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Received: 13 December 2021 | Accepted: 2 March 2022

DOI: 10.1111/jfb.15034

SPECIAL ISSUE PAPER-REGULAR

JOURNAL OF FISH BIOLOGY fsbi

Patterns of mother–embryo isotope fractionation in batoids vary within and between species

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Abstract

Patterns of mother–embryo fractionation of ^{13}C and ^{15}N were assessed for their predictability across three species of batoids caught as by-catch in south-eastern Australia. Stable isotope analysis of 24 mothers and their litters revealed that isotope ratios of embryos were significantly different from their corresponding mothers and that the scale and direction of the difference varied within and across species. The range of variation across species was 3.5‰ for $\delta^{13}C$ and 4‰ for $\delta^{15}N$, equivalent to a difference in trophic level. In one species (*Urolophus paucimaculatus*) litters could be significantly enriched or depleted in ^{13}C and ^{15}N relative to their mothers' isotope signatures. These results suggest that patterns of mother–embryo isotope fractionation vary within and between species and that these patterns may not be explained only by developmental mode. Contrasting patterns of fractionation between and within species make it difficult to adjust mother–embryo fractionation with broad-scale correction factors.

KEYWORDS
batoids, fractionation, histotrophy, isotopes, maternal, provisioning

1 | INTRODUCTION

The use of stable isotope analyses to answer questions relating to diet preference (Plass-Johnson *et al*., 2013), carbon provisioning (Phillips *et al*., 2014), trophic relationships (Raoult *et al*., 2015; Chan *et al*., 2022) and anthropogenic impacts (Vizzini and Mazzola, 2006) or to examine movement patterns (Shimada *et al*., 2014; Raoult *et al*., 2020) of elasmobranchs is increasing (Fisk *et al*., 2002; Speed *et al*., 2012; Shipley *et al*., 2017a; Bird *et al*., 2018). This field of research has expanded due to the relatively low cost of analysis and the comparatively reliable data that can be obtained for larger sample sizes, especially in comparison to labour-intensive gut content examination that can provide accurate species-specific prey information, but is not as effective at examining food-web-level interactions (Martínez del Rio *et al*., 2009). One area where isotope analysis can be beneficial to elasmobranch research is the determination of nursery site contributions to diet (Dale *et al*., 2011; Kinney *et al*., 2011; Belicka *et al*., 2012; Carlisle *et al*., 2015), where isotopes can provide information on long-term habitat use and diet of neonates and young-of-the-year. Although the use of stable isotope analysis has proved reliable and the technique is now widely accepted by ecologists, one aspect that is still poorly understood and has been identified as requiring further investigation is the effect of maternal provisioning on neonate or young-of-the-year isotope ratios, which leads to a maternal isotope signature in the offspring (Olin *et al*., 2011; Shipley *et al*., 2017a).

The concept of a maternal isotope signature is not novel (Doucett *et al*., 1999; Grey, 2001) and stipulates that neonates or young-of-the-year generally exhibit isotopic signatures that relate more to the isotope values of their mother rather than their own diet preference (Olin *et al*., 2011). Because many but not all species of elasmobranchs exhibit ontogenetic shifts in diet to higher trophic levels (de la Morinière *et al*., 2003; Estrada *et al*., 2006; Knoff *et al*., 2008), maternal isotope signatures could incorrectly suggest that neonates have diets at a higher trophic level than their true diet. In elasmobranchs, this pattern has been confirmed in neonates of some species (Matich *et al*., 2010; Olin *et al*., 2011), but direct examination of embryonic isotope signatures has provided conflicting evidence in some sharks with some apparent mother–embryo fractionation (McMeans *et al*., 2009; Vaudo *et al*., 2010; Kim *et al*., 2012c; Le Bourg *et al*., 2014; Olin *et al*., 2018). In marine mammals, foetuses were generally enriched relative to their mothers (Borrell *et al*., 2016), whereas lactating young that should theoretically be a trophic level above their mother were generally less enriched than predicted (Jenkins *et al*., 2001; Cherel *et al*., 2015). The ability to correct for these patterns of mother–embryo fractionation on population scales would enable researchers to determine more precisely the potential diets of neonates and juveniles, as well as the habitats they rely on for nutrition (Shiffman *et al*., 2012). This is especially important given work suggesting that muscle stable isotope values can reflect their mothers' values for years after parturition (Niella *et al*., 2021), possibly preventing this tool from being used to answer questions relating to ecology or management for these size classes.

In many cases it appears that separate isotopic fractionation occurs within the embryos, which leads to isotopic signatures that do not necessarily reflect the values of their mother. Nonetheless, these studies generally have small sample sizes (*i.e*., only one adult in Vaudo *et al*., 2010, and a maximum of five in Olin *et al*., 2018), are generally conducted on a single species or species with similar developmental modes (Vaudo *et al*., 2010) and are thus unlikely to depict patterns on larger scales that could be applied across species. Some research suggests that developmental mode is a possible explanation for patterns in mother–embryo fractionation (McMeans *et al*., 2009; Olin *et al*., 2018; Broadhurst *et al*., 2019); nonetheless, this has not been explicitly tested because of small sample sizes or comparisons of similar species.

This study determined whether maternal isotope signatures were observable in the embryos of three species of batoids frequently caught as by-catch in trawl fisheries in south-eastern Australia: the sparsely spotted stingaree (*Urolophus paucimaculatus*), the greenback stingaree (*Urolophus viridis*) and the Tasmanian numbfish (*Narcine tasmaniensis*). Because urolophid species exhibit aplacental viviparous histotrophy (White and Potter, 2005; Last and Stevens, 2009; Yick *et al*., 2011), and Tasmanian numbfish are aplacental with yolk provisioning only (like other members of the genus, De Carvalho *et al*., 2002; Last and Stevens, 2009), it was hypothesised that patterns of mother–embryo fractionations would differ between genera but remain similar within each genus.

2 | MATERIALS AND METHODS

2.1 | Fieldwork and sample processing

Sparsely spotted stingarees (*U. paucimaculatus*), banded stingarees (*U. viridis*) and Tasmanian numbfish (*N. tasmaniensis*) were collected as by-catch from research trawls conducted by the University of Tasmania vessel *FTV Bluefin* in November and December 2014 and in December 2015 along the north-eastern coastline of Tasmania, Australia (40° 18.101 S, 148° 33.596 E). These were exploratory trawls at depths of *c*. 30 m using a 70 mm mesh demersal fish net and a speed of *c*. 3 kts. Individuals were snap frozen on board the vessel on capture and transported to Macquarie University for analysis. Trawling for research purposes was permitted in accordance with ethical guidelines number A0015366, UTAS AEC. All procedures performed were in accordance with the ethical standards of the University of Newcastle and Macquarie University.

Once thawed, individuals were weighed and measured for total weight and length. Reproductive stage was determined by assessing the development of ovaries and the thickness of the uterine wall for females or by the degree of calcification of claspers for males (Awruch *et al*., 2008). If females were gravid, embryos were extracted and underwent the same morphological assessments. In total, the authors captured and processed 82 *N. tasmaniensis*, including 9 mothers; 122 *U. paucimaculatus*, including 14 mothers; and 8 *U. viridis*, including 1 mother. Thus, 24 gravid females and 62 associated embryos were examined, with litter sizes that ranged between 1 and 5 pups.

Muscle tissue was removed from each individual, dried in an oven at 65°C for 24 h and ground to a fine powder using a mortar and pestle. Muscle was removed from wings by separating off the muscle from the underlying ceratotrichia using a sterilised scalpel. About 1–2 mg of ground muscle tissue from each individual was weighed in a separate tin capsule for analysis. The samples were analysed for stable carbon (^{13}C) and nitrogen (^{15}N) isotopes with a Europa EA GSL Elemental analyser coupled to a Hydra 20–22 automated Isoprime isotope ratio mass spectrometer (Sercon Ltd.) at Griffith University (Queensland, Australia). The ratio of isotopes was expressed as relative per thousand difference (‰) between the sample and the standards, which were Pee Dee Belemnite for carbon and atmospheric nitrogen for nitrogen (IAEA-NA, IAEA-N2 for ^{15}N and IAEA-CH-6 for ^{13}C). Ten standards per plate at minimum were treated as samples, with precision for $\delta^{15}N$ and $\delta^{13}C$ that was <0.1‰.

2.2 | Analyses

Stable isotope ratios can be misleading if tissues demonstrate a high C:N ratio indicative of high lipid content (Hussey *et al*., 2012a; Hussey *et al*., 2012b). In addition, the high urea content of elasmobranch tissues can affect stable isotope ratios (Kim and Koch, 2012; Carlisle *et al*., 2016; Li *et al*., 2016; Shipley *et al*., 2017b). Because relative comparisons between mothers and embryos within species were

being made rather than the absolute ecological values per Carlisle *et al*. (2016), lipid or urea extractions were not believed to be necessary. Intraspecific lipid and urea content variations in muscle tissue have not been widely explored, because muscle tissue is generally not used as an energy reserve (Pethybridge *et al*., 2014) and the authors did not expect significant mother–embryo muscle tissue differences in lipid or urea content. In addition, some studies on mother–embryo fractionation have conducted lipid and urea extractions (McMeans *et al*., 2009; Olin *et al*., 2018), whereas others have not (Vaudo *et al*., 2010; Broadhurst *et al*., 2019). Because chemical lipid or urea extractions were not conducted for this study, results should be interpreted within that context.

Authors' interpretation of lipid and urea content effects relied on the common assumption that within-species C:N ratios do not vary significantly. To verify whether this was indeed the case, they ran an ANOVA with C:N ratios as the response variable, with fixed factors stage of maturity (embryo, juvenile and adult) and an interaction with species, including all sampled individuals (not just mothers and embryos). To determine whether differences in C:N ratios are driving the differences in stable isotope values, the authors ran general linear models with $\delta^{13}C$ and $\delta^{15}N$ values as response variables, C:N ratio as a determinant and an interaction with species.

To determine whether there were patterns of mother–embryo fractionation in embryos extracted from corresponding mothers (*i.e*., assuming the isotope values did not differ between the two), $\delta^{13}C$ and $\delta^{15}N$ values were analysed using separate linear mixed-effect models for each species with one isotope as the response variable, where samples were a mother or embryo as a fixed factor, and embryos nested within their associated parent to separate individual clutches according to their parent.

Although there may be significant differences between mothers and their clutches, the direction of these patterns may not be consistent on a species scale, because the patterns of fractionation may vary interspecifically between litters. Separate ANOVAs were run for each species and for ^{13}C and ^{15}N isotopes. The difference in isotope values between litters and the stable isotope values of their associated mothers was a continuous variable and each mother and associated litter as a fixed factor.

Previous research has indicated that there is a relationship between the size of embryos and stable isotope ratios (McMeans *et al*., 2009). The range of embryo sizes was variable in this study due to species-specific differences in seasonal reproduction; therefore, the isotope ratios of the embryos were tested in a linear regression with their lengths for each species and isotope.

All analyses were conducted in R (v. 3.3.3) (R Development Core Team, 2013) and RStudio with the lmer and ggplot2 packages (Wickham, 2009; Bates *et al*., 2014).

3 | RESULTS

There were no significant differences in C:N ratios between adults, juveniles and embryos for *U. paucimaculatus* and minor but untestable (due to sample size) differences between embryos and adult *U. viridis*. Nonetheless, there were significant differences between C:N ratios of embryos and both juvenile and adult *N. tasmaniensis* (TukeyHSD, $P < 0.001$, Figure 1). There was a significant negative relationship between C:N ratios and $\delta^{13}C$ values that was approximately thrice weaker in *U. paucimaculatus* ($df = 1_{62}$, $F = 12.34$, $P < 0.001$, $R^2 = 0.16$) than for *N. tasmaniensis* ($df = 1_{49}$, $F = 43.51$, $P < 0.001$, $R^2 = 0.46$, Figure 3). There were weak but significant opposite relationships between C:N ratios and $\delta^{15}N$ values for *U. paucimaculatus* ($df = 1_{62}$, $F = 6.77$, $P = 0.01$, $R^2 = 0.08$) and *N. tasmaniensis* ($df = 1_{49}$, $F = 10.42$, $P = 0.002$, $R^2 = 0.16$, Figure 2) (Appendix S1).

Embryos had isotopic ratios that were distinct from those of their mothers, suggesting that mother–embryo fractionation was occurring. Linear mixed models with embryos nested within parents showed significant differences in $\delta^{13}C$ muscle tissue values between adults and the embryos for *N. tasmaniensis* and *U. paucimaculatus* but not for *U. viridis* (Table 1). Additional models showed significant differences in $\delta^{15}N$ muscle tissue values between adults and their embryos across all species (Table 1). *U. paucimaculatus* and *U. viridis* embryos were generally enriched in ^{13}C relative to their mothers, but $\delta^{15}N$ maternal differences were highly variable. *N. tasmaniensis* embryos were generally depleted in ^{13}C and ^{15}N relative to their mothers (Figure 3).

Significant within-species differences between mothers and their pups were observed. Mother-isotope stable isotope values between litters of pups differed significantly in $\delta^{13}C$ and $\delta^{15}N$ for both *N. tasmaniensis* ($df = 8$, $F = 8.72$, $P < 0.001$; $df = 8$, $F = 9.56$, $P < 0.001$) and *U. paucimaculatus* ($df = 13$, $F = 8.97$, $P < 0.001$; $df = 13$, $F = 14.6$, $P < 0.001$). Because only one litter of *U. viridis* was caught, this species was not included in this analysis.

No significant relationship between the total length of embryos and their $\delta^{15}N$ or $\delta^{13}C$ values was observed, suggesting there is no isotope enrichment with size (Table 2; Figure 4).

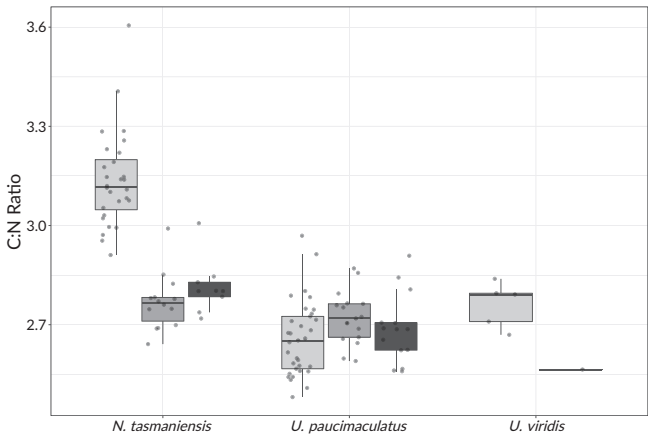

FIGURE 1 Boxplots overlayed with raw data of C:N ratios of batoid species at different life stages, including gravid and non-gravid adults. Stage (▭) Embryo, (▭) Juvenile, and (▭) Adult

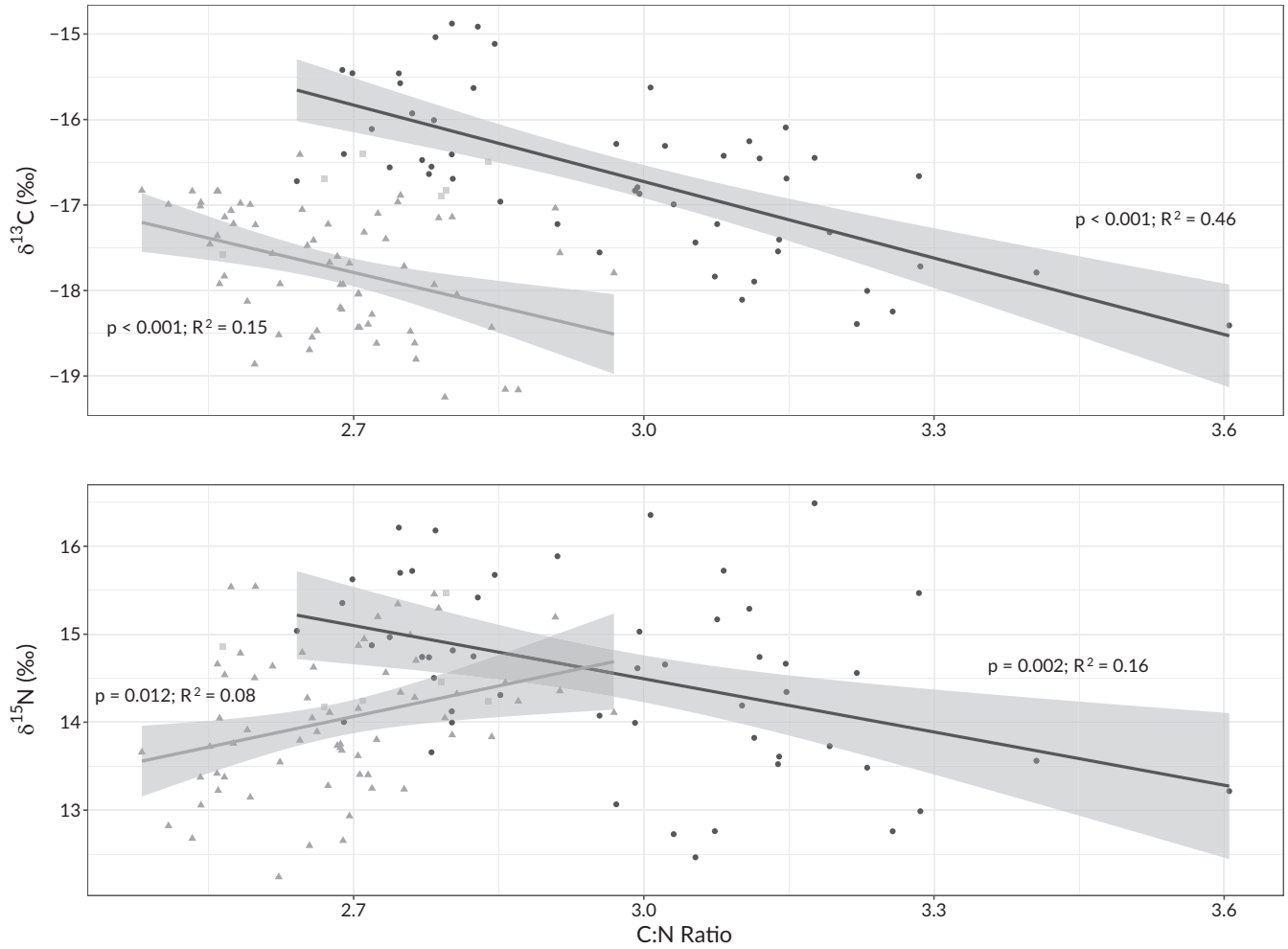

FIGURE 2 Relationships between C:N ratios and stable isotope values in batoids examined in this study, overlayed with significant linear relationships. Species (╍) *N. tasmaniensis*, (┄) *U. paucimaculatus*, and (┈) *U. viridis*

Species	Value	Factor	Estimate	S.E.	*T*-value	*P*-value
Narcine tasmaniensis	$\delta^{13}C$	Stage (embryo)	−1.16	0.12	−9.61	<0.001
	$\delta^{15}N$	Stage (embryo)	−0.99	0.36	10.46	<0.001
Urolophus paucimaculatus	$\delta^{13}C$	Stage (embryo)	0.76	0.08	8.99	<0.001
	$\delta^{15}N$	Stage (embryo)	0.39	0.14	2.74	0.012
Urolophus viridis	$\delta^{13}C$	Stage (embryo)	1.11	0.23	4.81	<0.01
	$\delta^{15}N$	Stage (embryo)	−0.34	0.59	−0.57	>0.05

TABLE 1 Results of linear mixed models for $\delta^{13}C$ and $\delta^{15}N$ with embryos nested within parents for batoid species and the fixed factor "stage" comparing adults from their embryos

4 | DISCUSSION

This is the first study to examine mother–embryo fractionation across numerous species of batoid. Embryos of the three species of elasmobranchs exhibited stable isotope signatures that were significantly different from their corresponding mothers' tissues. Both species of urolophid had embryos with significantly higher $\delta^{13}C$ values, but *U. viridis* did not significantly differ in $\delta^{15}N$ values, but the sample size for this species was small (just one mother and its five pups). Conversely, *N. tasmaniensis* embryos were depleted in ^{13}C and ^{15}N relative to their mothers' isotopic signature. The patterns of differences between mothers and offspring were also significantly different between clutches within *N. tasmaniensis* and *U. paucimaculatus*. Ratios of C:N, traditionally assumed to be constant within a species, were significantly different between embryos, and juveniles and adults of *N. tasmaniensis*, suggesting novel ways to store energy in embryos. Together, these results reiterate that patterns of mother–embryo fractionation and provisioning vary within and between species and that there are species-specific trends in isotope enrichment or depletion of embryos relative to the maternal isotope signatures.

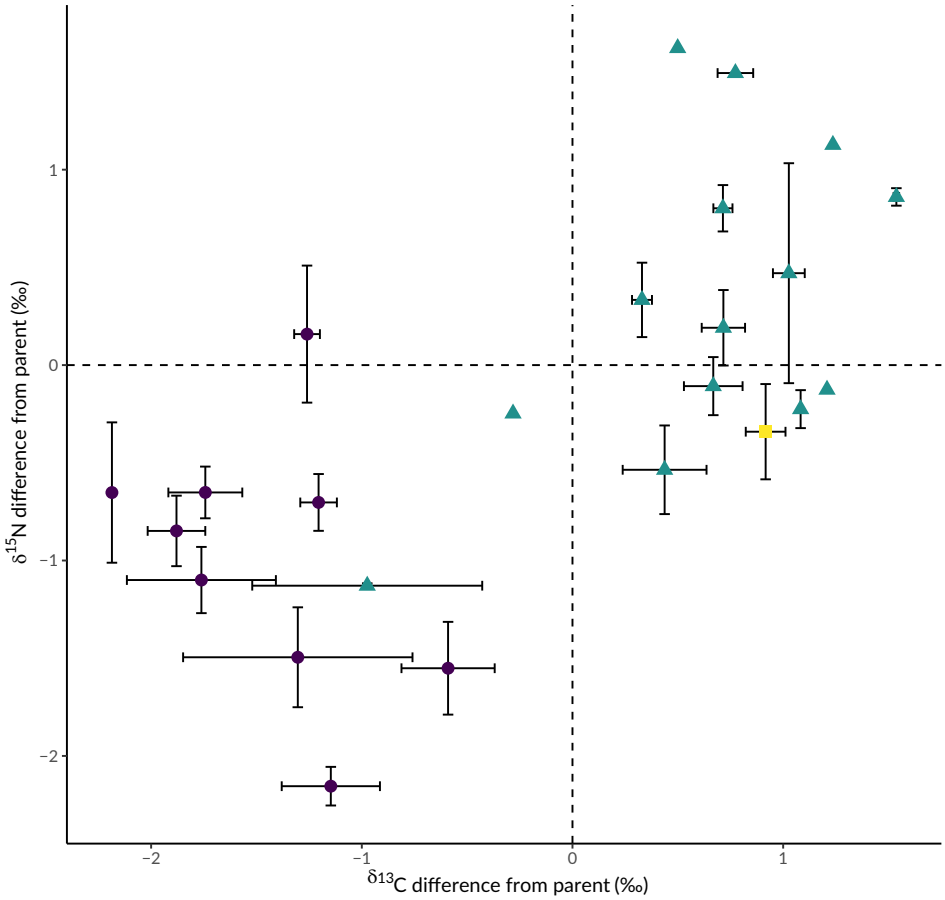

FIGURE 3 Mean ± s.e. $\delta^{13}C$ and $\delta^{15}N$ isotopic ratio difference in litters of embryos from each parent *Narcine tasmaniensis*, *Urolophus paucimaculatus* and *Urolophus viridis* from this study. Species (●) *N. tasmaniensis*, (▲) *U. paucimaculatus*, and (■) *U. viridis*

TABLE 2 Results of linear regressions examining the relationship between the total length of embryos and $\delta^{13}C$ and $\delta^{15}N$

Species	Value	*df*	*F*-statistic	*P*-value
Narcine tasmaniensis	$\delta^{15}N$	1, 26	3.72	>0.05
	$\delta^{13}C$	1, 26	0.16	>0.05
Urolophus paucimaculatus	$\delta^{15}N$	1, 31	2.67	>0.05
	$\delta^{13}C$	1, 31	2.09	>0.05
Urolophus viridis	$\delta^{15}N$	1, 3	0.41	>0.05
	$\delta^{13}C$	1, 3	1.08	>0.05

These opposed isotope enrichment and depletion trends between mothers and their litters for the species examined were surprising. The authors predicted that if enrichment or depletion occurred, it would relate to the type of embryonic development (*e.g.*, placental, aplacental) due to the differences in how nutrients are allocated for each developmental mode. Unlike other studies, all species tested in this study have aplacental histotrophic viviparous development, suggesting that only the mode of embryonic development may not explain the differences in patterns of fractionation, because opposite patterns were detected between species with identical developmental modes. Rate of histotrophy has been suggested as impacting mother–embryo differences in stable isotope values (Broadhurst *et al.*, 2019), and authors' results corroborate this hypothesis given *N. tasmaniensis* does not have histotrophy, whereas the *Urolophus* rays do. Controlled experiments that focus on the stable isotope values of yolk, histotroph and placental inputs would help determine the interaction between these factors. These patterns are further complicated by a lack of knowledge on trophic fractionation patterns in batoids, which should receive broader focus so more isotope research can be conducted on these charismatic and widespread animals.

Trends of fractionation were also highly variable within species and ranged from 0‰ to 2‰ for both $\delta^{13}C$ and $\delta^{15}N$, respectively. Trophic fractionation or enrichment factors are generally assumed to be 1‰ for ^{13}C and 2‰ for ^{15}N in sharks (Hussey *et al.*, 2010a), although they can range up to 3.5‰ for ^{13}C and 5.5‰ for ^{15}N (Kim *et al.*, 2012a; Kim *et al.*, 2012b). Due to the intraspecific variability of mother to embryo fractionation observed in this study across species, trophic assessments of neonates could result in falsely attributing higher or lower trophic levels or misidentifying the sources of carbon.

Studies on stable isotope ratios of placentatrophic Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*) found similar trends as those observed in the present urolopid species, with offspring enrichment in ^{13}C and ^{15}N (McMeans *et al.*, 2009). The placentatrophic scalloped hammerhead shark (*Sphyrna lewini*) and its offspring also displayed similar trends (Vaudo *et al.*, 2010); nonetheless, only one individual was examined in this study, making rigorous comparisons difficult. Aplacental viviparous *Squalus megalops* and *Centrophorus moluccensis* mothers and embryos had no difference in $\delta^{13}C$ values but displayed significantly depleted $\delta^{15}N$ values (Le Bourg *et al.*, 2014). Thus, it appears

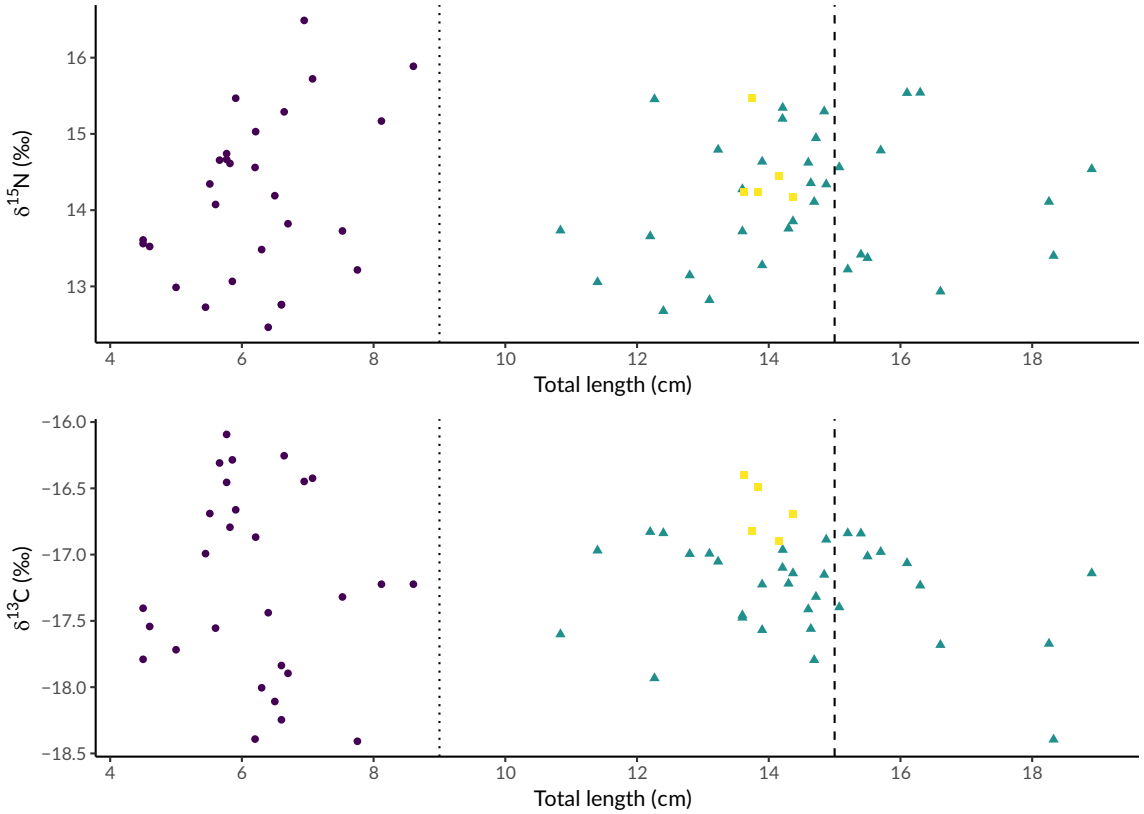

FIGURE 4 Relationship between total length and $\delta^{13}C$ and $\delta^{15}N$ of *Narcine tasmaniensis*, *Urolophus paucimaculatus* and *Urolophus viridis* embryos from this study Species (●) *N. tasmaniensis*, (▲) *U. paucimaculatus*, and (■) *U. viridis*. Dotted and dashed vertical lines are the expected minimum size at birth of *N. tasmaniensis* and *U. paucimaculatus*, respectively, sourced from Last and Stevens (2009). Species (●) *N. tasmaniensis*, (▲) *U. paucimaculatus*, and (■) *U. viridis*

that the depletion of both ^{13}C and ^{15}N observed in *N. tasmaniensis* is a novel pattern to date, though interactive effects of urea and lipids on the stable isotope values, which demonstrate a high degree of species-specific effects (Kim and Koch, 2012; Carlisle *et al*., 2016), should be tested to determine if they impacted the present results. The present results taken in the context of other findings underline that mother–embryo isotope fractionation can occur positively or negatively for both isotopes, may be independent of developmental regime and can vary substantially within and across species. Thus, no broad batoid correction can be applied, and species-specific maternal patterns need to be determined before any isotopic corrections can occur.

The present research demonstrates the difficulties in extrapolating species-wide trends for isotopic differences from small numbers of litters. Most studies have access to only a handful of mothers and pups, and this reduces the possibility of assessing effect size. Explaining the observed variability in embryonic isotope ratios within and across species of elasmobranchs without additional controlled experiments is difficult. Perhaps the most parsimonious explanation is maternal provisioning of higher-quality nutrients through higher lipid content in yolk, histotroph or livers, which in this case possibly explained the differences between embryo and juvenile/adult C:N ratios in *N. tasmaniensis*. McMeans *et al*. (2009) suggest that this may occur in sharks; nonetheless, their studies had higher relative $\delta^{15}N$ in embryos, which was not the case in *N. tasmaniensis*, occurred in only some *U. paucimaculatus* and is not observed in other studies (Le Bourg *et al*., 2014). In cases where embryos are depleted in ^{15}N, it suggests that in those species the maternal provisioning of embryos is of lower quality, which is known to have transgenerational negative fitness consequences for offspring of invertebrates within species (Frost *et al*., 2010). Maternal provisioning also implies an energetic cost to mothers, however, and there was no evidence of change in the mothers' isotope ratios compared to other adults for both *N. tasmaniensis* and *U. paucimaculatus* in the present study.

Studies examining trophic fractionation often assume that C:N ratios and by association lipid and urea content are relatively constant within species. Authors' results show that within species, there can be significant differences in C:N ratios between adults and embryos, a difference in ratio as large as *c*. 0.5. These differences may be explained by a lack of lipid and urea extractions in this study, which can impact stable isotope measurements in elasmobranchs (Kim and Koch, 2012; Carlisle *et al*., 2016), but by association suggest that lipid and urea extractions may mask differences in tissue composition between embryos and their mothers. The associated implication with higher C:N ratios is that embryos have higher lipid and urea content in their muscles than their mothers, and because the mothers had similar C:N ratios as other adults and juveniles, this was not a result of lipid

depletion in mother muscle tissues. This effect could be a means for newborns to have higher stores of energy post parturition, typically considered to be associated with internalisation of yolk (Rodda and Seymour, 2008) or enlarged livers (Gilmore *et al*., 1983; Hussey *et al*., 2010b), and should be investigated further.

The location of the muscle tissue may also affect the results, although this has not been tested. Because the present analyses were on white muscle tissue, which is not a primary source of energy reserves in elasmobranchs, it is possible that authors did not detect the effects of maternal provisioning on the mothers' muscle tissue. Livers are the main form of energy storage in elasmobranchs and are twice as energy dense as muscle (Pethybridge *et al*., 2014). The few studies that have examined maternal isotope signatures relative to maternal muscle and liver found conflicting results, with embryo signatures having a greater difference relative to maternal liver tissue and liver tissue with little difference relative to maternal muscle (McMeans *et al*., 2009; Le Bourg *et al*., 2014). These results suggest that although maternal liver tissue may be where nutrients are sourced for embryonic development, the higher temporal variability of the tissue may make it less effective at predicting embryo isotope signature relative to muscle tissue in species that exhibit diet shifting, which may be a better indicator of maternal isotope ratio over the gestation term.

The degree of embryonic isotope fractionation can vary with length of embryos during development (Grey, 2001; McMeans *et al*., 2009; Olin *et al*., 2018). The results of the present study do not corroborate these previous studies, as no significant relationships between embryo size and stable isotope values in any of the species were observed. Because the species examined here are aplacental, differences between these results and previous research could be due to the lack of a shift from yolk to placental feeding (as suggested for placentatrophic species in Olin *et al*., 2018) in these species. Future controlled experiments should measure the effects of maternal provisioning on mothers and offspring over the pregnancy, which would also aid in understanding the relationship between maternal muscle and liver tissues during embryonic development.

This study provides a multispecies comprehensive assessment of the isotopic relationship between mothers and their embryos in multiple species of elasmobranchs. Due to the differences in fractionation patterns across species, it is improbable that a single estimate of isotopic fractionation can be applied across species of elasmobranchs to correct for maternal isotope signatures. This conclusion is in line with the suggestion from Olin *et al*. (2011) that species-specific assessments are necessary. The authors recommend that any research that focuses on isotope ratios in elasmobranch neonates or young-of-the-year use caution when extrapolating from such data.

ACKNOWLEDGEMENTS

We thank the crew of the *FTV Bluefin* for all their assistance and good nature in challenging conditions. We thank Associate Professor Chris Scarlett for allowing us to use his chartered vessel. This research was supported by funding and facilities provided by the University of Newcastle and Macquarie University. Open access publishing facilitated by The University of Newcastle, as part of the Wiley - University of Newcastle agreement via the Council of Australian University Librarians.

AUTHOR CONTRIBUTIONS

V.R., J.-M.P., J.E.W. and T.F.G. designed the study. V.R., J.E.W. and T.F.G. conducted fieldwork and collected the samples. V.R., J.-M.P., C.S. and V.D. processed the samples. V.R. analysed the data. V.R., J.-M.P., J.E.W. and T.F.G. contributed to the manuscript. J.E.W. and T.F.G. contributed to and sourced funding for this project. All authors provided final approval for the publication of this manuscript.

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SUPPORTING INFORMATION

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How to cite this article: Raoult, V., Gaston, T. F., Smith, C., Dolfo, V., Park, J.-M., & Williamson, J. E. (2022). Patterns of mother–embryo isotope fractionation in batoids vary within and between species. *Journal of Fish Biology*, 1–9. https://doi.org/10.1111/jfb.15034