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Trophic niche of Australian cownose rays (*Rhinoptera neglecta*) and whitespotted eagle rays (*Aetobatus ocellatus*) along the east coast of Australia

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Abstract

Australian cownose rays (*Rhinoptera neglecta*) and whitespotted eagle rays (*Aetobatus ocellatus*) are large myliobatiform rays that co-occur off temperate eastern Australia. Here, we performed stable-isotope analyses (δ^{13}C, δ^{15}N and δ^{34}S) on fin clips of both species to gain insights into their trophic interactions and isotopic niches, and assess the effect of preservation (ethanol-stored versus frozen) on isotopic values of fin-clip tissue of *R. neglecta*. Linear mixed models identified species as the main factor contributing to variation among δ^{15}N and δ^{34}S values, and disc width for δ^{13}C. Bayesian ecological niche modelling indicated a 57.4% to 74.5% overlap of trophic niches, with the niche of *R. neglecta* being smaller and more constrained. Because values of δ^{13}C were similar between species, variation in isotopic niches were due to differences in δ^{15}N and δ^{34}S values. Linear mixed models failed to detect differences in isotopic values of ethanol-stored and frozen fin tissue of *R. neglecta*. This study provides the first examination of the trophic ecology of *R. neglecta* and the comparison of isotopic niche with *A. ocellatus*, which will facilitate future research into the trophic interactions of these species and aid better resource management.

KEYWORDS
batoid, foraging ecology, isotopic niche, Myliobatiformes, stable isotopes, tissue storage

1 | INTRODUCTION

The trophic ecologies and resource use of elasmobranchs have been extensively explored and attributed to various intrinsic and extrinsic factors, including body size or ontogenetic stage (Ajemian & Powers, 2012; Heithaus *et al*., 2013; Sommerville *et al*., 2011), morphology (Yemişken *et al*., 2018; Yick *et al*., 2011), prey availability (Armstrong *et al*., 2016; Frixione *et al*., 2020; Stewart *et al*., 2017), geographic location (Ajemian & Powers, 2012; Bird *et al*., 2018) and resource partitioning or competition with co-occurring species (Kinney *et al*., 2011; Papastamatiou *et al*., 2006; Rangel *et al*., 2019; Raoult *et al*., 2015). However, much of this existing research is biased towards sharks, with ray species considerably underrepresented in ecological studies, especially those involving trophic interactions or diet.

Dietary information is only available for ~30% of all ray species (Chondrichthyes: Batoidea; Flowers *et al*., 2020), despite the taxa being morphologically diverse, occupying various demersal and pelagic habitats, and having a reportedly large impact on the structure and function of marine ecosystems (Flowers *et al*., 2020; Ruocco & Lucifora, 2017; Vaudo & Heithaus, 2011). This lack of information is of concern, considering some understanding of intra- and interspecific resource partitioning is a prerequisite to management guidelines (Madigan *et al*., 2021), especially where species interact with commercial fisheries.

Within Myliobatiformes, the Rhinopteridae (cownose rays), Aetobatidae (pelagic eagle rays) and Mobulidae (devil and manta rays) families are often referred to as pelagic rays due to their transient behaviour between coastal inshore and epipelagic offshore habitats (Last & Stevens, 1994). Among these three families, rhinopterids and aetobatids have jaws and tooth plates that are morphologically adapted to durophagy (Aschliman, 2014; Kolmann *et al*., 2015; Summers, 2000) and primarily consume benthic hard-shelled prey such as molluscs (bivalves and gastropods) and crustaceans (Bade *et al*., 2014; Collins *et al*., 2007; Schluessel *et al*., 2010; Serrano-Flores *et al*., 2019). Some species have been implicated in the decimation of commercially important shellfish populations given their propensity to aggregate in vast numbers at productive locations (Peterson *et al*., 2001; Smith & Merriner, 1985; Yamaguchi *et al*., 2005). Cownose rays, *Rhinoptera bonasus* (Mitchill 1815), and potentially other cownose ray species are considered opportunistic generalists and seemingly operate along a dietary spectrum depending on the availability of prey (Ajemian & Powers, 2012; Collins *et al*., 2007). In comparison, aetobatids such as the spotted eagle ray, *Aetobatus narinari* (Euphrasen 1790), are hard-prey specialists and often prefer a single prey type or species (Ajemian *et al*., 2012; Serrano-Flores *et al*., 2019; Yamaguchi *et al*., 2005).

Despite their cosmopolitan coastal distributions, the level of niche partitioning and dietary separation among pelagic myliobatiform rays has not been thoroughly explored. Off temperate eastern Australia, two common, disparate yet co-occurring species are the Australian cownose ray (*Rhinoptera neglecta* Ogilby 1912) and whitespotted eagle ray (*Aetobatus ocellatus* (Kuhl 1823); Jacobsen & Stevens, 2015; Kyne *et al*., 2016; Last & Stevens, 1994). *Aetobatus ocellatus* is categorized as a hard-prey specialist that exhibits minor dietary shifts as body size increases (Last & Stevens, 1994; Schluessel *et al*., 2010). Currently no trophic information exists for *R. neglecta*, and their ecology has only been assumed from congenerics, for example *R. bonasus*, whose diet varies with ontogeny and across populations (Ajemian & Powers, 2012; Bade *et al*., 2014; Collins *et al*., 2007), and the Pacific cownose ray (*R. steindachneri* Evermann & Jenkins 1891), a species that can switch diets depending on the availability of prey and has been observed feeding among planktivorous whale sharks (*Rhincodon typus* Smith 1828; Ehemann *et al*., 2019; Frixione *et al*., 2020). *Rhinoptera neglecta* and *A. ocellatus* co-occur along the coast of New South Wales (NSW), Australia, where they are often concurrently caught in bather-protection gillnets and observed during aerial surveys (Broadhurst & Cullis, 2020; Dalton *et al*., 2021; Kelaher *et al*., 2020; Tagliafico *et al*., 2019; Tobin *et al*., 2014). Both species likely exhibit similar patterns of habitat use in this region, but their diet and impacts on regional prey populations remain unknown.

Stable-isotope analysis (SIA) is a modern approach for examining the diet and trophic niche of a species to quantify intra- and interspecific patterns of resource use and estimate resource overlap across time and space (Hussey *et al*., 2012; Swanson *et al*., 2015). Tissues including muscle, liver, blood or cartilage can be used for SIA, but fin clips are often easier to obtain and can be archived in ethanol for future use or genetic analysis (Hussey *et al*., 2011). Traditionally, isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) have been used to estimate isotopic niche and overlap (Connolly *et al*., 2004; Swanson *et al*., 2015). δ^{13}C values inform the primary productivity pathway being used by the organism whilst δ^{15}N values can be used to estimate the trophic position of a species, with apex predators having higher δ^{15}N values than species at lower trophic levels (Connolly *et al*., 2004; Hussey *et al*., 2012; Post, 2002; Rossman *et al*., 2016). The addition of more isotope tracers in SIA, such as sulphur (^{34}S), increases niche dimensionality, making isotopic variation more discernible and robust (Costa-Pereira *et al*., 2019). δ^{34}S values distinguish between pelagic and benthic productivity pathways in estuarine or nearshore coastal communities (Post, 2002; Raoult *et al*., 2019), and thus offer another axis on which to examine resource use.

Freezing samples at −20°C is the recommended preservation method for tissues used in SIA (Burgess & Bennett, 2017; Kim & Koch, 2012; Vizza *et al*., 2013). However, this methodology is not always feasible because the logistics of sampling and archiving of samples often results in tissues being stored in chemical preservatives such as ethanol (Kim & Koch, 2012). The effects of ethanol on ^{13}C and ^{15}N in tissues of marine organisms can vary between animal group and tissue type (Burgess & Bennett, 2017; Kelly *et al*., 2006; Olin *et al*., 2014; Vizza *et al*., 2013), but these effects have not been assessed for other isotopic tracers such as ^{34}S.

Our objectives were to use SIA (isotopic values of δ^{13}C, δ^{15}N and δ^{34}S) to document the trophic niches of *R. neglecta* and *A. ocellatus* along the coast of NSW, discern the level of isotopic overlap between these species and provide a baseline for future efforts to describe their ecological niches and roles in the region. Furthermore, because the majority of fin clips were stored in ethanol, a secondary aim was to investigate whether preservation method (ethanol-stored versus frozen at −20°C) had any effect on derived isotopic values.

2 | MATERIALS AND METHODS

2.1 | Specimen capture and tissue collection

Specimens were collected from government-sanctioned bather-protection gillnets, and their collection was approved by the New South Wales Animal Care and Ethics Committee (NSW ACEC no. 08–06). Each bather-protection gillnet measured 150 m long × 4–6 m deep, comprised 600 or 800 mm stretched mesh openings and was deployed 500 m off the beach, parallel to the coast (see Broadhurst & Cullis, 2020 for details of gear). Gillnets were set 2 m below the surface and at least 0.5 m above the seabed and inspected every 12–72 h, depending on logistics.

Twenty-nine *R. neglecta* and 21 *A. ocellatus* were caught between December 2016 and May 2018 in gillnets deployed off five beaches in northern NSW, from Lennox Head (28.77°S, 153.60°E) to Evans Head (29.10°S, 153.44°E), as part of the north coast shark-meshing trial (NCST) to address a cluster of shark–human interactions (Broadhurst & Cullis, 2020; Figure 1). A further six *R. neglecta* were collected between September 2019 and April 2020 from gillnets deployed in the NSW shark meshing (bather protection) program (SMP), which involves 51 gillnetted beaches from Newcastle (32.89°S, 151.82°E) to Wollongong (34.47°S, 150.93°E; Reid *et al.*, 2011).

During sampling, individuals were sexed and their disc width measured (to the nearest centimetre). For individuals caught in the NCST, a fin clip was sampled from either the left or right pectoral fin and stored in 70% ethanol. Individuals were tagged with a conventional spaghetti tag and released 500 m away from the gillnet if alive or disposed of if deceased. For the six specimens caught in the SMP, replicate fin clips were sampled (from either the left or right pectoral fin); one sample was stored in 70% ethanol and the other was frozen at −20°C.

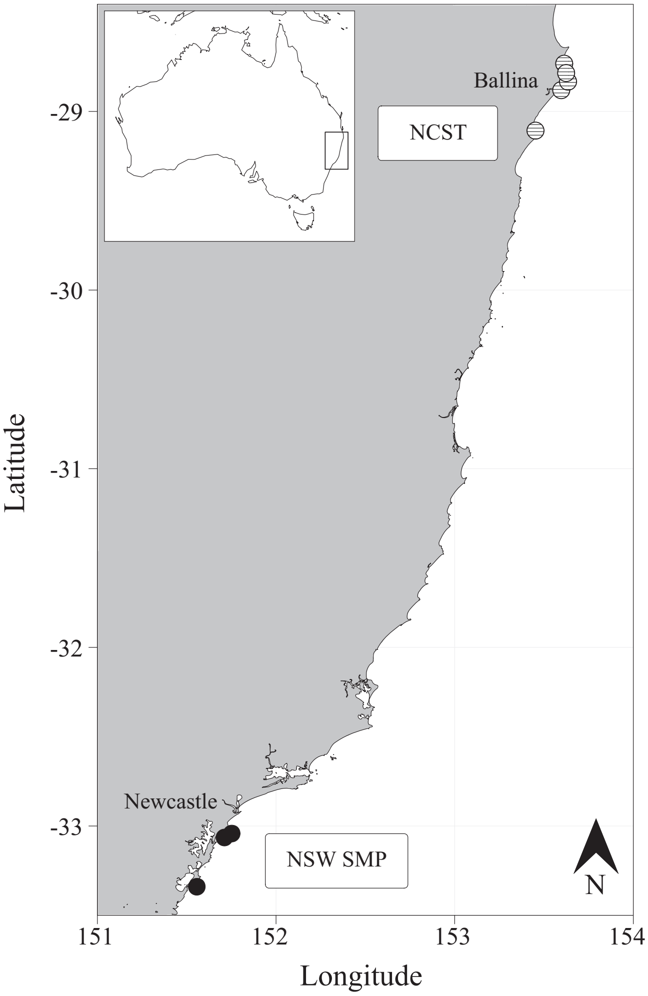

FIGURE 1 Locations in New South Wales, Australia, where fin clips of *Rhinoptera neglecta* and *Aetobatus ocellatus* were collected. Both species were sampled in the north coast shark-meshing trial (NCST; striped dots), but only *R. neglecta* was sampled in the NSW shark meshing (bather protection) program (SMP; black dots)

2.2 | Sample preparation

All fin clips were treated in an identical manner throughout subsequent preparation and processing. Fin clips stored in ethanol were left at room temperature for 60 h to allow residual ethanol to evaporate. Ethanol-evaporated and frozen fin clips were dried at 60°C in a Binder BD 53 oven (Binder, www.binder-world.com) for 48 h before being homogenized using a Retsch MM400 ball mill (Retsch, www.retsch.com). Samples were considered adequately homogenized when no large particles (>2 mm) remained. Lipid extractions were not conducted under the premise of low lipid content in batoids (Crook *et al.*, 2019; Hussey *et al.*, 2011; Olin *et al.*, 2014). Additionally, although these fin clips are comprised of skin, ceratotrichia and other tissues (Hussey *et al.*, 2011), acid washes were not conducted because they would have resulted in excessive loss of tissue for some smaller samples.

Urea extractions were conducted as required for SIA of elasmobranch tissues (Carlisle *et al.*, 2017; Kim & Koch, 2012). Homogenized samples were soaked overnight in 15 ml of deionized water. The following day, samples were rinsed with deionized water and centrifuged for 90 s at 350 *g* in an Eppendorf 5810R (Eppendorf, www.eppendorf.com). Rinsing and centrifugation of samples was repeated three times with at least 30 min between rinses (modified methodology of Raoult *et al.*, 2019). Urea-extracted samples were dried at 60°C for at least 72 h or until fully dry. Dried pellets were ground to a very fine powder using stainless-steel grinding cylinders and balls in a Retsch MM200 ball mill.

Powdered samples were sent to the commercial Griffith University Stable Isotope Laboratory in Brisbane, Australia. There, ~9 mg of prepared sample was placed into tin capsules. Stable isotopes of ^{13}C, ^{15}N and ^{34}S were analysed using a Europa EA-GSL Elemental analyser (Europa Scientific Inc., Cincinnati, OH, USA) and Sercon Hydra 20–22 isotope ratio mass spectrometer (Sercon Limited, www.serconlimited.com). Standards were Pee Dee belemnite for ^{13}C, atmospheric nitrogen for ^{15}N and Vienna-Canyon diablo troilite for ^{34}S. Elemental precision (standard error) for standards was 0.0‰ for δ^{13}C, 0.1‰ for δ^{15}N and 0.7‰ for δ^{34}S. Isotope values are reported in standard delta (δ) notation in parts per thousand (‰).

2.3 | Data analyses

Variability among δ^{13}C, δ^{15}N and δ^{34}S values was investigated using two groups of linear mixed models (LMMs) testing the null hypotheses of no effects of (1) the species and their sex or disc width and (2) fin-sample preservation (ethanol versus frozen at −20°C). The first group of LMMs were done using the ethanol-preserved fin clips from the 29 *R. neglecta* and 21 *A. ocellatus* caught off northern NSW (during the NCST), and comprised the fixed effects of 'species', 'sex' and 'disc width', and appropriate interactions. Random blocking effects included sampling 'day' and 'site/location netted'. In the second group of models, data were limited to replicate fin samples from the six *R. neglecta* collected off central NSW (as part of the SMP), with 'storage method' the only fixed effect, while 'specimen' and sampling 'day' were random ('site/location netted' was not included because these were unique to days).

For all LMMs, normality of residuals was assessed using Q–Q plots and data were analysed raw (no data transformation was required). For the first group of models, a backward-selection algorithm was employed with nonsignificant fixed terms removed until the remaining fixed terms were significant. The statistical significance of fixed effects was evaluated at the 5% level using exact Wald *F*-tests derived from a conditional sum of squares. The LMMs were fitted using ASReml-R in R (R Core Team, 2021).

Bayesian ecological niche models were implemented in R (R Core Team, 2021) to compare the isotopic niches of *R. neglecta* and *A. ocellatus*. The nicheROVER package (Lysy *et al*., 2014) was used to quantify niche overlap and hypervolume niche size, where niche overlap represents the probability that an individual of one species would occur within the niche of the other. To generate these metrics, 1000 Monte Carlo draws and an α-level of 0.95 were specified. Niche volume, calculated using the standard ellipsoid volume function developed by Rossman *et al*. (2016), was used to compare the ecological niche volume (based on 95% probability of the posterior distribution) of the two species in three dimensions.

3 | RESULTS

3.1 | Isotopic niche of *R. neglecta* and *A. ocellatus*

The 29 sampled *R. neglecta* were substantially smaller than the 21 *A. ocellatus*, with disc widths ranging from 67 to 155 cm and 96 to 220 cm, respectively (Supporting Information Table S1). Both species were dominated by males, with only seven *R. neglecta* and four *A. ocellatus* females sampled.

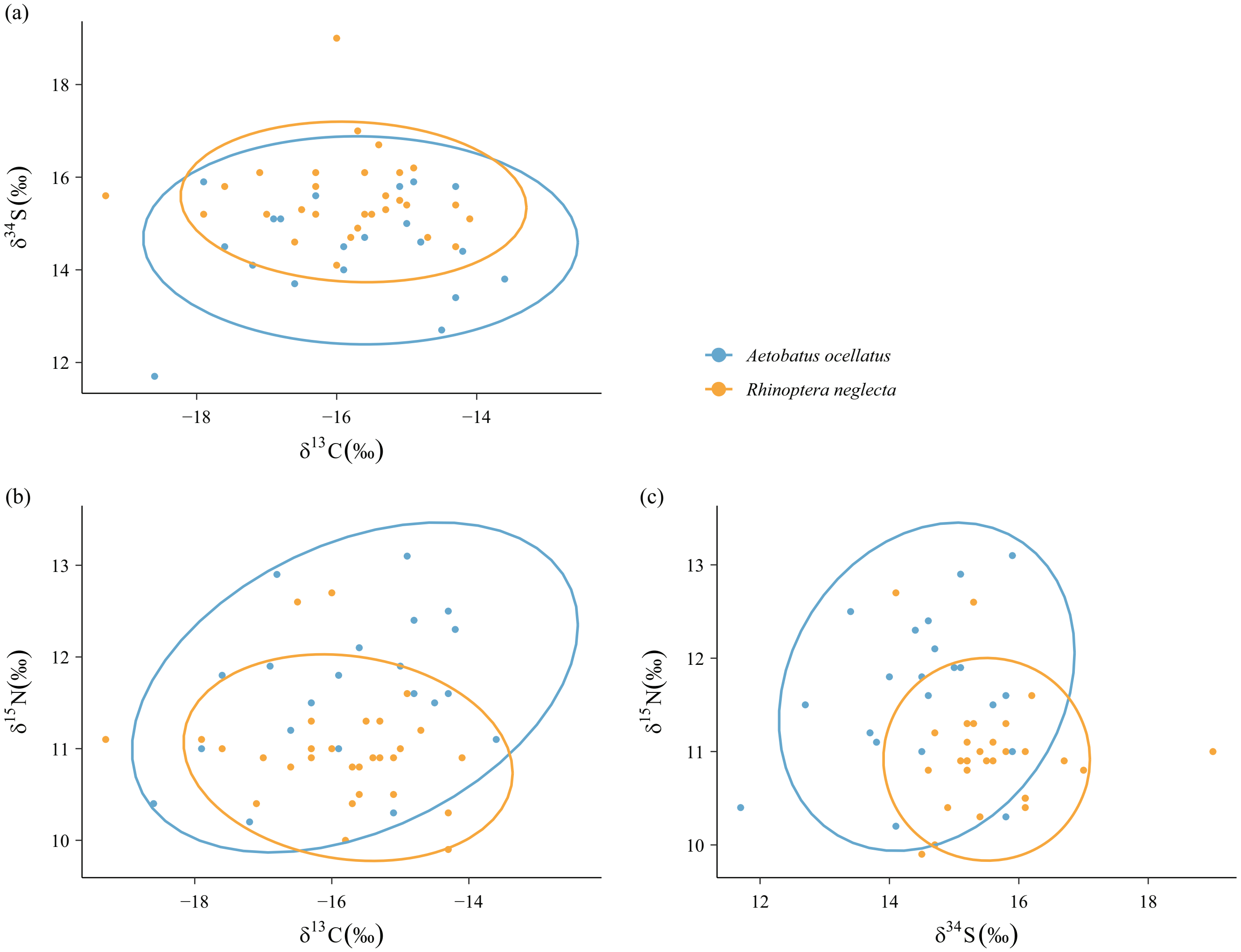

FIGURE 2 Stable isotope biplots for (a) δ^{13}C, δ^{34}S, (b) δ^{13}C, δ^{15}N and (c) δ^{34}S, δ^{15}N values obtained from fin clips of *Rhinoptera neglecta* (orange, $n = 29$) and *Aetobatus ocellatus* (blue, $n = 21$). Points represent values for individual rays and ellipses represent the species' isotopic niche. -●- *A. ocellatus*; -●- *R. neglecta*

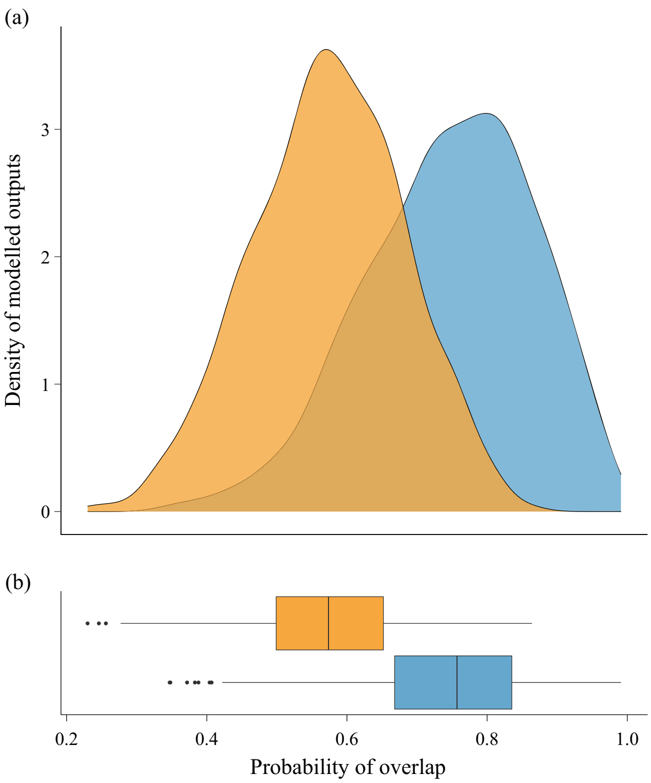

FIGURE 3 Estimated niche overlap of *Rhinoptera neglecta* (orange) and *Aetobatus ocellatus* (blue) based on posterior distributions calculated with the nicheROVER package (Lysy *et al*., 2014). (a) Density of modelled outputs of the probability that an individual from one species would occur within the niche of the other and (b) corresponding boxplots to assist with interpreting distributions. 🟦 *A. ocellatus*; 🟧 *R. neglecta*

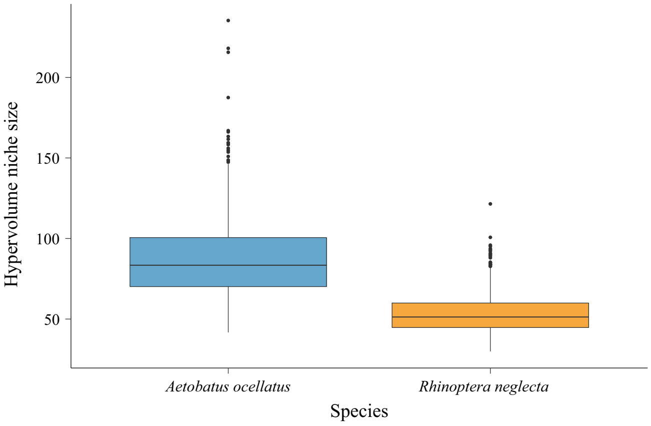

FIGURE 4 Estimated hypervolume niche size of *Rhinoptera neglecta* and *Aetobatus ocellatus* based on posterior distributions calculated with the nicheROVER package (Lysy *et al*., 2014)

The LMMs assessing variability among isotopic values of *R. neglecta* and *A. ocellatus* were reduced to the main effect of species for δ^{15}N (LMM, $F = 15.69$, $P = <0.01$) and δ^{34}S (LMM, $F = 10.59$, $P = <0.01$). Predicted mean values (± s.e.) of δ^{15}N were lower in *R. neglecta* (11.0 ± 0.2‰) than *A. ocellatus* (11.6 ± 0.2‰), but δ^{34}S values were the opposite (15.5 ± 0.2‰ and 14.6 ± 0.2‰, respectively). Regardless of the species or their sex, disc width positively affected δ^{13}C values (LMM, $F = 6.64$, $P = <0.05$).

Bayesian ecological niche modelling revealed substantial overlap in the isotopic niches of *R. neglecta* and *A. ocellatus* (Figure 2). The mean posterior probability that an individual *R. neglecta* would be found within the niche of *A. ocellatus* was estimated to be 74.53%, whilst the mean probability of an individual *A. ocellatus* being present within the niche of *R. neglecta* was lower, at 57.40% (Figure 3). This overlap was mostly due to similarities in values of δ^{13}C as differences in mean δ^{15}N and δ^{34}S values were statistically significant between the two species. Niche modelling also indicated differences in estimates of niche size and volume for *R. neglecta* and *A. ocellatus*. The mean posterior distribution of niche size (± s.e.) was substantially smaller for *R. neglecta* (53.16 ± 11.96) than *A. ocellatus* (87.35 ± 24.02; Figure 4). These means are corroborated by standard ellipsoid volumes, where the mean ellipsoid volume (25% and 95% credible interval) was 3.1‰^{3} (1.9, 4.4) for *R. neglecta* and 5.1‰^{3} (3.0, 7.6) for *A. ocellatus*.

3.2 | Effect of ethanol storage on stable isotope values of fin tissue

Linear mixed models failed to detect differences in isotopic values between the two preservation methods for fin tissue of *R. neglecta* on any of the assessed stable isotopes (LMM, δ^{13}C $F = 5.94$, $P = 0.06$; δ^{15}N $F = 1.36$, $P = 0.30$; δ^{34}S $F = 0.05$, $P = 0.83$). Predicted mean values of ethanol-stored and frozen samples were similar for δ^{15}N (11.0 ± 0.5‰ and 11.3 ± 0.5‰, respectively) and δ^{34}S (14.8 ± 0.3‰ and 14.9 ± 0.3‰, respectively), while predicted mean values for δ^{13}C were slightly greater in the frozen samples (−14.7 ± 0.4‰ *vs*. −15.8 ± 0.4‰). Variation in δ^{13}C and δ^{34}S values for ethanol-stored samples was greater than for those stored at −20°C (Figure 5).

4 | DISCUSSION

R. neglecta is classified as 'Data Deficient' in the IUCN Red List of Threatened Species, with many aspects of the species' biology and ecology undocumented or inferred from congenerics (Jacobsen & Stevens, 2015). This study provides the first examination of *R. neglecta* trophic ecology and isotopic niche in temperate eastern Australian waters, with the data indicating a more constrained isotopic niche than the co-occurring *A. ocellatus*. Because the smaller niche of *R. neglecta* suggests the species uses a narrower range of resources, we propose the resource use and diet of *R. neglecta* may be more susceptible to perturbations impacting prey populations than *A. ocellatus*.

A high level of isotopic niche overlap was observed between *R. neglecta* and *A. ocellatus*, suggesting similarities in the resource use of these two species along the coast of NSW. Comparable values of δ^{13}C were expected for these morphologically similar species that have concomitant regional fidelity along the coast of eastern Australia (Broadhurst & Cullis, 2020; Kelaher *et al*., 2020; Tagliafico *et al*., 2019;

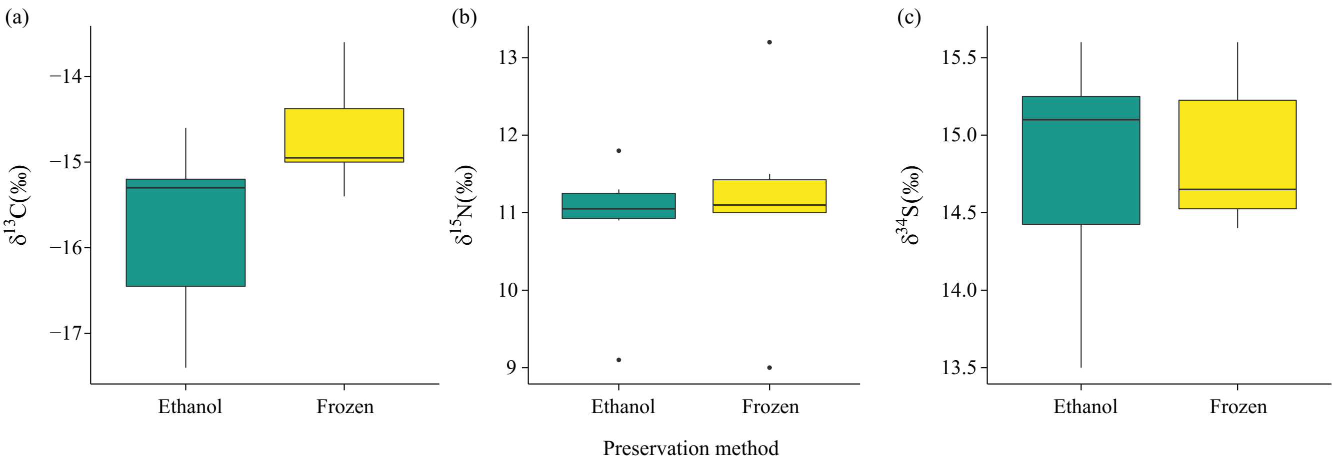

FIGURE 5 Comparison of (a) δ^{13}C, (b) δ^{15}N and (c) δ^{34}S values of fin clips from *Rhinoptera neglecta* preserved in 70% ethanol (green) and frozen at -20°C (yellow) ($n = 6$ per preservation method)

Tobin *et al*., 2014). Therefore, it is not surprising that these species are foraging within the same habitats and using the same, or very similar, primary productivity pathways. Whilst δ^{15}N values of the two species differed, the ecological significance of this result may not be profound because the observed difference did not exceed the typical discrimination factor of nitrogen (3.4‰; Post, 2002) or the reported discrimination factor of other elasmobranchs (2.3‰; Hussey *et al*., 2010). Similarly, the small difference in δ^{34}S values may not bear substantial ecological significance (mean difference <1‰), suggesting both species rely on similar bentho-pelagic sources.

High levels of niche overlap have been observed for manta ray species coexisting in the Philippines and Sri Lanka, which was hypothesised to reflect convergence on highly abundant patches of prey (Stewart *et al*., 2017). It is possible *R. neglecta* and *A. ocellatus* are also converging on the same or similar prey sources along the coast of NSW, with the diet of *R. neglecta* being a subset of *A. ocellatus*' diet. The larger niche space and volume of *A. ocellatus* suggests this species uses a wider range of resources and could be considered an opportunistic or generalist predator, especially considering the intraspecific variability of isotopic values (Rossman *et al*., 2016). In other parts of their range, *A. ocellatus* also had the largest isotopic niche area among sympatric elasmobranchs (Vaudo & Heithaus, 2011). Although the isotopic niche size and volume of *R. neglecta* suggests the species uses a narrower range of resources, the diet and trophic ecology of *Rhinoptera* spp. are complex. There is evidence *R. bonasus* employs different foraging ecologies throughout its range (Bade *et al*., 2014; Collins *et al*., 2007) and across life stages and habitat type, for example in the northern Gulf of Mexico, the diet of adult *R. bonasus* in coastal and barrier island regions were dominated by crustaceans whilst juveniles in estuarine areas preferred bivalves (Ajemian & Powers, 2012). Therefore, the possibility that the resource use and trophic interactions of *R. neglecta* may vary spatially and with ontogeny warrants further investigation.

Based on the δ^{13}C and δ^{15}N values of benthic and pelagic primary producers and consumers in south-eastern Australia (Supporting Information Table S2), it is possible *R. neglecta* and *A. ocellatus* are using both benthic and pelagic resources. Clarification on resource use can be strengthened if δ^{34}S values are taken into consideration, but this information is not available for many species. There are also difficulties associated with making inferences about a species' trophic ecology with SIA alone. In addition to being unable to identify specific prey items with SIA, there is the potential for taxonomically and functionally distinct prey sources to produce isotopically similar signatures (Rohner *et al*., 2017; Stewart *et al*., 2017). As such, differences in isotopic signatures may not reflect differences in resource use, nor do similar isotopic values directly imply similar prey sources. Because no gut-content data were available and no primary producers were analysed, the specific diet composition of these two species can neither be described nor used to make inferences regarding their trophic level or their interactions with prey. To better understand a species' resource use and trophic interactions, analyses should include examples of prey obtained from stomachs, or the isotopic values of potential prey or primary producers (*e.g*., Couturier *et al*., 2013; Raoult *et al*., 2019).

Although there is very limited understanding of *R. neglecta* biology and ecology, there are assumed parallels in the life history, reproductive and foraging strategies of *R. neglecta* and *A. ocellatus* (Jacobsen & Bennett, 2013; Neer & Thompson, 2005; Schluessel *et al*., 2010). However, differences in maximum size and feeding apparatus of *R. neglecta* and *A. ocellatus* may be driving the separation of isotopic niche. Sexually mature *R. neglecta* have a disc width of 100 cm whereas *A. ocellatus* reach 300 cm (Weigmann, 2016). For many marine species, body size determines mouth gape and potentially mouth protrusion, which can limit the size of consumable prey, affect foraging efficiency (Fisher *et al*., 2011; Park *et al*., 2017) and drive trophic relationships (Hayden *et al*., 2019). Furthermore, differences in head, jaw and tooth plate morphology of rhinopterids and aetobatids (McEachran & Aschliman, 2004; Summers, 2000) may affect diet and contribute to the observed isotopic niches. While both cownose and eagle rays have cephalic lobes, there are disparities in the function of these appendages during foraging that may affect prey handling and selection (Mulvany & Motta, 2014; Sasko *et al*., 2006;

Wilga *et al*., 2012). Future research examining the effects of size and morphology on the trophic ecology of these rays is required to further decipher interspecific variation of resource use.

Although ethanol is not the recommended method of preservation for stable isotope samples, it is often unavoidable where freezing is difficult or if using historical samples (Burgess & Bennett, 2017; Kelly *et al*., 2006; Kim & Koch, 2012; Olin *et al*., 2014). When using alcohol preservation, the effect of ethanol on the isotopic values of tissues should be assessed and sources of error identified to accurately interpret the results of SIA (Kim & Koch, 2012; Olin *et al*., 2014). Although the sample size for this analysis was small, the results generally align with those from a previous study on *R. bonasus* fin clips that found no significant difference in the mean $\delta^{13}C$ and $\delta^{15}N$ values for the two preservation methods (Olin *et al*., 2014). Whilst there was no effect of ethanol preservation detected here, Kim and Koch (2012) reported altered $\delta^{13}C$ values in ethanol-stored elasmobranch muscle. Variability in results of preservation-effect studies warrants further research to determine the impact of chemical preservation on isotopic values in different tissues and taxonomic groups.

Another consideration for interpreting the results here is the use of fin clips. Fin clips are an amalgamation of skin, ceratotrichia, muscle and connective tissue, and are typically enriched in ^{13}C (Hussey *et al*., 2011). To mediate the influence of cartilage on the isotopic signatures, acid washes are routinely conducted to remove inorganic carbon (Kim & Koch, 2012). Such procedures were not possible here because the small size of most samples and loss of tissue during washing would have resulted in many nonviable samples.

Elasmobranch tissues, especially those of batoids, are often assumed to have low lipid content and typically do not require lipid correction if C:N ratios are <3.5 (Carlisle *et al*., 2017; Crook *et al*., 2019; Li *et al*., 2016; Post *et al*., 2007). Lipid content in the present study ranged from 3.29 to 4.67 (*R. neglecta* 3.81 ± 0.01, *A. ocellatus* 4.01 ± 0.01). While the lack of lipid correction is an issue if comparing the results from this study to others on the same species, the focus of the research here was to compare trophic niches between the two species. Therefore, we are confident that the interspecific niche comparisons presented here are robust, if not the absolute comparisons. Further research into the effect of lipids on the isotopic values for these species should be conducted across several tissue types to understand the confounding effects of this compound.

5 | CONCLUSIONS

This study presents the first insights into the trophic ecology of *R. neglecta*. The findings are compared to those of *A. ocellatus*, a co-occurring but more researched species along the NSW coast. Examination of isotopic niche revealed a high level of overlap between the two species, primarily due to similarities of $\delta^{13}C$ values, which suggests these species are using similar resources along the coast of eastern Australia. Differences in isotopic niche were apparent, with *R. neglecta* fin clips being slightly more enriched in ^{34}S whilst those of *A. ocellatus* were more enriched in ^{15}N. Based on the isotopic values of benthic and pelagic primary producers in the region, *R. neglecta* and *A. ocellatus* may be using benthic and pelagic resources. However, limited isotopic values for sulphur in the scientific literature restrict the assumptions that can be made regarding bentho-pelagic resource use, thus further dietary studies are required to elucidate the trophic interactions of these species and determine whether they have diverging affinities for benthic and pelagic prey. Future research is also required to discern the ecological role of *R. neglecta* in ecosystems along the east coast of Australia. These aspects of the species' biology and ecology would assist in understanding the dynamics and connectivity of ecosystems, and aid in the development of effective species and ecosystem management strategies.

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AUTHOR CONTRIBUTIONS

Conceived and designed the study: J.E.W., F.R.A.J., V.M.P. and V.R. Contributed samples: M.K.B. and V.M.P. Preparation of samples and data analysis: A.J.C. and M.K.B. A.J.C. led the writing and all co-authors contributed to manuscript preparation.

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