JOURNAL OF **FISH** BIOLOGY

REGULAR PAPER

Resource partitioning between two young-of-year cownose rays *Rhinoptera bonasus* and *R. brasiliensis* within a communal nursery inferred by trophic biomarkers

Bianca de Sousa Rangel[1] | Nigel E. Hussey[2] | Aline D. Gomes[1] | Alexandre Rodrigues[3] | Luiz A. Martinelli[4] | Renata Guimarães Moreira[1]

[1]Departamento de Fisiologia, Laboratório de Metabolismo e Reprodução de Organismos Aquáticos, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil

[2]University of Windsor – Biological Sciences, Windsor, Ontario, Canada

[3]Laboratório de Biologia e Genética de Peixes, Instituto de Biociências de Botucatu, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil

[4]Departamento de Ecologia Isotópica, Centro de Energia Nuclear na Agricultura (CENA), Universidade de São Paulo, Piracicaba, São Paulo, Brazil

Correspondence
B.S. Rangel, Departamento de Fisiologia, Laboratório de Metabolismo e Reprodução de Organismos Aquáticos, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, travessa 14, 321, CEP 05508-090, Cidade Universitária, São Paulo, SP, Brazil.
Email: biarangel.sharks@gmail.com

Although interspecific trophic interactions plays a principal role within elasmobranch communal nurseries, little is known over variation in foraging strategies adopted by young-of-year of sympatric species. To test the hypothesis of dietary resource partitioning between batoids within a communal nursery, we investigated two cownose ray species, *Rhinoptera bonasus* and *R. brasiliensis*, which occur in heterospecific groups, a strategy predicted to increase survival and foraging success. Using two biochemical tracers, fatty acids (FA) and stable isotopes (δ^{15}N and δ^{13}C), the combined effects of maternal investment and the formation of heterospecific groups implying competition for, or partitioning of available food resources were investigated. Through univariate and multivariate analyses of biochemical tracers in several tissues (fin clip, muscle, liver, red blood cells; RBC) and plasma, our results revealed significant interspecific differences in tracers between the two species. Total FAs ($\sum$saturated FA, $\sum$monounsaturated FA and $\sum$polyunsaturated FA) and trophic biomarkers (*i.e.*, docosahexaenoic acid, arachidonic acid, oleic acid and δ^{15}N) were the principle tracers responsible for the differences detected. These data revealed that *R. brasiliensis* was less enriched in physiologically important essential FAs than *R. bonasus*. Our findings suggest that these congeneric species differ in maternal investment strategy and moderately partition food resources over relatively fine spatial scales within a single nursery habitat to limit competition. These results provide further knowledge on the foraging strategies adopted by batoids in communal nursery areas, information that is required for improving spatial conservation and management planning.

KEYWORDS
elasmobranch, fatty acids, life history strategy, maternal investment, stable isotopes, trophic ecology

1 | INTRODUCTION

Elasmobranch nurseries are habitats (commonly shallow protected coastal waters) where juveniles reside to maximise their fitness and survival during this early and high-risk life history stage (reviewed by Heupel *et al.*, 2007; Heupel *et al.*, 2019; Martins *et al.*, 2018). Of the two strategies proposed for how elasmobranchs utilise a nursery ground (*i.e.*, single or multi-species occupancy), multi-species occupancy

or a communal nursery involves the co-occurrence of juveniles of more than one species within a defined area (Heupel *et al*., 2019; Simpfendorfer & Milward, 1993). This communal strategy is considered to offer benefits through increased protection against predators, but equally may incur a cost or trade-off in terms of increased competition for available prey resources (Simpfendorfer & Milward, 1993). Accordingly, understanding the dynamics and consequences of interactions among juveniles within nurseries allows predictions of resource

availability *v.* resource needs and development of foraging abilities, which are essential components of ecological processes linked to habitat use, behaviour and overall energetic demands (Hussey *et al.*, 2017; Kinney *et al.*, 2011).

In rays, the formation of heterospecific groups has been observed, for example, in sub-adult stingrays *Pastinachus sephen* (Forsskål 1775) and *Himantura uarnak* (Gmelin 1789) (Semeniuk & Dill, 2006) and juvenile cownose rays *Rhinoptera bonasus* (Mitchill 1815) and *Rhinoptera brasiliensis* (Müller, 1836) (Figure 1; Rangel *et al.*, 2017, 2018). Co-occurrence has been hypothesised as a strategy adopted to increase survival, by reducing predation pressure per individual and increased foraging success to meet energetic requirements (Semeniuk & Dill, 2006). Foraging success however, is dependent on trophic interactions among congeneric rays, with individuals either competing for the same resources (*e.g.*, prey at the same trophic level) or partitioning prey to minimise competition (*e.g.*, consuming different prey at the same trophic level or at different trophic levels; Kinney *et al.*, 2011).

Biochemical tracers provide a promising tool to explore trophic interactions among juvenile organisms through allowing, for example, exploration of rapid ontogenetic shifts in resource use and competition among conspecifics over time (*e.g.*, Beckmann *et al.*, 2014; Matich *et al.*, 2015; Pethybridge *et al.*, 2018). However, when examining the biochemical signatures of juveniles, several key implications must be considered: (1) values in tissues, specifically for neonatal animals, reflect the maternal signal as a consequence of prenatal maternal investment (*i.e.*, through yolk, histotroph, placenta; *e.g.*, Olin *et al.*, 2011); (2) the timescale needed for juveniles to develop foraging skills (*i.e.*, the transfer rate from prenatal to postnatal resource use; *e.g.*, Belicka *et al.*, 2012; Matich *et al.*, 2015) and (3) the turnover rate of the tracer in the tissue analysed (*i.e.*, muscle *v.* liver or plasma; slow *v.* fast; Beckmann *et al.*, 2013; Kim *et al.*, 2012; MacNeil *et al.*, 2005).

In this study, both fatty acids (FA) and stable isotopes (SI, δ^{15}N and δ^{13}C) were used to explore the combined effects of maternal investment and if the formation of heterospecific groups implies competition or resource partitioning of available food resources between *R. bonasus* and *R. brasiliensis*. Given that co-occurring juvenile sharks can exhibit a degree of dietary resource partitioning to avoid or reduce competitive interactions (*e.g.*, Kinney *et al.*, 2011), we predict that resource partitioning of food resources will also occur among young-of-the-year (YOY) of the two cownose ray species within a communal nursery (Figure 1). To assess the temporal scale of the maternal influence effectively and adopted foraging strategy in the two species, plasma and multiple tissues (fin clip, muscle, liver, red blood cells; RBC) with different turnover rate were analysed. These data will improve knowledge on the use of communal nursery areas by batoids and interspecific resource partitioning strategies adopted by sympatric congeneric species, information which is required for long-term management (Martins *et al.*, 2018).

2 | MATERIALS AND METHODS

All research was conducted under appropriate research permits provided from the SISBIO (ICMBIO/SISBIO # 48572–1) and the Animal Ethics Committee (CEUA; # 258/2016) of the Institute of Biosciences, University of São Paulo.

2.1 | Sampling site and capture

Young-of-the-year (< 12 months old, 32–70 cm disc width, W_D; Fisher *et al.*, 2013; Rangel, 2018; B. Rangel, A. Gomes, N. Hussey, L. Martinelli & R. Moreira, unpubl. data) of *R. bonasus* and *R. brasiliensis* were collected in Bertioga, south-eastern Brazil (23° 49′ 35.02″ S, 46° 5′ 41.69″ W) between March 2016 and February 2017.
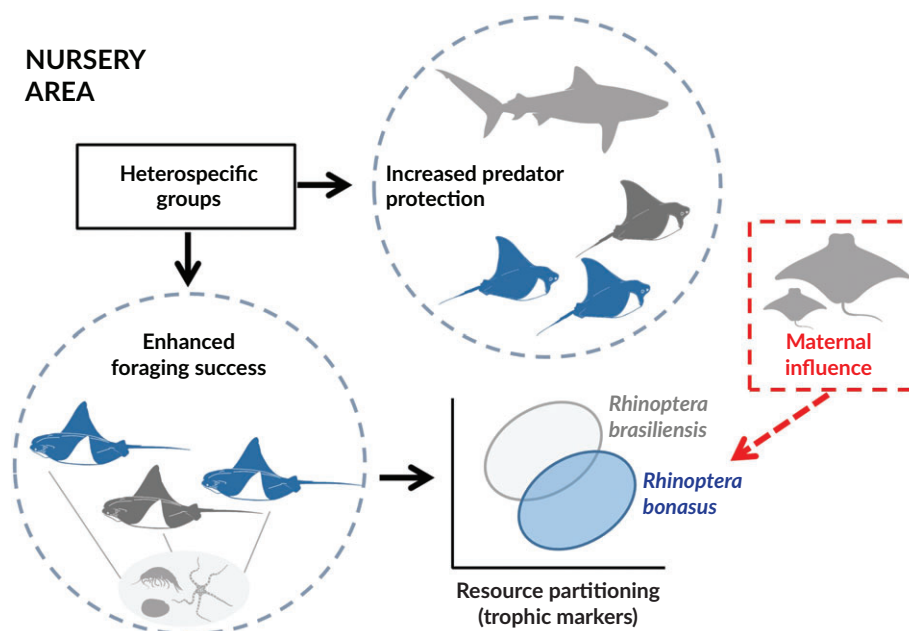

FIGURE 1 Schematic showing the formation of heterospecific groups of congener species of *Rhinoptera bonasus* and *R. brasiliensis* related to proposed benefits of occupying a single nursery area (Rangel *et al.*, 2017), including a prediction of expected dietary resource partitioning (Kinney *et al.*, 2011), but also accounting for maternal resource allocation

The region functions as a nursery area for the two cownose ray species (Rangel *et al.*, 2018), according to the criteria proposed by Heupel *et al.* (2007). All specimens were sampled following incidental capture by fishermen using beach seine nets targeting snook *Centropomus* spp. (Rangel *et al.*, 2018). Sampling was limited to these opportunistic fisheries captures as *R. bonasus* and *R. brasiliensis* are classified as Near Threatened and Endangered in the IUCN Red List assessment, respectively (Barker, 2006; Vooren & Lamónaca, 2004), while *R. brasiliensis* is categorised as Critically Endangered in the Brazilian national assessment (ICMBio, 2016).

Following the recording of biometric data (W_D, cm), a snip of the trailing edge of the dorsal fin (*c.* 100 mg), a muscle plug (*c.* 50 mg, using a 6 mm biopsy punch) and blood were taken from each individual using minor invasive approaches prior to their release. Blood samples (*c.* 1 ml) collected by caudal venipuncture were centrifuged for 10 min (655.2 *g*) to RBC and plasma (Rangel, 2018). Individuals that died during the capture process were also sampled, with additional liver samples taken. All tissue and blood samples were stored at −80°C until analysis.

2.2 | Analytical approach

To examine FA profiles, total lipids were extracted from muscle and liver samples using a chloroform–methanol–water solution (2.0:1.0:0.5, v:v:v), according to Folch *et al.* (1957) and then methylated with acetyl chloride (5% HCl in methanol; Christie, 2003). Blood plasma samples were directly methylated following the same protocol (Christie, 2003). FA analysis was carried out in a Varian gas chromatograph (GC; Model 3900; www.varian.com) coupled with a flame ionisation detector (FID) and a CP-8410 autosampler, as described by Gomes *et al.* (2016). The data are presented as % of total FA methylesters based on peak area analyses.

For stable isotope (δ^{15}N and δ^{13}C) analysis, RBC and fin-clip samples were lyophilised, homogenised and 400–600 µg of material weighed into tin capsules. The isotopic composition was determined by on-line combustion of samples by continuous flow–isotope ratio mass spectrometry (CF-IRMS), using an elemental analyser (Model 1110; Carlo Erba' www.carloerbareagents.com) interfaced to an isotope ratio mass spectrometer (IR-MS; ThermoQuest-Finnigan, Delta Plus; Thermo Fisher; www.thermofisher.com).

2.3 | Data analysis

To explore differences in muscle, liver and plasma FA profiles between the two congeneric ray species, non-parametric multi-dimensional scaling (nMDS) and analysis of similarities (ANOSIM) were conducted. To identify which FAs contributed to average dissimilarity between species, similarity percentage (SIMPER) tests were performed. For the multivariate analysis, twelve FAs were selected based on their abundance in all samples; C17:0, C16:0, C18:0, C16:1n7, C18:1n9, C18:1n7, C20:5n3, C22:5n3, C22:6n3 (docosahexaenoic acid; DHA), C20:4n6 (arachidonic acid; ARA), C22:4n6, C22:5n6. All above analyses were based on Bray–Curtis similarity matrices. Individual FAs (muscle, liver and plasma) and SI values (δ^{15}N and δ^{13}C; fin and RBC) were then compared between the two cownose ray species using

Student's *t*-test. For data with a non-normal distribution and small sample sizes (liver and plasma), a non-parametric Mann–Whitney Rank Sum test was used. We specifically analysed biochemical tracers in multiple tissues with different turnover rates to assist in differentiating between the effects of maternal influence (high turnover tissue) and foraging strategy adopted (slow turnover tissue) by the two species. Statistical significance was declared at *P* < 0.05. and all analyses were conducted in SigmaStat 3.10 (SystatSoftware, Inc.; www.systat.com) and PAST 3.12 (EFB; www.essential-freebies.de).

3 | RESULTS

Multivariate analysis revealed that muscle FAs for *R. bonasus* (*n* = 19; 44.2 ± 5.68 cm W_D; mean ± SD) and *R. brasiliensis* (*n* = 8; 47.3 ± 6.83 cm W_D) were significantly different, but a high degree of overlap was observed (ANOSIM *R* = 0.30, *P* < 0.01; SIMPER = 80.3%; Figure 2a and Supporting Information Table S2). SIMPER analysis found that ARA was the primary FA contributing 70.9% to the observed dissimilarity between the two species (Supporting Information Table S2). Univariate analysis revealed that *R. brasiliensis* muscle exhibited higher values of saturated FA (SFA; *P* < 0.05) and monounsaturated FA (MUFA; *P* < 0.01), including C18:1n9 (*P* < 0.05) and C18:1n7 (*P* < 0.01) when compared with *R. bonasus* (Figure 2a,c). In contrast, *R. bonasus* had higher values of polyunsaturated FA (PUFA; *P* < 0.001), n3 PUFA (*P* < 0.01) and n6 PUFA (*P* < 0.001), including DHA (*P* < 0.01) and ARA (*P* < 0.001) than *R. brasiliensis* (Figure 2c and Supporting Information Table S1).

For liver, multivariate analysis also found distinct FA profiles for *R. bonasus* (*n* = 6; 42.6 ± 6.10 cm W_D; mean ± SD) and *R. brasiliensis* (*n* = 5; 46.2 ± 6.79 cm W_D) and a lesser degree of overlap was observed (ANOSIM *R* = 0.62, *P* < 0.01; SIMPER = 22.9%; Figure 2b and Supporting Information Table S2). SIMPER analysis revealed that C16:0, DHA, C18:0 and ARA were the main FAs contributing to 56.8% of the observed dissimilarity between the two species (Supporting Information Table S2). Identical to muscle, *R. bonasus* liver tissue exhibited higher values of PUFA (*P* < 0.001), n3 PUFA (*P* < 0.01) and n6 PUFA (*P* < 0.01) than *R. brasiliensis* (*n* = 4; Figure 2b, d), which had higher values of SFA (*P* < 0.01). With regard to SFA, higher values of C16:0 (*P* < 0.01), C18:0 (*P* < 0.05) and C14:0 (*P* < 0.01), to MUFA, C16:1n7 (*P* < 0.01), C18:1n7 (*P* < 0.05) and lower values of DHA (*P* < 0.05) and C22:4n6 (*P* < 0.05) were recorded for *R. brasiliensis* when compared with *R. bonasus* (Figure 2d and Supporting Information Table S1).

There were no significant differences in blood plasma FA profiles between the two species (Figure 3), but this was a result of the low sample size analysed (*n* = 4, 50.5 ± 7.94 cm W_D and *n* = 3, 52.8 ± 0.76 cm W_D, mean ± SD; *R. bonasus* and *R. brasiliensis*, respectively). Variation in the blood plasma FA profiles, however, followed similar trends to those recorded in muscle and liver (Figure 2).

The δ^{15}N and δ^{13}C in *R. brasiliensis* fin tissue (*n* = 14, 51.4 ± 6.57 cm W_D, mean ± SD) were higher than those in RBC (*n* = 8; 52.1 ± 3.19 cm W_D, *P* < 0.001; Figure 4). When comparing the two species, ^{15}N values were enriched in dorsal fin and RBC of *R. bonasus* compared with *R. brasiliensis* (*P* < 0.01, *P* < 0.01,
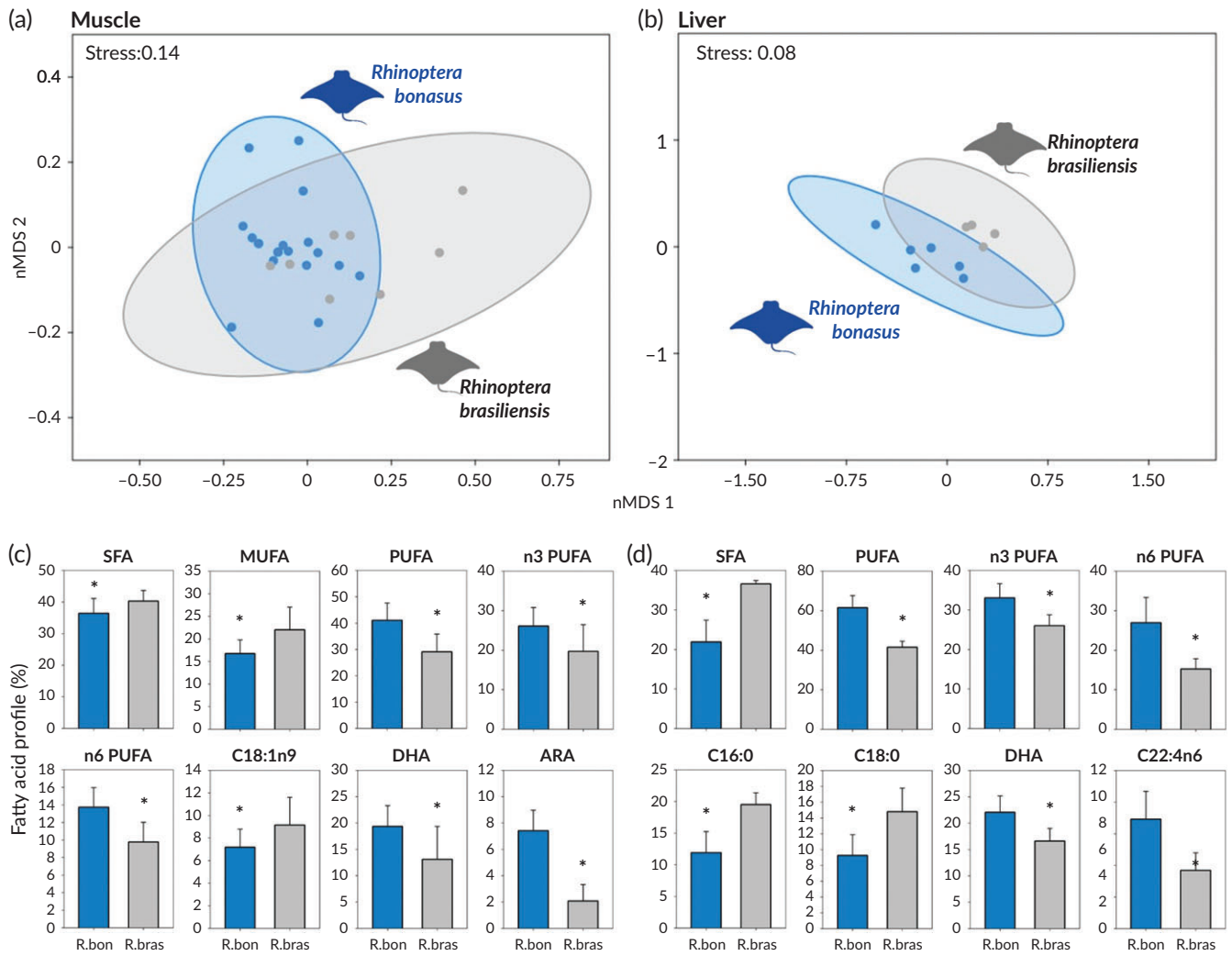
FIGURE 2 (a) Bray–Curtis non-metric multidimensional scaling (nMDS) ordinations of fatty acid (FA) profile in muscle and (b) liver and the mean (± SD) FA profiles in (c) muscle and (d) liver of *Rhinoptera bonasus* (■) and *R. brasiliensis* (□). * Statistically significant difference between species (*P* < 0.05). SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; DHA, docosahexaenoic acid. (Only FA with values of >5% are presented; for full data see Supporting Information Table S1)
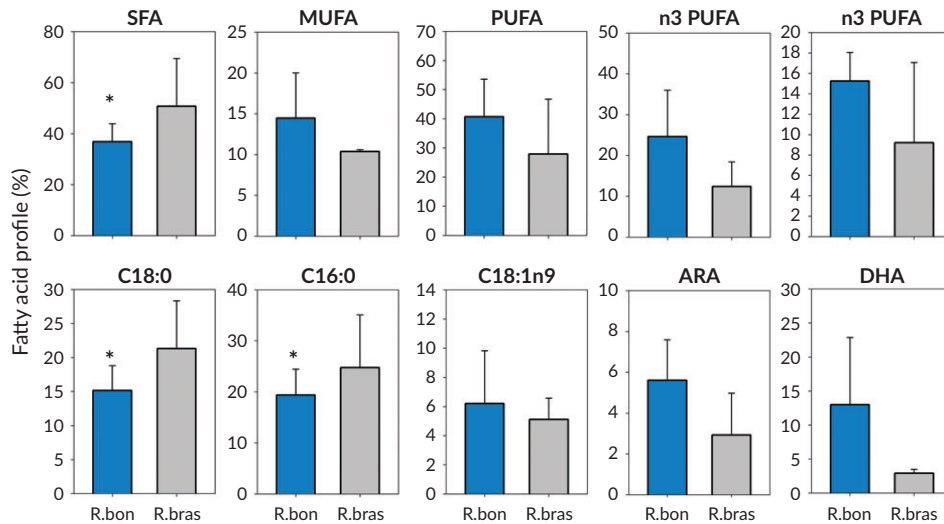

FIGURE 3 Mean (± SD) of the main blood plasma fatty acid profiles (contributing >5%) for *Rhinoptera bonasus* (■) and *R. brasiliensis* (□). SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; DHA, docosahexaenoic acid; ARA, arachidonic acid

respectively). For δ^{13}C, RBC values were also higher in *R. bonasus* compared with *R. brasiliensis* ($P < 0.05$), but no significant difference in fin δ^{13}C values was found between the two ray species (Figure 4).

4 | DISCUSSION

Through a comparison of nutritional, trophic and habitat biomarkers (FAs and SI) in several tissues (fin clip, muscle, liver and RBC) and plasma of two sympatric cownose rays, this study explored differences in maternal investment and foraging strategies and assessed resource partitioning within a batoid nursery. Our results demonstrate that these congeneric species differ in maternal investment strategy (*i.e.*, energy resource allocation) and moderately partition food resources, supporting our prediction of reduced competitive interactions. This appears to be a strategy generally adopted by sympatric elasmobranch species inhabiting communal nursery areas (*e.g.*, Kinney *et al.*, 2011; Matich *et al.*, 2017). Our multi-tracer, multi-tissue approach examining different sized individuals for the two species provides a comprehensive framework to explore ontogenetic switches in resource use from maternal to individual foraging in YOY animals (Figure 5).

The $\sum$FAs (*i.e.*, $\sum$SFA, $\sum$MUFA and $\sum$PUFA) and trophic markers (*i.e.*, DHA, ARA and δ^{15}N) were the main tracers responsible for the differences detected between the two cownose ray species across all tissues. Overall, *R. brasiliensis* was less enriched in physiologically important essential FAs (*e.g.*, DHA and ARA; Sargent *et al.*, 1999; Tocher, 2003; Arts & Kohler, 2009) and depleted in ^{15}N compared with *R. bonasus*. These consistent trends across all tissues indicate that the two cownose ray species vary in maternal and trophic relationships (*i.e.*, YOY and mothers).

While liver, muscle and plasma FA profiles are considered to directly reflect that of diet or maternal-foetal transfer (*e.g.*, Beckmann *et al.*, 2014; McMeans *et al.*, 2012), interpreting FA profiles of juveniles is dependent on the size of the animal and tissue turnover rate and consequently can reflect either the maternal signature, actual
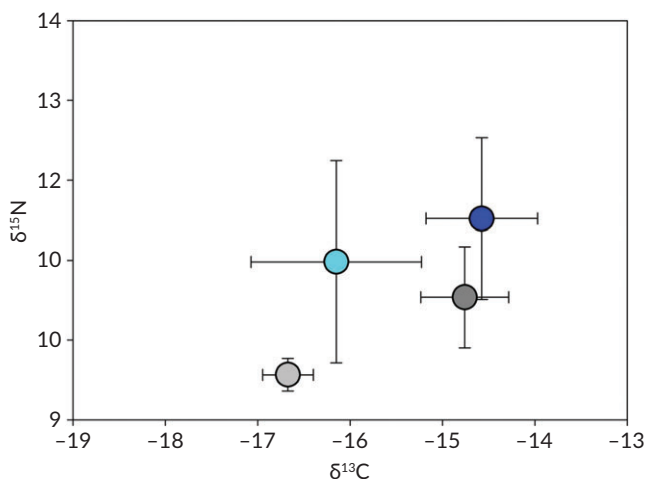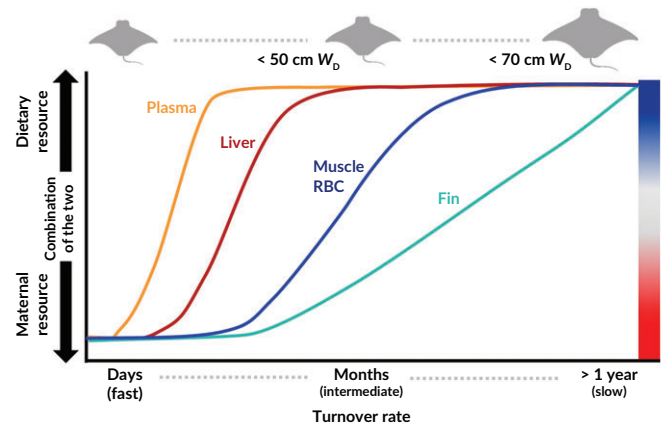

FIGURE 5 Conceptual model of the dynamic state of fatty acids (FA) and stable isotopes (SI) in different tissues and the maternal resource dependency or actual diet during the growth of young-of-the-year (< 1 year old) cownose ray species. The model acknowledges that turnover rates among FAs and SIs vary and are dependent on animal size and growth rate (Vander Zanden *et al.*, 2015), but provide a reasonable approximation

juvenile diet or a combination of both (Belicka *et al.*, 2012; Figure 5). When considering only FA profiles, liver tissue showed the strongest difference between species, with up to 18% less PUFA in liver of *R. brasiliensis* when compared with *R. bonasus*. Despite this, *R. brasiliensis* did not show an essential FA deficiency in liver and muscle tissue as previously reported for young placental sharks at birth (*i.e.*, DHA and ARA < 6%; Belicka *et al.*, 2012; Wai *et al.*, 2012). Considering that liver tissue was principally analysed for YOY < 50 cm W_D *R. brasiliensis* (< 4 months old, Fisher *et al.*, 2013; Rangel *et al.*, 2018) and it has a relatively fast turnover rate (Figure 5; *c.* 3 weeks; Beckmann *et al.*, 2013), differences in liver FA profiles between the two species suggest access to different resources, most likely during the prenatal phase (*i.e.*, variable maternal resources; Figure 5). These data consequently provide evidence for females of the two cownose ray species either consuming different prey resources during gestation, or that their maternal strategy differs through variable allocation of essential FAs during gestation.

The size of YOY *R. braziliensis* for which muscle tissue was analysed ranged between 35.5 and 56 cm W_D (birth size 43–48 cm W_D; Vooren & Lamónaca, 2004). This size range suggests that the muscle FA profiles represent a combination of maternal resource dependency and actual diet given its intermediate turnover rate (Figure 5; Beckmann *et al.*, 2013; Pethybridge *et al.*, 2015). Similarly, FA profiles of *R. bonasus* muscle tissue of YOY < 50 cm W_D reflected the maternal signature, whereas YOY > 50 cm W_D represented actual diet (B. Rangel, A. Gomes, N. Hussey, L. Martinelli & R. Moreira, unpubl. data). With the fastest turnover rate (and hence shorter-term indicator; Figure 5), plasma FAs of YOY individuals of *R. bonasus* and *R. brasiliensis*, provide complementary information to muscle and confirm dietary partitioning between the two cownose rays.

Combined, plasma and tissues (fin clip, muscle, liver and RBC) results suggest that YOY *R. brasiliensis* access maternal resources and prey of different trophic levels and with a lower nutritional quality (*i.e.*, lower percentages of DHA and ARA) compared with *R. bonasus*. These essential FAs play important physiological roles, for example,

FIGURE 4 Mean (± SD) δ^{15}N and δ^{13}C values in fin tissue and red blood cells (RBC) of young-of-the-year (YOY) cownose rays *Rhinoptera bonasus* (○, RBC $n = 10$; ●, fin, $n = 15$) and *R. brasiliensis* (○, RBC $n = 8$; ●, fin $n = 14$); n = sample size

DHA is important for the structure and function of cellular membranes, especially in brain tissue and retina (Sargent *et al.*, 1999; Stoknes *et al.*, 2004) and ARA is a precursor of eicosanoids, which act as signalling molecules or local hormones, involved in processes of inflammation and immune response and reproduction (Arts & Kohler, 2009; Tocher, 2003). Although *R. brasiliensis* did not show an essential FA deficiency in any tissue analysed, lower nutritional quality, potentially linked to factors such as low population size, may result in its increased vulnerability to anthropogenic influences such as fishing and pollution when compared with *R. bonasus* (Birnie-Gauvin *et al.*, 2017; Semeniuk *et al.*, 2007).

For both cownose rays, the habitat biomarker (*i.e.*, higher percentages of DHA and $\delta^{13}C$ values between -14 and -16‰) indicated that YOY rays were feeding on marine resources (Colombo *et al.*, 2016; Hussey *et al.*, 2012; Sardenne *et al.*, 2017), in addition, the $\delta^{13}C$ values and ARA levels together, indicate benthic foraging (Sardenne *et al.*, 2017). Despite this, $\delta^{15}N$ values (used as a proxy for absolute trophic level) of RBC (faster turnover rate; Kurle, 2002) and fin (slower turnover rate; Matich *et al.*, 2010) were variable and depleted in ^{15}N in *R. brasiliensis* compared with *R. bonasus*. These findings indicate the two cownose ray species feed on prey that occur in the same environment, but occupy a different trophic position, in agreement with FA profiles. In addition, $\delta^{15}N$ results of fin tissue (slow turnover rate) suggest that mature females of the two species partition resources during gestation (Figure 5). Currently, there are no comparable-isotopic data for *R. brasiliensis* and only preliminary analysis of stomach contents identifying a diet of molluscs (Vooren & Lamónaca, 2004). *Rhinoptera bonasus*, however, is known to be an opportunistic generalist, feeding on bivalves, polychaetes, crustaceans and echinoderms with numbers consumed dependent on location and availability of prey (Ajemian & Powers, 2012; Collins *et al.*, 2007). Previous isotopic data for *R. bonasus* reported $\delta^{15}N$ values of mean % SD 8.5 ± 0.3‰ (Olin *et al.*, 2014) and 9.4 ± 0.5‰ (Shaw *et al.*, 2016), slightly higher than those found in this study (and more similar to *R. brasiliensis*), but isotopic baseline data are not available to make direct comparisons.

In conclusion, cownose rays appear to partially conform to the framework of partitioning resources over relatively fine spatial scales within a single nursery habitat to limit competition. By analysing multiple biomarkers in several tissues with varying turnover rates and considering the size of juveniles, we provide a robust approach to assess maternal resources, nutritional condition and trophic interactions of YOY rays. Further research to assess the shifts in FA and SI profiles across early-life stages of *R. brasiliensis*, *i.e.*, through examining a larger sample size of different size individuals, is needed to investigate the neonatal nutritional strategy adopted by this species when compared with *R. bonasus* (B. Rangel, A. Gomes, N. Hussey, L. Martinelli & R. Moreira, unpubl. data). Identifying foraging strategies such as resource partitioning in communal nurseries is key for improving spatial conservation and management planning for batoid species (Martins *et al.*, 2018), especially when the ecological similarity of congeneric species may imply competition and reduced access to food resources that oversimplify food web interactions.

ORCID

Bianca de Sousa Rangel [ID] https://orcid.org/0000-0002-4029-765X

REFERENCES

Ajemian, M. J., & Powers, S. P. (2012). Habitat-specific feeding by cownose rays (*Rhinoptera bonasus*) of the northern Gulf of Mexico. *Environmental Biology of Fishes*, *95*, 79–97. https://doi.org/10.1007/s10641-011-9858-3.

Arts, M. T., & Kohler, C. C. (2009). Health and condition in fish: the influence of lipids on membrane competency and immune response. In *Lipids in aquatic ecosystems* (pp. 237–256). New York, NY: Springer.

Barker, A. S. (2006) *Rhinoptera bonasus*. In IUCN red list of threatened species version 2018. 2. Retrieved from https://www.iucnredlist.org/species/60128/12310195

Beckmann, C. L., Mitchell, J. G., Seuront, L., Stone, D. A. J., & Huveneers, C. (2014). From eggs to hatchling: Preferential retention of fatty acids biomarkers in young-of-the-year Port Jackson sharks *Heterodontus portusjacksoni*. *Journal of Fish Biology*, *85*, 944–952. https://doi.org/10.1111/jfb.12451.

Beckmann, C. L., Mitchell, J. G., Stone, D. A., & Huveneers, C. (2013). A controlled feeding experiment investigating the effects of a dietary switch on muscle and liver fatty acid profiles in Port Jackson sharks *Heterodontus portusjacksoni*. *Journal of Experimental Marine Biology and Ecology*, *448*, 10–18. https://doi.org/10.1016/j.jembe.2013.06.009.

Belicka, L. L., Matich, P., Jaffé, R., & Heithaus, M. R. (2012). Fatty acids and stable isotopes as indicators of early-life feeding and potential maternal resource dependency in the bull shark *Carcharhinus leucas*. *Marine Ecology Progress Series*, *455*, 245–256. https://doi.org/10.3354/meps09674.

Birnie-Gauvin, K., Peiman, K. S., Raubenheimer, D., & Cooke, S. J. (2017). Nutritional physiology and ecology of wildlife in a changing world. *Conservation Physiology*, *5*, cox030. https://doi.org/10.1093/conphys/cox030.

Christie, W. W. (2003). *Lipids analysis*. Bridgwater, England: The Oily Press.

Collins, A. B., Heupel, M. R., Hueter, R. E., & Motta, P. J. (2007). Hard prey specialists or opportunistic generalists? An examination of the diet of the cownose ray, *Rhinoptera bonasus*. *Marine and Freshwater Research*, *58*, 135–144. https://doi.org/10.1071/MF05227.

Colombo, S. M., Wacker, A., Parrish, C. C., Kainz, M. J., & Arts, M. T. (2016). A fundamental dichotomy in long-chain polyunsaturated fatty acid abundance between and within marine and terrestrial ecosystems. *Environmental Reviews*, *25*, 163–174. https://doi.org/10.1139/er-2016-0062.

Fisher, R. A., Call, G. C., & Grubbs, R. D. (2013). Age, growth and reproductive biology of cownose rays in Chesapeake Bay. *Marine and Coastal Fisheries*, *5*, 224–235. https://doi.org/10.1080/19425120.2013.812587.

Folch, J., Less, M., & Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry*, *226*, 497–503.

Forskål, P. (1775). Descriptiones animalium, avium, amphibiorum, piscium, insectorum, vermium / quae in itinere orientali observavit Petrus Forskål. Post mortem auctoris edidit Carsten Niebuhr. Adjuncta est

materia medica kahirina atque tabula maris Rubri geographica. Post mortem auctoris edidit Carsten Niebuhr. Hauniae. Descriptiones animalium quae in itinere ad Maris Australis terras per annos 1772 1773 et 1774 suscepto, 1–20 + i–xxxiv + 1–164, map. pp. x–xix and 22–76.

Gmelin, J. F. (1789). Caroli a Linne. Systema Naturae per regna tria naturae, secundum classes, ordines, genera, species; cum characteribus, differentiis, synonymis, locis. Editio decimo tertia, aucta, reformata, 3 vols in 9 parts. Lipsiae, 1788-93. Systema Naturae Linné, 1, 1033–1516.

Gomes, A. D., Tolussi, C. E., Boëchat, I. G., Pompêo, M. L., Cortez, M. P., Honji, R. M., & Moreira, R. G. (2016). Fatty acid composition of tropical fish depends on reservoir trophic status and fish feeding habit. Lipids, 51, 1193–1206. https://doi.org/10.1007/s11745-016-4196-z.

Heupel, M. R., Carlson, J. K., & Simpfendorfer, C. A. (2007). Shark nursery areas: Concepts, definition, characterization and assumptions. Marine Ecology Progress Series, 337, 287–297. https://doi.org/10.3354/MEPS337287.

Heupel, M. R., Kanno, S., Martins, A. P., & Simpfendorfer, C. A. (2019)). Advances in understanding the roles and benefits of nursery areas for elasmobranch populations. Marine and Freshwater Research In press. https://doi.org/10.1071/MF18081.

Hussey, N. E., DiBattista, J. D., Moore, J. W., Ward, E. J., Fisk, A. T., Kessel, S., … Gruber, S. H. (2017). Risky business for a juvenile marine predator? Testing the influence of foraging strategies on size and growth rate under natural conditions. Proceedings of the Royal Society B, 284(1852), 20170166. https://doi.org/10.1098/rspb.2017.0166.

Hussey, N. E., MacNeil, M. A., Olin, J. A., McMeans, B. C., Kinney, M. J., Chapman, D. D., & Fisk, A. T. (2012). Stable isotopes and elasmobranchs: Tissue types, methods, applications and assumptions. Journal of Fish Biology, 80, 1449–1484. https://doi.org/10.1111/j.1095-8649.2012.03251.x.

Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). (2016). Avaliação do risco de extinção dos elasmobrânquios e quimeras no Brasil: 2010–2012. Itajaí: CEPSUL.

Kim, S. L., del Rio, C. M., Casper, D., & Koch, P. L. (2012). Isotopic incorporation rates for shark tissues from a long-term captive feeding study. Journal of Experimental Biology, 215, 2495–2500. https://doi.org/10.1242/jeb.070656.

Kinney, M. J., Hussey, N. E., Fisk, A. T., Tobin, A. J., & Simpfendorfer, C. A. (2011). Communal or competitive? Stable isotope analysis provides evidence of resource partitioning within a communal shark nursery. Marine Ecology Progress Series, 439, 263–276. https://doi.org/10.3354/MEPS09327.

Kurle, C. M. (2002). Stable-isotope ratios of blood components from captive northern fur seals (Callorhinus ursinus) and their diet: Applications for studying the foraging ecology of wild otariids. Canadian Journal of Zoology, 80, 902–909. https://doi.org/10.1139/z02-069.

MacNeil, M. A., Skomal, G. B., & Fisk, A. T. (2005). Stable isotopes from multiple tissues reveal diet switching in sharks. Marine Ecology Progress Series, 302, 199–206. https://doi.org/10.3354/meps302199.

Martins, A. P. B., Heupel, M. R., Chin, A., & Simpfendorfer, C. A. (2018). Batoid nurseries: Definition, use and importance. Marine Ecology Progress Series, 595, 253–267. https://doi.org/10.3354/MEPS12545.

Matich, P., Heithaus, M. R., & Layman, C. A. (2010). Size-based variation in inter-tissue comparisons of stable carbon and nitrogen isotopic signatures of bull sharks (Carcharhinus leucas) and tiger sharks (Galeocerdo cuvier). Canadian Journal of Fisheries and Aquatic Sciences, 67, 877–885. https://doi.org/10.1139/F10-037.

Matich, P., Kiszka, J. J., Heithaus, M. R., Mourier, J., & Planes, S. (2015). Short-term shifts of stable isotope (δ^{13}C, δ^{15}N) values in juvenile sharks within nursery areas suggest rapid shifts in energy pathways. Journal of Experimental Marine Biology and Ecology, 465, 83–91. https://doi.org/10.1016/j.jembe.2015.01.012.

Matich, P., Kiszka, J. J., Mourier, J., Planes, S., & Heithaus, M. R. (2017). Species co-occurrence affects the trophic interactions of two juvenile reef shark species in tropical lagoon nurseries in Moorea (French Polynesia). Marine Environmental Research, 127, 84–91. https://doi.org/10.1016/j.marenvres.2017.03.010.

McMeans, B. C., Arts, M. T., & Fisk, A. T. (2012). Similarity between predator and prey fatty acid profiles is tissue dependent in Greenland sharks (Somniosus microcephalus): Implications for diet reconstruction. Journal of Experimental Marine Biology and Ecology, 429, 55–63. https://doi.org/10.1016/j.jembe.2012.06.017.

Mitchill, S. L. (1815). The fishes of New York described and arranged. Transactions of the Literary and Philosophical Society of New York, 355–492.

Müller, J. (1836). Vergleichende Anatomie der Myxinoiden, der Cyclostomen mit durchbohrtem Gaumen. Erster Theil. Osteologie und Myologie. Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlin, 1834: 65–340, Pls. 1–9.

Olin, J. A., Hussey, N. E., Fritts, M., Heupel, M. R., Simpfendorfer, C. A., Poulakis, G. R., & Fisk, A. T. (2011). Maternal meddling in neonatal sharks: Implications for interpreting stable isotopes in young animals. Rapid Communications in Mass Spectrometry, 25, 1008–1016. https://doi.org/10.1002/rcm.4946.

Olin, J. A., Poulakis, G. R., Stevens, P. W., DeAngelo, J. A., & Fisk, A. T. (2014). Preservation effects on stable isotope values of archived elasmobranch fin tissue: Comparisons between frozen and ethanol-stored samples. Transactions of the American Fisheries Society, 143, 1569–1576. https://doi.org/10.1080/00028487.2014.954055.

Pethybridge, H. R., Choy, C. A., Polovina, J. J., & Fulton, E. A. (2018). Improving marine ecosystem models with biochemical tracers. Annual Review of Marine Science, 10, 199–228. https://doi.org/10.1146/annurev-marine-121916-063256.

Pethybridge, H. R., Parrish, C. C., Morrongiello, J., Young, J. W., Farley, J. H., Gunasekera, R. M., & Nichols, P. D. (2015). Spatial patterns and temperature predictions of tuna fatty acids: tracing essential nutrients and changes in primary producers. PLoS One, 10, e0131598. https://doi.org/10.1371/journal.pone.0131598.

Rangel, B. S. (2018) Lipids and stable isotopes as indicators of maternal investment and neonatal nutritional strategies in histotrophic stingrays (Master Dissertation). Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil.

Rangel, B. S., Cruz, V. P., Rodrigues, A., Araujo, M. L. G., Oliveira, C., Foresti, F., & Moreira, R. G. (2017). Sympatric and syntopic occurrence of cownose rays: Neonatal strategies for survival? Journal Applied Ichthyology, 33, 542–545. https://doi.org/10.1111/jai.13343.

Rangel, B. S., Rodrigues, A., & Moreira, R. G. (2018). Use of a nursery area by cownose rays (Rhinopteridae) in Southeastern Brazil. Neotropical Ichthyology, 16, e170089. https://doi.org/10.1590/1982-0224-20170089.

Sardenne, F., Hollanda, S., Lawrence, S., Albert-Arrisol, R., Degroote, M., & Bodin, N. (2017). Trophic structures in tropical marine ecosystems: A comparative investigation using three different ecological tracers. Ecological Indicators, 81, 315–324. https://doi.org/10.1016/j.ecolind.2017.06.001.

Sargent, J., Bell, G., McEvoy, L., Tocher, D., & Estevez, A. (1999). Recent developments in the essential fatty acid nutrition of fish. Aquaculture, 177, 191–199. https://doi.org/10.1016/S0044-8486(99)00083-6.

Semeniuk, C. A., & Dill, L. M. (2006). Anti-predator benefits of mixed-species groups of cowtail stingrays (Pastinachus sephen) and whiprays (Himantura uarnak) at rest. Ethology, 112, 33–43. https://doi.org/10.1111/j.1439-0310.2006.01108.x.

Semeniuk, C. A., Speers-Roesch, B., & Rothley, K. D. (2007). Using fatty-acid profile analysis as an ecologic indicator in the management of tourist impacts on marine wildlife: A case of stingray-feeding in the Caribbean. Environmental Management, 40, 665–677. https://doi.org/10.1007/s00267-006-0321-8.

Shaw, A. L., Frazier, B. S., Kucklick, J. R., & Sancho, G. (2016). Trophic ecology of a predatory community in a shallow-water, high-salinity estuary assessed by stable isotope analysis. Marine and Coastal Fisheries, 8, 46–61. https://doi.org/10.1080/19425120.2015.1121940.

Simpfendorfer, C. A., & Milward, N. E. (1993). Utilization of a tropical bay as a nursery area by sharks of the families Carcharhinidae and Sphyrnidae. Environmental Biology of Fishes, 37, 337–345. https://doi.org/10.1007/BF00005200.

Stoknes, I. S., Økland, H. M., Falch, E., & Synnes, M. (2004). Fatty acid and lipid class composition in eyes and brain from teleosts and elasmobranchs. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 138, 183–191. https://doi.org/10.1016/j.cbpc.2004.03.009.

Tocher, D. R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science*, *11*, 107–184. https://doi.org/10.1080/713610925.

Vander Zanden, M. J., Clayton, M. K., Moody, E. K., Solomon, C. T., & Weidel, B. C. (2015). Stable isotope turnover and half-life in animal tissues: A literature synthesis. *PLoS One*, *10*, e0116182. https://doi.org/10.1371/journal.pone.0116182.

Vooren, C. M., & Lamónaca, A. F. (2004). *Rhinoptera brasiliensis. The IUCN Red List of Threatened Species* Version 2018. 2. Retrieved from https://www.iucnredlist.org/species/44595/10912274.

Wai, T. C., Yeung, J. W., Lam, V. Y., Leung, K. M., Dudgeon, D., & Williams, G. A. (2012). Monsoons and habitat influence trophic pathways and the importance of terrestrial-marine linkages for estuary sharks. *Ecosphere*, *3*, 1–31. https://doi.org/10.1890/ES11-00276.1.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Rangel BS, Hussey NE, Gomes AD, Rodrigues A, Martinelli LA, Moreira RG. Resource partitioning between two young-of-year cownose rays *Rhinoptera bonasus* and *R. brasiliensis* within a communal nursery inferred by trophic biomarkers. *J Fish Biol*. 2019;1–8. https://doi.org/10.1111/jfb.13958