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

Alysha J. Chan1 | Vincent Raoult2 | Fabrice R. A. Jaine1,3 | Victor M. Peddemors4 | Matt K. Broadhurst5,6 | Jane E. Williamson1

1School of Natural Sciences, Macquarie University, Sydney, New South Wales, Australia
2School of Environmental and Life Sciences, University of Newcastle, Ourimbah, New South Wales, Australia
3Sydney Institute of Marine Science, Mosman, New South Wales, Australia
4New South Wales Department of Primary Industries, Fisheries Research, Sydney Institute of Marine Science, Mosman, New South Wales, Australia
5New South Wales Department of Primary Industries, Fisheries Conservation Technology Unit, National Marine Science Centre, Southern Cross University, Coffs Harbour, New South Wales, Australia
6Marine and Estuarine Ecology Unit, School of Biological Sciences, University of Queensland, Brisbane, Queensland, Australia

Correspondence
Alysha J. Chan, School of Natural Sciences, Macquarie University, 205b Culloden Road, Macquarie Park, NSW, 2109, Australia.
Email: alysha.chan@hdr.mq.edu.au

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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 (δ13C, δ15N and δ34S) 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 δ15N and δ34S values, and disc width for δ13C. 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 δ13C were similar between species, variation in isotopic niches were due to differences in δ15N and δ34S 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 (Chondrichthyens: 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; Schlussel 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; Schlussel et al., 2010). Currently no trophic information exists for R. neglecta, and their ecology has only been assumed from congeners, 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 ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have been used to estimate isotopic niche and overlap (Connolly et al., 2004; Swanson et al., 2015). $\delta^{13}C$ values inform the primary productivity pathway being used by the organism whilst $\delta^{15}N$ values can be used to estimate the trophic position of a species, with apex predators having higher $\delta^{15}N$ 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 ($\delta^{34}S$), increases niche dimensionality, making isotopic variation more discernible and robust (Costa-Pereira et al., 2019). $\delta^{34}S$ values distinguish between pelagic and benthic productivity pathways in estuarine or nearshore coastal communities (Post, 2002; Raoul et al., 2019), and thus offer another axis on which to examine resource use.

Freezing samples at $\sim$20°C is the recommended preservation method for tissues used in SIA (Burruss & 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 (Burruss & 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 $\delta^{34}S$.

Our objectives were to use SIA (isotopic values of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) 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 $\sim$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 $\times$ 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.

### 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 Raoul 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 13C, 15N and 34S 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 13C, atmospheric nitrogen for 15N and Vienna-Canyon diablo troilite for 34S. Elemental precision (standard error) for standards was 0.0‰ for δ13C, 0.1‰ for δ15N and 0.7‰ for δ34S. Isotope values are reported in standard delta (δ) notation in parts per thousand (‰).

### 2.3 Data analyses

Variability among δ13C, δ15N and δ34S 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

![FIGURE 1](https://example.com/figure1.png)  
**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).
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 $\alpha$-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.

![Stable isotope biplots](image-url)

**Figure 2** Stable isotope biplots for (a) $\delta^{13}$C, $\delta^{34}$S, (b) $\delta^{13}$C, $\delta^{15}$N and (c) $\delta^{34}$S, $\delta^{15}$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*
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 δ13C 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 δ13C as differences in mean δ15N and δ34S 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, δ13C $F = 5.94$, $P = 0.06$; δ15N $F = 1.36$, $P = 0.30$; δ34S $F = 0.05$, $P = 0.83$). Predicted mean values of ethanol-stored and frozen samples were similar for δ15N (11.0 ± 0.5‰ and 11.3 ± 0.5‰, respectively) and δ34S (14.8 ± 0.3‰ and 14.9 ± 0.3‰, respectively), while predicted mean values for δ13C were slightly greater in the frozen samples (−14.7 ± 0.4‰ vs. −15.8 ± 0.4‰). Variation in δ13C and δ34S 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 congeners (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 δ13C 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;...
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 δ15N 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 δ34S values may not bear substantial ecological significance (mean difference <1‰), suggesting both species rely on similar benthic-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 δ13C and δ15N 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 δ34S 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 not being able 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; Schlüssel 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 acotobatids (McCann & 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;

**FIGURE 5** Comparison of (a) δ13C, (b) δ15N and (c) δ34S values of fin clips from Rhinoptera neglecta preserved in 70% ethanol (green) and frozen at −20°C (yellow) (n = 6 per preservation method)
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 δ13C and δ15N 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 δ13C 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 13C (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 δ13C 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 34S whilst those of A. ocellatus were more enriched in 15N. 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.

ORCID

Alysha J. Chan https://orcid.org/0000-0001-6236-9717
Vincent Raout https://orcid.org/0000-0001-9459-111X
Fabrice R. A. Jaine https://orcid.org/0000-0002-9304-5034
Victor M. Peddemors https://orcid.org/0000-0002-8743-9782
Matt K. Broadhurst https://orcid.org/0000-0003-0184-7249
Jane E. Williamson https://orcid.org/0000-0003-3627-4508

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Additional supporting information may be found in the online version of the article at the publisher’s website.

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