators to coexist (Speed et al. 2011). Resources may also be partitioned temporally, i.e. different species using the same habitat or consuming the same prey but at different times (Schoener 1974). Resource partitioning is therefore expected to occur in assemblages containing several predators with similar diets and overlapping distributions, even if on a fine scale.

The stable isotope compositions of carbon and nitrogen in animal tissues are routinely used as tracers for tropho-spatial ecology (Domi et al. 2005). Carbon isotope ratios vary between sources of primary production, which frequently differ between habi-

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tats, such as inshore and offshore zones, and can therefore be used to infer feeding provenance (Craig 1953, DeNiro & Epstein 1978, Hobson et al. 1994, Burns et al. 1998). Variations in carbon isotope ratios among locations occur due to different modes of photosynthesis by primary producers, differences in the carbon isotope ratios in dissolved organic carbon or differential fractionation during photosynthetic uptake and assimilation (Barnes et al. 2009). The concentration and isotopic composition of dissolved inorganic carbon and phytoplankton growth rates are also influenced by temperature, which leads to predictable latitudinal gradients in $\delta^{13}C$ values of marine phytoplankton (Barnes et al. 2009, Trueman et al. 2012, Magozzi et al. 2017).

Nitrogen isotope ratios in tissues of marine consumers also vary spatially and are controlled by the nitrogen pool, the nutrient source at the base of the food web and the mechanism by which nitrogen isotopes are integrated into the tissues (Jennings & Warr 2003, Trueman et al. 2012). Nitrate availability is also influenced by temperature and therefore has an effect on nitrogen isotope ratios. Other influences on nitrogen ratios include coastal proximity and pollution (Cabana & Rasmussen 1996, Cole et al. 2004).

Preferential excretion of ^{12}C and ^{14}N leads to enrichment of consumer tissues in the heavier isotope (^{13}C and ^{15}N) compared to their prey. The combination of spatial and physiological differences in isotopic ratios at the base of the food web, and relatively predictable levels of trophic enrichment (of around 1.0 and 3.0‰ in carbon and nitrogen, respectively, though this can vary depending on the host tissue; Pinnegar & Polunin 1999), provide the basis for reconstruction of trophic level, diet sources and food web structure from stable isotope compositions (DeNiro & Epstein 1981, Burns et al. 1998, Bearhop et al. 2004, Inger & Bearhop 2008). However, the combination of spatial and trophic influences on consumer tissue isotopes also complicates their interpretation. The term 'trophic geography' (Bird et al. 2018) refers to the integration of space use and diet type as drivers for resource separation. Two animals with different tissue isotopic compositions may therefore be separated by diet type, area of foraging or both — and can be defined as showing different trophic geographies, implying resource and/or habitat partitioning.

As tissues grow and turnover at different rates, the selection of different tissues for isotope analysis can provide information about an individual's trophic geography at various time intervals (Hobson & Clark 1992, Bearhop et al. 2004). Muscle tissue for example can provide information about foraging location and trophic level over longer periods ranging from weeks to years, while liver or blood samples typically integrate information over shorter time periods (Tieszen et al. 1983, Malpica-Cruz et al. 2012). Metabolically inert tissues retain the isotopic composition of the organism at the time of tissue formation (Schell et al. 1989, Hobson 1999, Wallace et al. 2014). Metabolically inert tissues that grow continually or in discrete increments across the lifetime of an animal can therefore provide a biochemical record of an individual's trophic geography throughout ontogeny, and tissues such as otoliths provide a wealth of biochemical information relating to fish growth, movement, stock structure and life history ecology (Gillanders & Kingsford 2000, Trueman et al. 2012). Otoliths are primarily biomineralised tissues with minor macromolecular framework, limiting the potential for recovery of time-resolved sequential samples for organic isotope analyses (but see McMahon et al. 2011, Grønkjær et al. 2013). In addition, groups such as the elasmobranchs do not possess large calcified otoliths (Cailliet et al. 1986) and most do not have fin spines that can be used as tissue samples in stable isotope analysis. While elasmobranch vertebrae have been used to recover isotopic life history records (Estrada et al. 2006), a sequential sampling of elasmobranch vertebrate can be challenging, particularly in smaller species (Quaeck 2017, Quaeck-Davies et al. 2018).

The eye lenses of vertebrates are also metabolically inert tissues, which grow over the lifetime of an animal by circumferential addition of layers of protein (laminae) to the lens surface (Smelser 1965, Horwitz 1992, Dove & Kingsford 1998). The lens begins to grow during the early days of embryonic development (Grainger et al. 1992), with subsequent layers added on top. This leaves a chronological biochemical record that can potentially be used to determine foraging behaviour throughout ontogeny, with the lens core being the earliest days of the animal's life (Hunsicker et al. 2010, Wallace et al. 2014). In oviparous species, the core of the eye lens therefore forms from proteins assimilated by the mother during egg provisioning (or gestation in the case of placental animals). After hatching or at the onset of independent feeding, the isotopic composition of subsequent layers of lens protein reflect the isotopic expression of foraging (Olin et al. 2011). The use of eye lens tissue is relatively novel in isotope analysis; however, eye lenses have been successfully used to determine the feeding ontogeny of the commander squid *Berryteuthis magister* (Hunsicker et al. 2010), and several teleost and chondrichthyan species (Wallace et al. 2014, Quaeck 2017).

The Rajidae (skates) are commercially valuable mesopredatory elasmobranchs that form sympatric assemblages in coastal regions and are generally considered to exhibit site fidelity (Steven 1936). Studies of the broad-scale spatial ecology of Rajidae in northeast Atlantic waters around the UK have provided important snapshots of their distribution (Pinnegar & Polunin 1999), and depth ranges for some species of Rajidae have been established using fishing surveys (Ellis et al. 2005). Most of the common species of Rajidae in UK waters appear to have an overlapping coastal distribution, with some species such as *Raja brachyura* exhibiting a more southerly distribution (Walker & Heessen 1996, Walker & Hislop 1998, Pinnegar & Polunin 1999, Ellis et al. 2005).

While broad-scale spatial patterns of distribution are relatively well established, fine-scale details of how sympatric Rajidae species utilise shared space are less clear (Wiens 1989). This fine-scale information is especially important given that Rajidae are mobile, have patchy distributions, and are thought to remain in relatively localised coastal areas (Steven 1936, Walker & Heessen 1996, Walker & Hislop 1998, Pinnegar & Polunin 1999, Ellis et al. 2005). Recently, an electronic tag-release study revealed habitat partitioning between 4 species of rajid skates in a small coastal area (Humphries et al. 2016). However, the archival pressure data-logging tags used provided only depth preferences and there may be further habitat partitioning among species within their preferred depth range which is missed by depth-only recording tags. Furthermore, habitat partitioning between juveniles and adults is often missed in tagging studies and fishing surveys due to limitations on the size of individuals that can be tagged, and because neonates can escape through fishing nets with wider meshes. Our understanding of the distribution of juvenile Rajidae is therefore limited (Ellis et al. 2005), even though rajids are known to be oviparous and are thought to deposit eggs in nursery areas, where the juveniles remain until adulthood. Locations of skate nurseries around the UK have been proposed to be broadly in shallower waters than adult distributions (Ellis et al. 2005).

With regard to feeding ecology and potential resource partitioning by diet, Rajidae are considered to be generalist opportunistic feeders and while some species have dietary preferences, there is a high degree of dietary overlap (Holden & Tucker 1974, Ellis et al. 1996). Ontogenetic shifts in diet are commonly reported in Rajidae: for juveniles, mysids and amphipods are important, but with increasing size and age, there is a shift from such smaller prey items to larger crustaceans, fish and cannibalism (Holden & Tucker 1974, Ajayi 1982, Ellis et al. 1996, Farias et al. 2006, Moura et al. 2008). Juvenile Rajidae species in UK waters are therefore assumed to occur in similar habitats and share similar diets, potentially overlapping with adult distributions and diets. Habitat and/or resource partitioning may therefore be an important factor limiting competition between juveniles and adults, and perhaps among species.

To identify trophic level and dietary preferences in Rajidae, studies have largely used stomach contents analysis (Holden & Tucker 1974, Ajayi 1982, Ellis et al. 1996, Farias et al. 2006, Moura et al. 2008). This method can work well for adults of a species; however, juveniles are often excluded, especially newly hatched individuals, as they may be missed by commercial fisheries and may be too small to perform reliable stomach contents analysis (Coelho & Erzini 2006, Moura et al. 2008). Ontogenetic differences are frequently analysed as broad groups (Ellis et al. 1996), often simply before and after maturity (Farias et al. 2006), which potentially misses ontogenetic shifts in the early life of a rajid. Establishing trophic level through stomach contents analysis also has several caveats including the identification of partially digested prey, differential rates of digestion of prey types and regurgitation of prey on capture (Estrada et al. 2006). To determine trophic ecology through ontogeny with stomach contents analysis, large numbers of individuals at different life stages must be sampled, which may be ethically problematic in species' populations that are considered threatened. The success of juveniles in a population is vital for the survival of a species and governs the rate at which a population may recover from exploitation pressure or environmental perturbation. This is especially the case for threatened skates which are late maturing and have relatively low fecundity (Field et al. 2009, Dulvy et al. 2014).

Stable isotope analysis of Rajidae eye lenses therefore has the potential to provide an insight into juvenile feeding ecology, an insight that has progressed slowly using traditional methods. Here, we investigated the use of eye lenses in the study of trophospatial ecology of Rajidae. We provide the first life-history isotope records for sympatric Rajidae derived from eye lens proteins, and test the hypothesis that resource partitioning occurs between the 4 species and between juveniles and adults within species. Our results therefore provide an insight into ontogenetic shifts in feeding ecology of 4 skate species that may have been missed by stomach content analysis studies.

2. MATERIALS AND METHODS

This study is based on 4 sympatric skate species, spotted ray *Raja montagui,* smalleyed ray *R. microocellata,* blonde ray *R. brachyura* and thornback ray *R. clavata,* which are relatively common Rajidae species within the study site off Plymouth, Western English Channel, UK (Fig. 1). As part of a tagging programme aimed at revealing skate movements and migrations, archival data-logging tags were returned via the local commercial trawl and net fisheries for a reward (£50 per tag), together with information concerning its capture location and date of recapture. Occasionally however, fishers returned a tag still attached to a dead skate, which serendipitously enabled us to sample eye lenses. Length, disc width and weight were recorded for returned skates and tissues were removed for isotope analysis. Whole eyes were removed from a total of 50 dead individuals and muscle samples were removed from 47 individuals (Table 1).

Muscle was removed from the base of the tail, avoiding the skin, and both muscle and eye tissues were stored at −20°C until processing took place. Despite described differences in the maximum attainable length for each of the 4 species (McCully et al. 2012), there were no significant differences in total length between individuals of species sampled in the current study (ANOVA on ranks, Kruskal-Wallis, $H = 6.71$, df = 3, p = 0.082).

In the laboratory, whole eyes were thawed and a small incision made in the cornea to remove the eye lens. The outer gelatinous layer of the lens could not be sampled systematically and was removed prior to analysis. Under a microscope, layers of the lens were removed (delamination) using forceps and a scalpel, which were cleaned using ethanol between each layer to avoid contamination. The lens diameter (excluding the hydrated outer layer) was measured using Vernier calipers before each layer of the lens was removed. Between 5 and 9 layers (mean = 6.46) were removed from each lens depending on the starting diameter. Hence, 50 eyes produced 323 layers which were stored individually in sample tubes and frozen. Eye lenses and muscle tissue largely came from the same individuals with some opportunistic sampling of tissues; however, all individuals were mature.

Samples were freeze-dried for at least 6 h, then both muscle and eye lens tissues were pulverised, weighed using a microbalance to between 0.65 and 0.75 mg and sealed into tin capsules. Lipid extraction was not required as C/N ratios for every sample were <3.23. Isotope ratios of carbon and nitrogen were determined at the SEAPORT stable isotope facility at the University of Southampton using an Isoprime100 isotope ratio mass spectrometer (Isoprime) coupled to a Vario ISOTOPE select elemental analyser (Elementar). All stable isotope data are reported in delta (δ) notation. The internationally certified standards (US Geological Survey), Glutamic acid (SGA40), VPDB (Vienna PeeDee Belemnite) and Protein (Casein) Standard OAS (Batch no. 114859), were run at intervals between the eye lens samples to determine analytical accuracy and precision (Table 2).

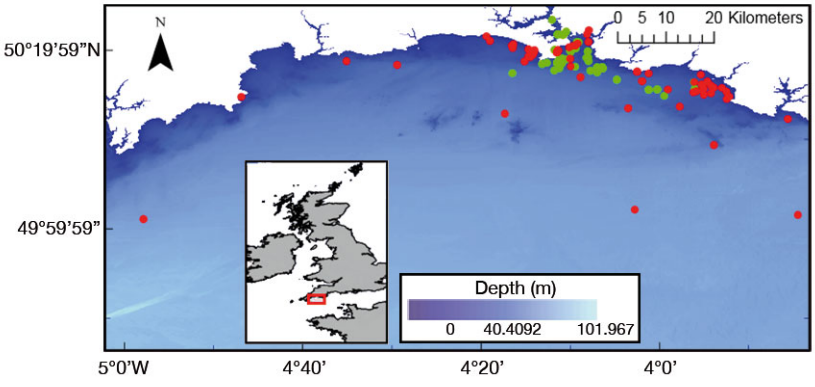

Fig. 1. Study location off Plymouth, south-west UK. Filled circles represent the results from a tagging study (●: release; ●: recapture), defining the spatial extent of the study area

Table 1. Summary of sample sizes and median capture lengths for each species of skate

Species	Muscle tissue samples	Male/female/ unknown (muscle tissue samples)	Eye lens, individuals	Eye lens, samples	Mean eye lens samples per individual	Median total length of mature individuals (mm)	Min.–max. lengths (mm)	Max. length (IUCN 2019) (mm)
Raja brachyura	12	6/6/0	14	97	6.92	743	487–1070	1200
R. microocellata	11	3/6/2	8	49	6.13	671	544–881	910
R. montagui	11	5/6/0	11	66	6	636	495–690	800
R. clavata	13	2/11/0	17	111	6.53	774	533–920	1047
Total	47	16/29/2	50	323	6.46			

Table 2. Standard deviation of 'Quality control' standard per run. VPDB: Vienna PeeDee Belemnite

Run	Normalised $^{15}N/^{14}N_{air}$ (‰)	Normalised $^{13}C/^{12}C_{VPDB}$ (‰)
Run1	0.21	0.08
Run2	0.19	0.05
Run3	0.14	0.04
Run4	0.16	0.09

Eleven eyes were also removed from neonate skates (6 *R. montagui*, 5 *R. microocellata*) that died after hatching from eggs laid in experimental aquaria by adult females tagged as part of a previous study (Humphries et al. 2016). Eye lenses were measured using Vernier calipers to provide an estimate of the eye lens diameter at hatching, which could be used to identify the likely position within each lens sampled from adult skates that corresponds to hatching. Juvenile eye lens tissues did not undergo stable isotope analysis.

The relationship between eye lens outer diameter and body length was tested by species using ANCOVA. An ANOVA on ranks (Kruskal-Wallis) was conducted to test for differences in isotopic compositions among species in 3 grouped ontogenetic phases (core, post-hatching and adult). The 'core' was classified as tissue from diameters smaller than the average diameter of the neonate eye lenses, the 'post-hatch' group contained the remaining eye lens tissue and the 'adult' group contained the muscle tissue data. Rajidae are relatively long-lived, slow-growing animals and muscle tissue was taken from mature individuals. Muscle tissue reflects diet over the most recent period of time, likely of the order of months up to a year (Logan & Lutcavage 2010) and therefore represents the adult phase. Fish eye lenses grow by essentially continued accretion to the external hydrated surface and dehydration within the lens to maintain a refractive index contrast at a relatively continuous proportion of the diameter of the lens. Consequently the outer hydrated layer represents a relatively large (but varying) proportion of the life history of the individual. We did not combine the outer lens sample with the sampled inner lens — discarding the outer hydrated layer therefore removes a substantial, but unknown, period of growth history.

By focussing our analyses on the dehydrated inner portions of the lens, at lens diameters inferred to correspond to body sizes of juvenile fish (based on the measured relationship between lens diameter and fish size), we avoided confounding our time-resolved retrospective samples with tissues formed recently in the life of the (adult) fish.

Within-group isotopic variability was quantified using Bayesian standard ellipse areas (SEAc) for each of the ontogenetic phases (core, post-hatch and adult) using the SIBER package in R (version 2.14.1) (Jackson et al. 2011, R Core Team 2015). Isotopic niche area and overlap (‰^{2}) were estimated based on 100 000 posterior draws of the SEAc. We calculated pairwise isotopic niche overlaps between species and used the mean proportional isotopic niche overlap among species as a metric (overlap indices) of overlap. Sequential samples of eye lens proteins recovered from eye lenses were analysed to generate isotopic time series (life history profiles). To characterise temporal patterns in $\delta^{15}N$ and $\delta^{13}C$ values across eye lens diameters, Generalized Additive Mixed Models (GAMM) were run in R with the mgcv package (version 3.5.3). The weighted Akaike's Information Criterion (wAIC) and the deviance explained was used to select the appropriate model. In the models, species and eye lens diameter were used as fixed effects and individual as a random effect to account for the repeated measures sample design. Convex hulls are calculated to illustrate the smallest convex polygon containing all the points for each species, providing a visual comparison of overlap between species for all samples.

To test for differences in the magnitude of ontogenetic variation in $\delta^{15}N$ values among species, the difference between maximum and minimum eye lens $\delta^{15}N$ values was calculated for each individual. All comparisons were explored using ANOVA with Tukey's post-hoc multiple comparison tests. All analyses were conducted in R (version 3.5.3) (R Core Team 2017).

3. RESULTS

3.1. Relationship between lens diameter and body length

Eye lens diameter appears to vary linearly with body size in all fish species so far investigated (Quaeck-Davies et al. 2018). In the 4 Rajidae species assessed here, there was a significant positive correlation between eye lens diameter and total length ($F_{4,56}$ = 28.84, p < 0.001) (Fig. 2). Nevertheless, an accurate measurement of the full diameter of lenses was difficult due to the hydrated gelatinous outer layer, particularly after freezing and thawing. Consequently, measured lens diameters showed a large

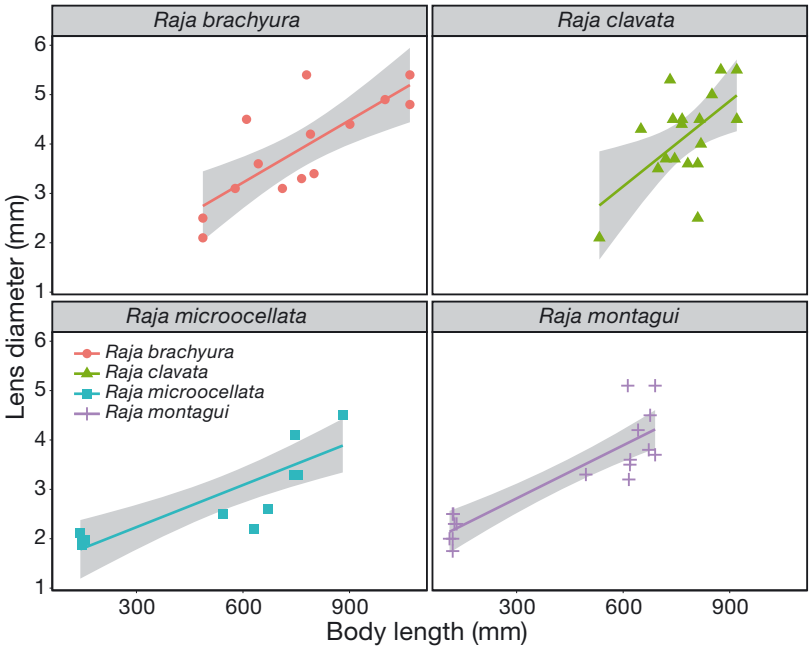

Fig. 2. The linear relationship between the total length (mm) of individual skates on recapture and their maximum eye lens diameter (mm). Standard error is represented by the grey areas. Each data point plotted denotes an individual skate

variation, and while it was clear that lens diameter increased consistently, and probably linearly with body size, it was not possible to accurately estimate adult size (or age) from lens diameters in the case of the 4 Rajidae species. The neonate eye lenses were less hydrated than the adults and the outer layers were retained; therefore, the observed range in eye lens diameters in neonates (1.75 to 2.5 mm) was likely more representative and provided a good estimate of eye lens diameter at hatching (ca. 2.1 mm). Eye lens diameter values <2.1 mm were therefore considered to be the 'core' of the eye, where the isotopic composition of the lens core represents maternal foraging during egg provisioning.

In general, eye lenses delaminated well with, on average, about 6 layers removed per individual. This provided a large enough sample for analysis as well as a relatively fine-scale isotopic life history.

3.2. Isotopic differences among species throughout ontogeny

No significant differences in $\delta^{15}N$ values were found among species during the adult phase ($F_{3,41}$ = 2.269, p = 0.10). There were, however, differences among species during the core and post-hatch phases (Fig. 3, Table 3).

Raja montagui in particular showed lower $\delta^{15}N$ values, especially in lens core regions. Lens core $\delta^{13}C$ values did not differ among species ($F_{3,26}$ = 1.372, p = 0.27), although there were differences detected between the adult and post-hatch phases (Table 3, Fig. 4).

3.3. SEAc analysis

In the lens core reflecting tissue synthesis before hatching, there were no significant differences in SEAc among the 4 species, but *R. montagui* had a distinct isotopic niche, with an index of 0 overlap with every other species (Fig. 5A, Table 4).

In the post-hatch phase, *R. clavata* had a larger SEAc value than *R. brachyura*, while *R. microocellata* had a larger SEAc than *R. montagui*. We also found greater isotopic overlap between all species during the post-hatch phase, the greatest difference between species was between *R. montagui* and *R. clavata* with an index of 0.23 (Fig. 5B, Table 4).

In the adult phase, isotopic SEAc values suggested that *R. clavata* had a significantly broader isotopic niche than *R. montagui* and *R. microocellata*. Overlap indices suggested distinct isotopic niches for all species, especially *R. brachyura* where no overlap with any other species was indicated (Fig. 5C, Table 4).

Table 3. Statistical differences in $\delta^{15}N$ and $\delta^{13}C$ values among skates at 3 ontogenetic stages. ns: not significant

Stable isotope and species comparison	Core p-values	Post-hatch p-values	Adult p-values
$\delta^{13}C$			
Raja clavata–R. brachyura	ns	0.05	<0.001
R. microocellata–R. brachyura	ns	ns	0.02
R. montagui–R. brachyura	ns	ns	0.01
R. microocellata–R. clavata	ns	ns	0.01
R. montagui–R. clavata	ns	<0.001	0.01
R. montagui–R. microocellata	ns	0.01	ns
$\delta^{15}N$			
R. clavata–R. brachyura	ns	<0.001	ns
R. microocellata–R. brachyura	ns	ns	
R. montagui–R. brachyura	ns	<0.001	
R. microocellata–R. clavata	ns	ns	
R. montagui–R. clavata	0.03	<0.001	
R. montagui–R. microocellata	0.01	<0.001	

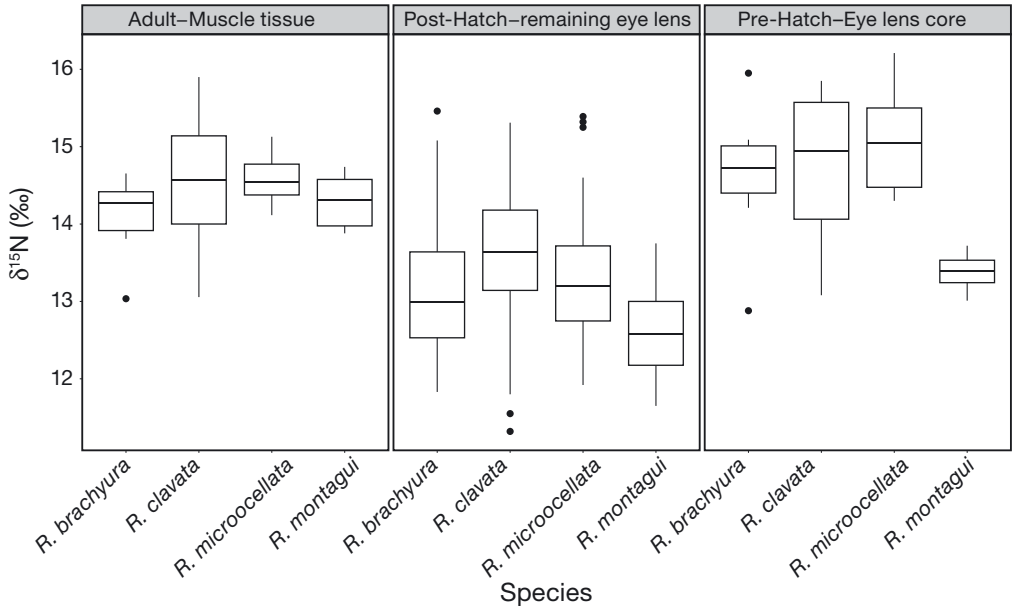

Fig. 3. Differences in $\delta^{15}N$ values among skate species at 3 ontogenetic stages. Box: 25th and 75th percentiles; whiskers: 5th and 95th percentiles; horizontal line: median; dots: outliers. No difference was found in the adult phase, but differences occurred in both the post-hatch and pre-hatch phases

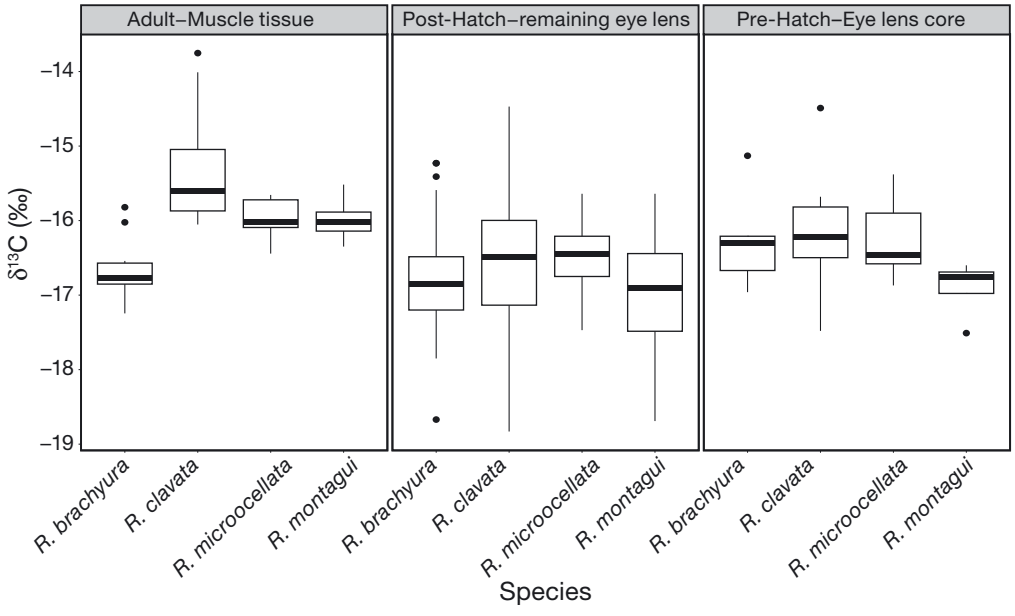

Fig. 4. Difference in $\delta^{13}C$ among skate species at 3 ontogenetic stages. Box: 25th and 75th percentiles; whiskers: 5th and 95th percentiles; horizontal line: median; dots: outliers. No significant differences were found in the pre-hatch phase, but differences were found in the adult and post-hatch phases

3.4. Isotopic time series across eye lenses

Time series of stable isotope compositions throughout ontogeny are given in Figs. 6 & 7. Species and eye lens diameter significantly influenced isotopic compositions in GAMM models: $\delta^{13}C$ ($p = 0.048$ and $p < 0.001$ respectively; 50.7% deviance explained) and $\delta^{15}N$ ($p < 0.001$ for both species and diameter; 73% deviance explained). $\delta^{15}N$ and $\delta^{13}C$ values in the core of the lens (reflecting maternal foraging during egg provisioning) were higher than in the 2 post-hatch phases for all species. Both $\delta^{15}N$ and $\delta^{13}C$ values

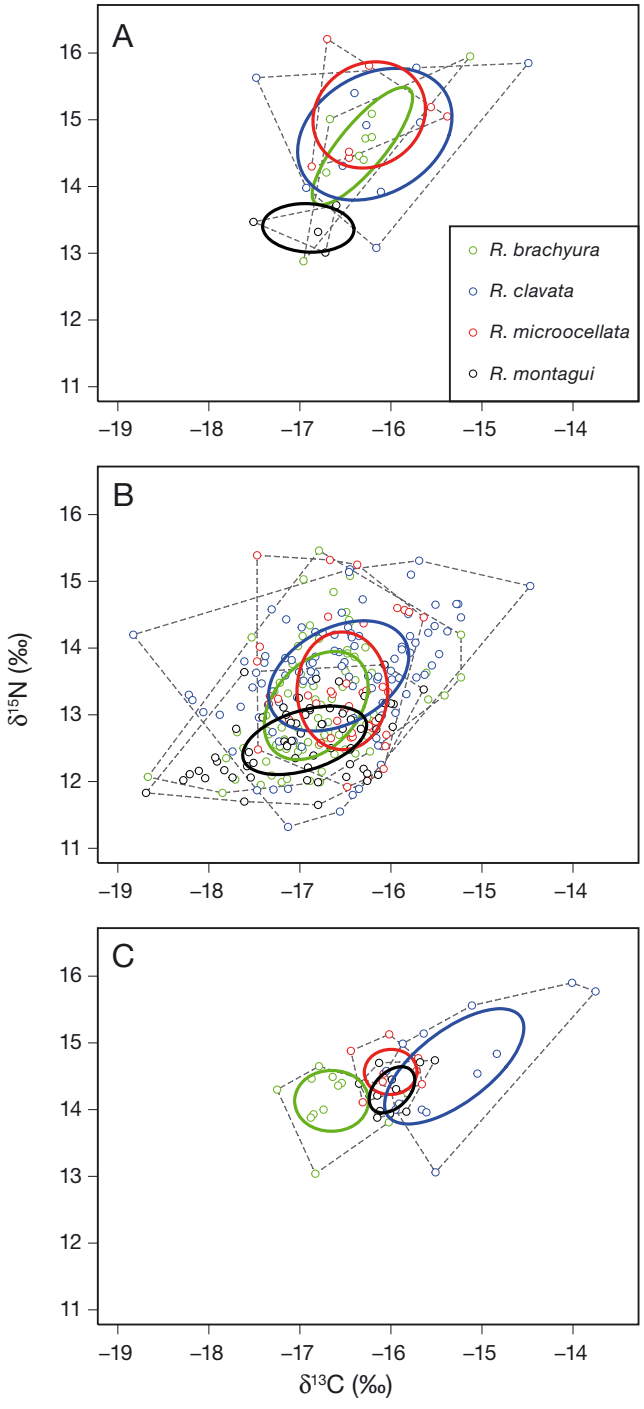

Fig. 5. Standard ellipse areas (solid lines) and convex hulls (dashed lines) by species of skate. (A) Pre-hatch/eye lens core, (B) post-hatch/eye lens tissue, (C) adults/muscle tissue

reached a lifetime minimum at around 2 to 2.5 mm lens diameter in every species ($\delta^{15}N$, Fig. 6; $\delta^{13}C$, Fig. 7) and this lifetime minimum was more pronounced in $\delta^{15}N$ values. After this minimum, values increased consistently with increasing eye diameter, and thus body size. Adult muscle tissue $\delta^{15}N$ values were not significantly different to lens core values in *R. microocellata*, *R. clavata* and *R. brachyura*, but were higher in *R. montagui*. For both isotopes, wAIC values were lower in the model with the individual as a random effect and were significant in both cases ($p < 0.001$).

3.5. Magnitude of $\delta^{15}N$ difference in eye lens tissue

R. montagui had the most consistent lifetime $\delta^{15}N$ values across eye lens tissue, with lifetime mean $\delta^{15}N$ values significantly lower than those seen in *R. brachyura* ($p < 0.001$) and *R. microocellata* ($p < 0.001$), which also showed the greatest ontogenetic change in $\delta^{15}N$ across the eye lens (Fig. 8, Table 5).

4. DISCUSSION

The use of eye lens tissues as a host for stable isotope analysis is relatively novel, but has been demonstrated in several species, including within teleost fish, squid and elasmobranchs (Hunsicker et al. 2010, Wallace et al. 2014, Quaeck 2017). The current study is the first to use eye lens proteins from Rajidae as a host for stable isotope analysis, which examined potential resource partitioning and juvenile life histories in 4 sympatric species from the north-east Atlantic.

Eye lenses of skates were, on average, delaminated into around 6 layers per individual, providing a relatively detailed isotopic life history, particularly during the juvenile stages. The core of the lens represents protein assimilated by the mother during egg provisioning and therefore provides a unique cross-generational biochemical link. Relating the position of a sample within a lens to the body size of the individual (and thus linking isotopic samples to individual body size and age) depends on recovery of precise allometric growth relationships between lens diameter and body size. Difficulties associated with measuring lens diameters in defrosted lenses precluded accurate reconstruction of body size from lens diameters. In the future, it would be beneficial if lens–body length calibrations were based upon fresh rather than frozen eye lens samples. Overall however, the eye lenses provided effective incremental tissues and have the potential to be used to compare trophic geographies of Rajidae across ontogeny.

Table 4. Difference and overlap in standard ellipse area (SEAc) for each stage of skate development

Stage	SEAc differences	p value	Overlap SEAc	Overlap indices
Muscle (Adult)	*R. clavata* & *R. montagui*	0.02	*R. montagui* & *R. brachyura*	0
			R. microocellata & *R. montagui*	0.13
	R. clavata & *R. montagui*	0.05	*R. montagui* & *R. clavata*	0.14
			R. clavata & *R. microocellata*	0.1
			R. clavata & *R. brachyura*	0
			R. montagui & *R. brachyura*	0
Eye lens (Post-hatch)	*R. brachyura* & *R. clavata*	0.03	*R. montagui* & *R. brachyura*	0.58
			R. microocellata & *R. montagui*	0.35
	R. microocellata & *R. montagui*	0.05	*R. montagui* & *R. clavata*	0.23
			R. clavata & *R. microocellata*	0.98
			R. clavata & *R. brachyura*	1.14
			R. brachyura & R. microocellata	0.85
Eye lens core <2.11 mm (Pre-hatching)	No significant differences		*R. montagui* & *R. brachyura*	0
			R. microocellata & *R. montagui*	0
			R. montagui & *R. clavata*	0
			R. clavata & *R. microocellata*	1.4
			R. clavata & *R. brachyura*	0.83
			R. brachyura & *R. microocellata*	0.60

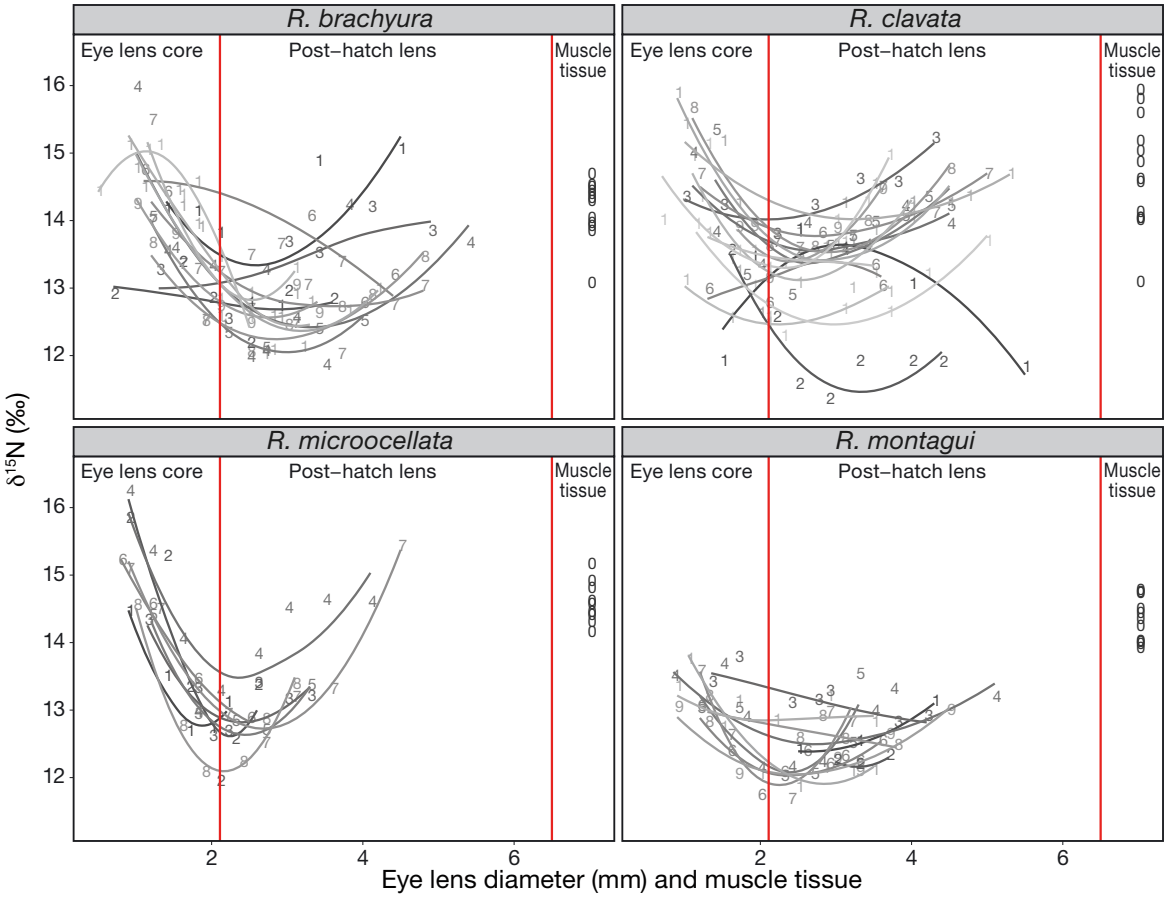

Fig. 6. $\delta^{15}N$ values across skate eye lens diameter (numbers and curves represent individual eye lenses) and in muscle tissue. Red lines delineate pre-hatch (core), post-hatch and adult (muscle) phases. Note the similar pattern across all species, where $\delta^{15}N$ reaches a low point shortly after hatching

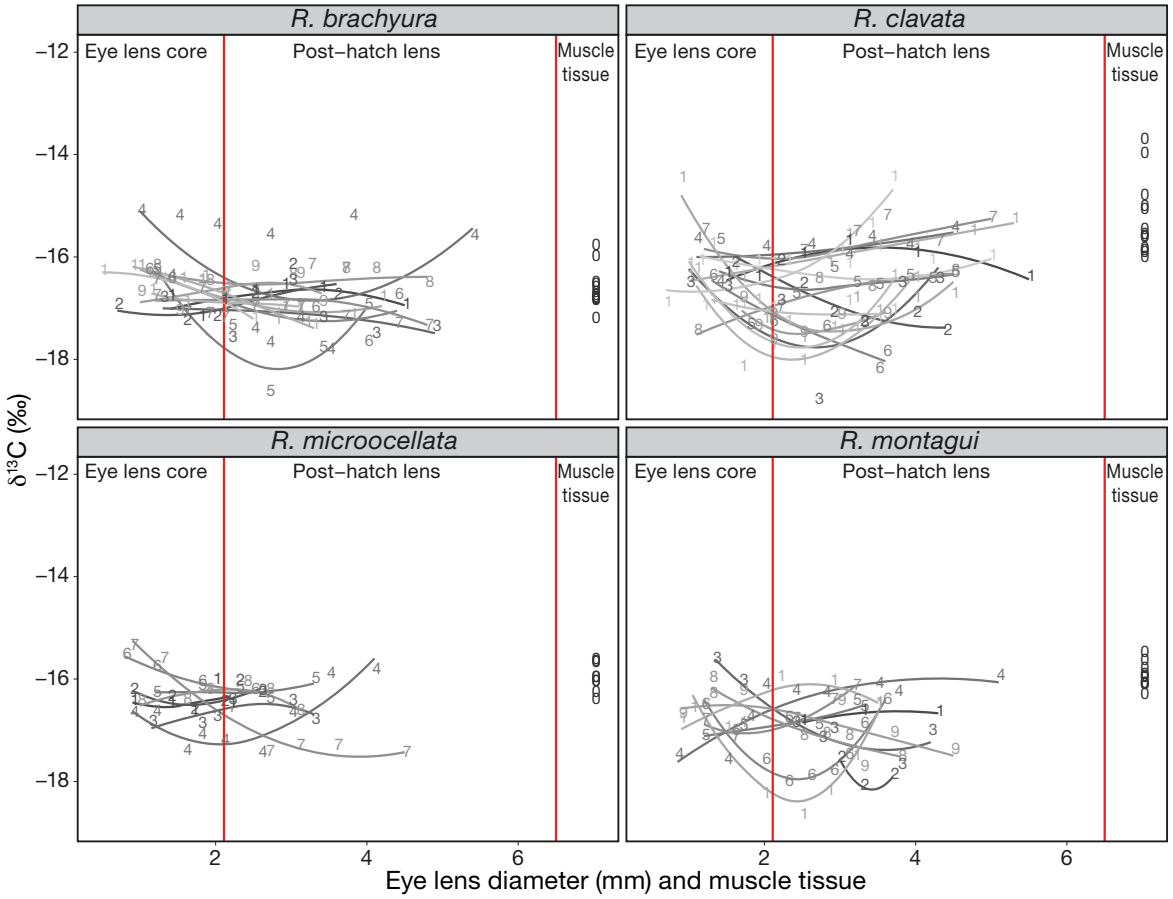

Fig. 7. $\delta^{13}C$ values across skate eye lens diameter and in muscle tissue (numbers and curves represent individual eye lenses). Red lines delineate pre-hatch (core), post-hatch and adult (muscle) phases

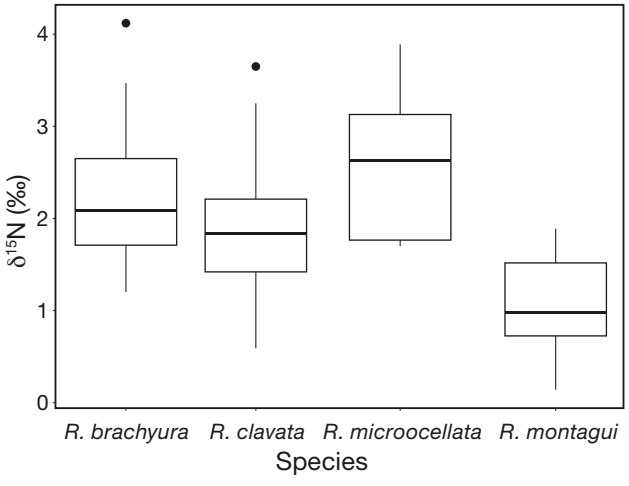

Fig. 8. The magnitude of maximum lifetime change in $\delta^{15}N$ values in skate eye lens tissues (i.e. $\Delta^{15}N_{max-min}$). Box: 25th and 75th percentiles; whiskers: 5th and 95th percentiles; horizontal line: median; dots: outliers. Note the higher magnitude of difference in *Raja microocellata* compared to *R. montagui*

Table 5. Average $\Delta^{15}N_{max-min}$ for each species of skate

Species	Mean	SD
Raja brachyura	2.338	0.792
R. clavata	1.777	0.712
R. microocellata	2.513	0.802
R. montagui	1.117	0.561

4.1. Comparative trophic geography and resource partitioning

Results from carbon and nitrogen isotope ratios of muscle tissue from adults of 4 Rajidae species imply distinct isotopic niches for all species, especially *Raja brachyura*, in which there was no overlap in SEAc. Carbon isotope ratios were more distinct among species, suggesting that the 4 species were partitioned by habitat rather than differences in their trophic level. A recent tagging study in the same location suggested separation of the 4 species into 2 pairs,

with a preferred shallower depth and inshore preference of *R. clavata* and *R. microocellata* and deeper depths or an offshore preference of *R. brachyura* and *R. montagui* (Humphries et al. 2016). Collectively, these findings provide support for the existence of fine-scale habitat partitioning between these 4 sympatric species. The current study however, suggests further partitioning between species than could be inferred from depth archival tags. *R. clavata* and *R. microocellata* had distinct isotopic niches, but used similar shallow-water habitats in the tagging study. The $\delta^{13}C$ values for *R. clavata* and *R. microocellata* were also higher (less negative) than the other species, which suggests shallower inshore feeding (e.g. Hobson et al. 1994), supporting the tagging studies. Of the 4 species investigated in this study, *R. clavata* is the only species that routinely associates with estuarine habitats (Hunter et al. 2005a,b), as well as marine and more varied substrates (Steven 1932). *R. clavata* also had a significantly wider adult isotopic niche than *R. microocellata* and *R. montagui*. This observed difference in the current study is supported by data of the reported prey of *R. clavata*, which ranges from entirely crustaceans in Carmarthen Bay (Ajayi 1982) to a more piscivorous diet off the coast of Africa (Ebert et al. 1991, Smale & Cowley 1992). In addition, studies have shown that, off the African coast, *R. clavata* has a more diverse diet than any of 14 other species of skate (Ebert et al. 1991). Combined isotopic and tagging methods provide strong evidence for a wider niche and more individual variation in trophic geography in adult *R. clavata* relative to the other rajid species studied here.

The eye lens tissues that represented the post-hatch phase indicated a greater overlap in isotopic niche between species than during the adult phase. This is perhaps a result of the large proportion of time that this portion of the eye lens represents, which also captures the greatest degree of change or shifts in trophic geography during ontogeny. Despite this, there were distinctions in SEAc between *R. montagui* and *R. clavata* during this phase. Significant differences were found in both $\delta^{15}N$ and $\delta^{13}C$ values among species. Most notably, $\delta^{15}N$ values in *R. montagui* were significantly lower than all other species during the juvenile phase. Differences in trophic ecology between species may be a result of differences in specialisation, preference or availability of prey items between species. Such differences may reduce competition for sympatric species whose young occupy overlapping habitats (Espinoza et al. 2012). Differences in spatial ecology between adult and juvenile Rajidae have previously been suggested, where nursery grounds are shallower than adult preferred habitat (Ellis et al. 2005). The current study suggests, however, that not all juveniles of these 4 species are foraging in the same habitat. Overall, the differences found in the current study suggest the occurrence of both habitat partitioning and trophic ecology separation play a role in partitioning these species at this life stage.

4.2. Maternal egg provisioning

In Rajidae, proteins in the eye lens core are deposited during embryonic development and provide a biochemical record of the isotopic composition of food consumed by mothers during egg provisioning. Consequently, all individuals retain a biochemical record of maternal foraging in the lens core and of their own foraging post-hatching or birth in subsequent layers of the eye lens (Olin et al. 2011). Short-term biochemical changes relating to diet, location and/or physiological status are more likely to be preserved in relatively rapidly assimilated egg tissue than in muscle tissue with a relatively long time window associated with growth and tissue turnover. The consistently lower $\delta^{15}N$ values found in the core eye lens tissues in *R. montagui* compared to the other species therefore represent a difference in the trophic geography of egg-provisioning females. This is especially interesting because there were no significant differences in $\delta^{15}N$ values in the adult tissues between species, suggesting that *R. montagui* females uniquely change their feeding behaviour or nutritional status during egg provisioning. Sex differences in skate feeding have been observed in barndoor skate *Dipturus laevis*, where large males consumed predominantly fish, while large females consumed equal amounts of fish and crustaceans (Schmitt et al. 2015). Potentially, *R. montagui* mothers may feed on lower trophic level prey while provisioning their eggs, or alternatively, may reduce nitrogen excretion rates (leading to lower isotopic fractionation) during this time. Future studies with expanded sample sizes are needed to explore the potential for sex-biased feeding differences in adult skates.

4.3. Trophic level across ontogeny

In all species, $\delta^{15}N$ values were higher in the eye lens core. Here, enriched values in the core of the eye represent the embryonic phase when individuals rely on the egg yolk provided by the mother for nutrition.

After hatching (lens diameters >2.11 mm), values decreased, reaching their lowest level at 2.0 to 2.5 mm, after which there was a gradual increase in $\delta^{15}N$ in all species across ontogeny. Several studies have demonstrated a dietary shift in Rajidae from amphipods and crustaceans to fish across ontogeny (Ajayi 1982, Smale & Cowley 1992, Ellis et al. 1996, Ebert & Bizzarro 2007), and the individual-level isotopic life histories recovered from lens proteins in the current study are consistent with such ontogenetic diet shifts in all 4 skate species. A commonly cited hypothesis to explain ontogenetic shifts in diet refers to the development of the feeding apparatus (MacNeill & Brandt 1990, Costalago et al. 2012). Rajidae gape size and the strength of the jaw both increase with ontogeny in the same way as with many elasmobranchs (Motta & Huber 2012). Dietary shifts across ontogeny can also be caused by behavioural shifts that change their prey type due to changing densities in the habitat they occupy (Tanaka et al. 2006, Costalago et al. 2012). It is also likely that larger fish are more experienced or are more efficient predators than juveniles (Espinoza et al. 2012). In Rajidae, there is some evidence that oviposition occurs in nursery areas separate from adults (Ellis et al. 2005), where the density of prey types is likely different. It is therefore likely that a combination of a difference in gape size between adult and juveniles, in addition to prey availability and the foraging experience of adults, that results in the differences across ontogeny found in the current study.

Across ontogeny, there was significant individual variation in $\delta^{15}N$, potentially representing the opportunistic or generalist nature of individuals from an early age. there were also significant differences among species in the magnitude of change in $\delta^{15}N$ values across ontogeny. *R. microocellata* had the largest difference in $\delta^{15}N$, followed by *R. brachyura*. Interestingly, both of these species are suggested to have an adult diet containing more fish, while the diets of the other 2 species contain more crab (Ajayi 1982, Ellis et al. 1996), indicating that this greater magnitude of change potentially reflects this shift to a more piscivorous diet by these 2 species.

4.4. Spatial ecology across ontogeny

Carbon isotope ratios also varied across eye lens diameter, in a similar pattern to nitrogen isotopes, starting at a high value in the core or egg phase, reaching minimum values on hatching and increasing across ontogeny. Differential habitat selection across ontogeny is common in elasmobranchs (Simpfendorfer et al. 2005, Grubbs 2010), where birthing or nursery areas are often visited seasonally by females for oviposition or parturition. These habitat selections by different age groups may reduce intra-species competition between adults and juveniles. Juveniles are likely to be more vulnerable to predators, due to their smaller size; therefore, habitat selection by juveniles may occur to avoid predators that adults of the species need not avoid (Lima & Dill 1990). There is also evidence that increasing body size increases activity space (McNab 1963) and adults of a species may also have different energy demands that can only be obtained in different habitat to the juveniles (Grubbs 2010). In our study, the change in carbon isotope ratios across the eye lens suggest that juveniles are partitioned by habitat from adults, and that juveniles disperse or migrate during development to different habitats.

4.5. Conclusions and implications

The stable isotope composition of eye lens proteins provides promising insight into trophic geography and resource partitioning in Rajidae. Retrospective analyses from eye lens proteins allowed a comparative assessment of tropho-spatial ecology during cryptic early juvenile phases and of foraging behaviour during maternal egg provisioning. With future analysis of the development of Rajidae eye lenses and the rate at which eye lens layers are deposited as the individual grows, a greater understanding of the time frame that a layer of the eye lens represents will help to improve predictions on temporal-ontogenetic shifts. Equally, a greater understanding of the complex fine-scale habitat mosaic within our and other study areas, and with the construction of dynamic isoscapes, will all help to determine the likely habitats of these species. Increasing sample sizes in terms of individuals would also allow one to test for individual specialisation, such as partitioning resources within species and age groups. The current data suggests that this might occur, but a larger sample size would help to fully explore this.

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