Running tittle: Trophic niches among tropical sharks

Interspecific variation in trophic niche metrics among euryhaline and coastal elasmobranchs in northern Australia

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Abstract

Tropical elasmobranchs are thought to play significant roles in many coastal and river ecosystems, yet few studies have explored their trophic ecology. We investigated the trophic niches of seven elasmobranch species in northern Australia, using stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes (SI) and fatty acid (FA) markers taken from muscle tissues sampled during the tropical monsoonal wet and dry seasons. Both SI and FA metrics suggested significant niche partitioning between the species; with two distinct guilds apparent from the SIs: a marine foodweb based on macroalgae and seagrass, and an estuarine/freshwater foodweb with a seston base. Fatty acid profiles showed contributions from both macroalgal and seston based food webs. Varying degrees of interspecific niche distinction were apparent: *Glyphis garricki*, *G. glyphis* and *Himantura dalyensis* had FAs from vascular plants and diatoms/macroalgae which separated them from *Carcharhinus leucas*, *C. amboinensis* and *Rhizoprionodon taylori* that had FA markers associated with marine dinoflagellates. Niche metrics differed between methods, where for example, *G. garricki* had the smallest SI niche area yet the largest FA niche space, but a generally low probability of overlap with other species. Differences in the niche metrics between SI and FA are likely due to their disparate turnover times. Our results suggest that coastal and euryhaline elasmobranchs provide trophic connections between tropical marine, estuarine and freshwater ecosystems.

Key words: niche metrics, sharks, *Glyphis*, *Carcharhinus*, *Rhizoprionodon*, biotracers, stable isotopes, fatty acids

Introduction

Resource partitioning, whereby species segregate habitats and dietary items over space and time (Ross 1986), is fundamental to our understanding of coastal ecosystem structure and function (Kitchell et al. 2002). Elasmobranchs (sharks and rays) are demographically vulnerable predators that are thought to play key roles in aquatic food webs (Stevens et al. 2000, Dulvy et al. 2014). As middle-order or apex predators, elasmobranchs can provide ecosystem stability by influencing the abundance and health of prey species at multiple trophic levels, and connect otherwise distinct food webs (Rooney et al. 2006, Heithaus et al. 2013).

Niche theory suggests that species have a set of specific resource requirements that form their unique space, an "*n* – dimensional niche hypervolume" (Hutchinson 1957), which may be measured and compared among locations, seasons and species. In some cases, species can share resources to produce niche overlap. Whether such overlap leads to competition is dependent on the spatial and temporal extent of shared resource use. In the case of trophic overlap, sympatric species may target different prey items (Yick et al. 2011), and/or have temporal or spatial differences in consumption that negate competitive interactions (Ross 1986). Sympatric elasmobranchs have been found to have variable trophic overlap, ranging from high (e.g. sympatric batoids, Banded Stingaree *Urolophus cruciatus* and Tasmanian Numbfish *Narcine tasmaniensis* (Yick et al. 2011)) to low (e.g. Caribbean Reef Shark *Carcharhinus perezi*, Nurse Shark *Ginglymostoma cirratum* and Southern Stingray *Dasyatis americana* (Tilley et al. 2013)) in a range of elasmobranch assemblages (Papastamatiou et al. 2006, Heithaus et al. 2013).

Euryhaline elasmobranchs are capable of tolerating a range of salinities and may complete different life history stages in marine, estuarine or freshwater habitats with tropical regions having the most diversity of euryhaline elasmobranch species (Lucifora et al. 2015). Due to their reliance on rivers and estuaries, euryhaline elasmobranchs must adapt to fluctuations in salinity and turbidity over tidal and seasonal cycles, both in terms of their own physiology, and the abundance of potential prey items. Such environmental changes are particularly extreme in tropical rivers due to the variation between high-flow monsoonal 'wet' seasons and long low-rainfall 'dry' seasons (Douglas et al. 2005, Warfe et al. 2011). Trophic resource use and partitioning within tropical elasmobranch assemblages is poorly understood at present, although recent studies have indicated the existence of overlap among juvenile *Carcharhinus amboinensis* and *C. leucas* in northern Australia (Tillett et al. 2014), and among *C. leucas* and *Pristis pristis* in Western Australia (Thorburn et al. 2014).

Analysis of biochemical tracers in animal tissue, such as stable isotopes (SI) and fatty acids (FAs), is an effective approach for estimating time-integrated trophic niches of aquatic species (Hussey et al. 2011, Jackson et al. 2011, Pethybridge et al. 2014). The predictable fractionation of stable isotopes (e.g., $\delta^{13}C$, $\delta^{14}N$) through food webs, allows isotopes to be used for a variety of applications, including estimation of niche area, resource partitioning and dietary overlap. Fatty acids can also be traced through food webs, and are particularly useful for detecting trophic markers via essential FAs (EFAs) that can only be obtained through dietary sources (Iverson 2009). These EFAs are synthesized by different primary producers such as dinoflagellates, diatoms or algae, after which they are accumulated up the food chain (Dalsgaard et al. 2003,

Parrish et al. 2013). Fatty acids often have faster turnover rates than isotopes (e.g. 14 weeks in shark muscle tissue (Beckmann et al. 2014), cf. 6 – 12 months for $\delta^{13}C$ and $\delta^{15}N$, (Malpica-cruz et al. 2012, Hussey et al. 2012), and have been used to reveal physiological and environmental changes over relatively fine scales, such as seasonal differences in the EFA trophic biomarker omega 3 [ω3] / omega 6 [ω6] (Jayasinghe et al. 2003, Pethybridge et al. 2015).

Northern Australia provides an excellent setting for studying tropical elasmobranch trophic ecology due to the relatively pristine state of the tropical estuarine and riverine ecosystems (Warfe et al. 2011) and a high diversity of sympatric elasmobranch species (Last & Stevens 2009). This includes euryhaline (*C. leucas, Glyphis garricki, G. glyphis, Himantura dalyensis, P. pristis)* and coastal (*C. amboinensis* and *Rhizoprionodon taylori*) species. In the current study, we use SI and FA biochemical tracers to evaluate the extent of trophic resource overlap or partitioning among seven euryhaline and coastal elasmobranchs during the wet and dry season in northern Australia. We also use these tracers to identify the basal sources of food webs (marine, estuarine, fresh water) and apply biochemical niche metrics (Jackson et al. 2011, Swanson et al. 2015) to determine the extent of dietary overlap within and among species across seasons.

Methods

Sample collection and preparation

Seven species of euryhaline and coastal elasmobranchs were collected in the South Alligator River, Northern Territory (NT), Australia from March 2013 to July 2014 (Table 1, Fig. 1). This time period included the two broad seasons, the ‘wet’ and

101 'dry'. The wet is characterized by high humidity, rainfall and temperature from
102 November to March followed by decreases in these parameters during the dry season
103 from April to October. Linked to the variation in rainfall, salinity also varies
104 throughout the seasons. Salinity was measured with an YSI (Xylem, USA) at each site
105 (Fig. 1), during each sampling event. The mouth and coastal region of the river ranged
106 from 32.4 – 34.5‰ (dry) and 17.1–24.7‰ (wet) whereas the mid-lower region of the
107 river ranged from 18.3 – 28.1‰ (dry) and 0.4 – 11.3‰ (wet).
108
109 *Rhizoprionodon taylori* and *Carcharhinus amboinensis* were captured with baited line
110 in the lower estuary and coastal region of the South Alligator River, Kakadu National
111 Park (Fig. 1). *Glyphis garricki*, *G. glyphis*, *C. leucas*, *Pristis pristis* and *Himantura*
112 *dalyensis* were caught in mid-lower estuarine reaches with a combination of fishing
113 methods including 4 – 6 inch gill nets and baited lines. A 5 mm biopsy punch (Stiefel,
114 USA) was used to collect muscle tissue from the caudal peduncle within 5 minutes of
115 capture, with the tissue sample immediately placed on liquid nitrogen (-196°C) for
116 preservation and initial storage in the field. Within one week, tissues (mean wet
117 weight 0.02 ± 0.01g) were transferred to a -20°C freezer, and then later freeze-dried
118 (Alpha 1-4 LSC, Christ, Germany) at -20°C for 21 hours and then -30°C for three
119 hours. To avoid degradation of the sample from defrosting and refreezing, frozen
120 muscle samples were dissected in the freezer to remove dermal layers and as much
121 connective tissue as possible. Where sample tissue masses were low, tissue was only
122 used for SIA and not FA analysis (Table 1).
123
124 *Stable Isotopes*

Muscle tissue was rinsed in milli-Q water and sonicated to remove excess urea as per Kim & Koch (2011). Tissues were then freeze-dried to a constant weight and pulverized using a combination of micro-scissors and micro-pestle or a coarse pestle and ceramic mortar. A subset of material was weighed to 400 – 1000 ug in tin cups for combustion in a Sercon Europa EA-GSL elemental analyzer (Sercon Ltd, UK), which were then gas analyzed with a Sercon Hydra 20 – 22 – isotope ratio mass spectrometer (Sercon Ltd, UK) at the Australian Rivers Institute, Griffith University. The international standards used to determine the relative $\delta^{13}C$ and $\delta^{15}N$ were Peedee Belemnite Carbonate and Atmospheric Nitrogen. We did not use mathematical models to correct $\delta^{13}C$ values for lipids as lipid content in all samples were deemed to be low as inferred by ratios of total organic carbon to total organic nitrogen (C:N) being < 3.5% (Post et al. 2007) and by the low lipids inferred from total FAs (Every et al. In press).

Trophic positions (TP) for each species were calculated using the narrowing discrimination with increasing dietary $\delta^{15}N$ values approach (Hussey et al. 2014). This combined two models: a meta-analytical Bayesian model of diet discrimination factors, and the $\delta^{15}N$ values with a von Bertalanffy growth model. This works on the concept that as trophic position (TP) increases, the amount of dietary discrimination factors (the increase of $\delta^{15}N$ at each TP) is not constantly added, but decreased at each TP (Hussey et al. 2014). To calculate this, an inverse relationship of dietary discrimination factors to $\delta^{15}N$ was determined, based on known discrimination factors in Hussey et al. (2014), which was then applied to data used in this study. Popeye mullet (*Rhinomugil nasutus*, $\delta^{15}N$ 6.62±1.19, trophic position 2.92 (Froese & Pauly 2015)) was used as the baseline consumer collected at the same location and over the

same time period as the elasmobranch tissue samples. The following equation was used with values from Hussey et al. (2014) meta analysis:

$$TP = \frac{\log(\delta^{15}N_{lim} - \delta^{15}N_{base}) - \log(\delta^{15}N_{lim} - \delta^{15}N_{TP})}{k} + TP_{base}$$

where TP_{base} is the trophic position of baseline consumer (*R. nasutus*), $\delta^{15}N_{TP}$ is the consumer $\delta^{15}N$ value and k is calculated from β_0 5.92 [4.55, 7.33] and β_1= -0.27 [-0.41, -0.14] after (Hussey et al. 2014):

$$k = -\log \frac{(\beta_0 - \delta^{15}N_{lim})}{\delta^{15}N_{lim}}$$

where term:

$$\delta^{15}N_{lim} = \frac{-\beta_0}{\beta_1}$$

Fatty acids

Lipid was extracted using the modified Bligh and Dyer (1959) method, which utilizes an overnight one-phase extraction process. The extracted lower layer was concentrated, blown down with nitrogen gas and dried to a constant weight. Approximately half of the total lipid extract was transmethylated (Parrish et al. 2015) to liberate the FAs from the lipid backbone. After the solution was prepared, 0.2 ml was injected into an Agilent Technologies 7890B gas chromatograph (GC) (Palo Alto, California, USA) equipped with an Equity-1 fused silica capillary column (15 x 60.1 mm i.d. and 0.1 mm film thickness), a flame ionization detector, a splitless injector and an auto-sampler. Peaks were quantified using Agilent Technologies ChemStation software (Palo Alto, California, USA). Confirmation of peak identifications was by

GC-mass spectrometry (GC-MS), using a column of similar polarity to that described above and a Finnigan Thermoquest DSQ GC-MS system.

Niche area calculations

A SI Bayesian ellipse model (SIBER) using the R package Stable Isotopes Analysis in R (SIAR) was used to measure two different metrics of isotopic niche areas, convex hull total area (TA) (Layman et al. 2007, Jackson et al. 2011) and the stable isotope ellipse area corrected for sample size (SEA_c) (Jackson et al. 2011). Although TA in a $\delta^{15}N$ – $\delta^{13}C$ biplot is strongly affected by sample size (Jackson et al. 2011, Syväranta et al. 2013) it was calculated to explore species variation and the potential overlap of individuals (Layman et al. 2012, Heithaus et al. 2013). Drawing standard ellipse areas (SEA) in isotopic space ($\delta^{13}C$ and $\delta^{15}N$) that incorporated 95% of the sampled individuals, is considered to provide a more accurate measure of niche width than TA and is less biased by sample size (Jackson et al. 2011, Syväranta et al. 2013). To further deal with small, uneven sample sizes, we applied a sample size-correction measure, calculated from the covariance matrix determined via Bayesian inference to enable the area to be measured (SEA_C). Confidence intervals of the ellipse size are calculated from Bayesian likelihoods, where by the probability of one ellipse being bigger than the other can be determined by the uncertainty in the probable values. Overlaps of (SEA_C) were calculated by establishing the percent difference between the ellipses. Only *C. leucas*, *G. garricki* and *R. taylori* had ellipses analyzed as *n* values were over 30, which is considered optimum for these analysis (Syväranta et al. 2013).

197 Fatty acid niche space and overlap was calculated using a multivariate extension on
198 the bivariate approach of Jackson et al. (2011). Here, Bayesian priors are used to
199 determine the hyper-volume and the probability of finding one species in another's
200 niche space (Swanson et al. 2015). To increase the confidence of this model the
201 amount of FAs used was reduced as the numbers of individuals were considered to be
202 too low for the number of variables (M. Lysy, personal communication, 6/11/2015).
203 Therefore for ~30 individuals of each species, five FAs were used and only *G.*
204 *garricki, C. leucas* and *R. taylori* were analyzed. Essential FAs were selected since
205 they are largely accumulated through diet. The most abundant EFAs were also used.
206 Each FA was then represented as an axis, calculated in FA hyperspace and projected
207 onto a two dimensional plot as a probabilistic projection to display FA niche space.
208 Overlap was calculated based on the probability that one species would be found in
209 the niche of another species and a mean value of niche space calculated.
210
211 *Statistical Analysis*
212 Analysis of variance (ANOVA) was used to determine differences in stable isotope
213 tracers among species and seasons. For *C. leucas* an Analysis of Covariance
214 (ANCOVA) was run with total length (TL) as a covariate to account for the effects of
215 length on isotopic values. Assumptions of normality, variance, homogeneity of slopes
216 and colinearity were tested through visual inspection of boxplots, calculating
217 Leverage, Residuals and Cook's D and graphed in a Residual vs Fitted, Normal Q-Q
218 and Residual vs Fitted values plots prior to accepting the models. No transformation
219 was necessary.
220

Fatty acids were converted to a percentage and those with group means less than 0.5% were not included in statistical analyses. To evaluate interspecific differences in FA profiles a one-way semi-parametric permutation multivariate analysis of variance (PERMANOVA) routine was applied to a Euclidean distance matrix, using a minimum of 9900 unique permutations. To confirm which FAs were most responsible for observed interspecific differences an analysis of similarity (SIMPER) was conducted. To test the effects of season on FA profiles, a second PERMANOVA was run only on *G. garricki*, as seasonal-based n-values were low in other species (Table 1). To compare FA profiles between species and season, a Principal Coordinate Analysis (PCO) was constructed from non-data arranged into a difference matrix based on Euclidean distances. Analysis of covariance was used to explore interspecific differences in ω3/ω6 between *G. garricki*, *G. glyphis*, *C. leucas* and *R. taylori*. As significant effects were found, we then used an ANCOVA with TL as a covariate, between each shark's ω3/ω6 ratio to consider possible allometric effects. This ratio has been linked to changes in environmental responses and may assist in explaining interspecific differences.

Univariate analyses were performed in R using the R core packages (R Development Core Team 2014), SIBER (Jackson et al. 2011) and one FA multivariate analysis used the package NicheROVER to determine overlap between sharks (Swanson et al. 2015) whilst the other multivariate tests were performed in PRIMER (v6) (Clarke & Gorley 2006).

Results

Stable isotopes

Muscle $\delta^{13}C$ and $\delta^{15}N$ values in seven elasmobranch species revealed two distinct trophic guilds (Fig. 2). The most enriched values were in *C. leucas, R. taylori, C. amboinensis* and *P. pristis* (Table 2, Fig. 2). The elasmobranch guild with the lowest range of $\delta^{13}C$ and $\delta^{15}N$ values included *G. glyphis*, *H. dalyensis* and *G. garricki* (Table 2, Fig. 2). When comparing all species there was a significant relationship between $\delta^{13}C$ and $\delta^{15}N$ ($p > 0.01$, $F_{df} = 33.2_{1, 117}$, $R^2 = 0.22$) and between the ratio of C:N and $\delta^{13}C$ ($p > 0.01$, $F_{df} = 14.81_{1,117}$, $R^2 = 0.11$). Total length was not significant in any species or isotope, with the exception of $\delta^{13}C$ values in *C. leucas* ($p > 0.01$, $F_{df} = 10.29_{1, 30}$, $R^2 = 0.25$).

Based on $\delta^{15}N$ values, the calculated TP ranged from 3.2±0.8 to 4.8±0.9 and was lowest in *G. glyphis* and highest in *C. leucas* (Table 2). Analysis of variance indicated significant differences among species for $\delta^{13}C$ and $\delta^{15}N$. The post hoc test for $\delta^{15}N$ showed no significant differences between *R. taylori* and *C. leucas,* and between *G. garricki* and *G. glyphis,* whereas all others species differed significantly. For $\delta^{13}C$, only *G. garricki* and *G. glyphis* were not significantly different from each other. Although seasonal effects for $\delta^{13}C$ were found in *C. leucas,* the inclusion of TL as a covariate negated the seasonal effect and revealed TL to be the only significant variable (Table 3).

Of the three species that convex hull total area (TA) was calculated for, *C. leucas* was the largest followed by *R. taylori* and *G. garricki*. *Carcharhinus leucas* overlapped strongly with *R. taylori,* although some individuals were closer in niche space to *G. garricki*. *Glyphis garricki* had the least overlap with other species: only the outliers of *R. taylori* and *C. leucas* shared their TA. Convex hull total areas were larger in the dry

271 season than the wet season for the three sharks. *Rhizoprionodon taylori* and *C. leucas*
272 had the biggest differences between the wet and dry season.
273
274 Size-sample corrected standard ellipse areas (SEA_C) of stable isotopes ranged from
275 6.86 to 18.46 (confidence intervals (CI) 97 – 99%) with *C. leucas* having the largest
276 area followed by *R. taylori* and *G. garricki* (Table 4, Fig. 1 in the Supplement 1).
277 *Carcharhinus leucas* and *R. taylori* SEA_C overlapped and were clearly separated from
278 *G. garricki* (Table 4, Fig. 1 in the Supplement 1). There was no overlap between *G.*
279 *garricki*, *C. leucas* and *R. taylori*. However, *C. leucas* and *R. taylori* had a partially
280 overlapping SEA_C. All species had seasonal differences in SEA_C, with higher values
281 in the dry season for *G. garricki*, and lower dry season values in *R. taylori* and *C.*
282 *leucas* (Fig. 2, Table 4). Seasonal differences were also evident in the overlap of the
283 SEA_C between *C. leucas* and *G. garricki* and, *G. garricki* and *R. taylori* with more
284 overlap in the dry than the wet season (Fig. 2, Table 4). The SEA_C of *C. leucas* in the
285 dry season slightly overlapped with that of *G. garricki*, although there was little niche
286 overlap with *G. garricki* over the whole sampling period (Table 4, Fig. 3).
287
288 *Fatty acids*
289 Sixty-five FAs were identified in the elasmobranch muscle tissue, of which 31 were
290 detected with mean values above 0.5% for any one species (Table 1, 2, and Table 1 in
291 the Supplement 2). Relative abundance of saturated FAs (SFAs) were similar in *C.*
292 *leucas*, *G. garricki*, *G. glyphis* and *H. dalyensis* ranging from 27.5±7.2 to 32.0±8.7%
293 but were higher in *R. taylori* and *C. amboinensis* (42.5 and 43.6±12.3%, respectively).
294 The monounsaturated FAs (MUFA) were relatively low in *G. glyphis*, *G. garricki* and
295 *R. taylori* (means ranging from 18.8% – 22.2%) compared to other elasmobranchs,

296 whose mean relative abundance ranged from 24.8% to 29.7%. Relative amounts of
297 polyunsaturated FAs (PUFA) were highly variable between species (means ranging
298 from 14.8% – 44.0%) with it lowest in *H. dalyensis* and highest in the *Glyphis*
299 species. Polyunsaturated FAs were similar between *C. leucas* and *R. taylori*
300 (30.3±2.2% and 30.9±3.4%, respectively) and were dominated by three EFAs:
301 20:4ω6, 22:6ω3 and 22:4ω6.
302
303 There were significant differences in FA profiles between species (PERMANOVA:
304 $F_{df} = 9.16_3$, $p < 0.01$) and there was no effect of season in the *G. garricki* FA profiles
305 ($F_{df} = 0.69_1$, $p = 0.60$). Elasmobranch FAs showed grouping between species, overlap
306 and some slight seasonal differences (Fig. 4). *Glyphis garricki* collected in the wet
307 season had similar FA profiles to those caught in the dry season. The greatest overlap
308 was between *G. glyphis* and *G. garricki* and one subgroup of *C. leucas*. The least
309 overlap was in *R. taylori*, separated by the SFAs: 17:0, 18:0 and 16:0. A large
310 subgroup of *C. leucas* was also separated from the other species by the FAs, 18:2c,
311 20:3ω9, 18:2b, 18:1ω9 and 20:2. Whereas the *Glyphis* spp. were largely separated by
312 EFAs: 20:3ω6, 22:6ω3, 22:4ω6, 22:5ω6 and 20:4ω6.
313
314 The highest mean ratio of ω3/ω6 was in *R. taylori* and the lowest was in *G. glyphis*
315 ranging from (0.9±1.4 – 0.4±0.8) (Table 2). An ANOVA of these taxa revealed
316 significant interspecific differences ($p = 0.01$, $F_{df} = 7.2_{3,76}$, $R^2 = 0.22$), however post
317 hoc tests found significant differences between *G. garricki* and *C. leucas*, and *G.*
318 *glyphis* and *C. leucas* and differences between *R. taylori* and the other sharks; *G.*
319 *garricki, G. glyphis* and *C. leucas*. Total length had a significant effect on ω3/ω6

ratios in *C. leucas* ($p = 0.05$, $F_{df} = 4.2_{1, 21}$, $R^2 = 0.18$), *G. garricki* ($p = 0.02$, $F_{df} = 6.2_{1, 23}$, $R^2 = 0.18$) but not in *R. taylori* or *G. glyphis*.

The largest FA niche size was *G. garricki* followed by *C. leucas* and *R. taylori* (Table 4, Fig. 5 and Fig. 3 in the Supplement 3). The chance of *G. garricki* being in another species' niche space was low (0.6% for *R. taylori* and 13.2% for *C. leucas*) yet *C. leucas* and *R. taylori* had larger probabilities for being in *G. garricki* niche space (79.9% for *R. taylori* and 61.7% for *C. leucas)*. Slight seasonal differences were evident in *G. garricki* FA niche space with a 15% probability of difference between the wet and dry (Table 4).

Trophic niche metric comparisons

Taxon and seasonal groupings were less prominent for FA profiles than for isotopic profiles, which highlighted two distinct guilds. Niche metric calculations supported these variations but there were differences between methods used, which meant they were not directly comparable. Between the two SI niche metrics (SEA_C and TA), TA was the largest and had the most overlap compared to SEA_C. Also the SEA*c* overlap in *G. garricki* was slightly larger during the wet than the dry, yet the reverse was true in TA overlap. *Carcharhinus leucas* showed the greatest difference between SI and FA niche metrics. In SEA_C, *C. leucas* had the most overlap with *R. taylori*, yet in FA niche space *C. leucas* had the most overlap with *G. garricki* (Table 4). As FA niche space is a probability projection rather than geometric (Swanson et al. 2015), different probabilities can be obtained for the same two species as each calculation is the likelihood of one species being in the others' niche space. For example *R. taylori* had a high probability of being in *G. garricki* niche space but the reverse was not true. This

345 was quite different from SEA*c* where *G. garricki* and *R. taylori* had little chance of
346 sharing resources except in TA. Seasonal differences between *G. garricki* SI and FA
347 niche probability appeared similar with a 15% difference between wet and dry values
348 and the overlap difference in SI was 9.5%.
349
350 **Discussion**
351 Analysis of SI and FA niche metrics demonstrated significant differences in the
352 trophic resource use of elasmobranch species, with indications of resource
353 partitioning within the South Alligator River ecosystem and surrounding coastal
354 waters. Although SI showed clear separation between coastal/marine and euryhaline
355 elasmobranchs, the difference was less pronounced between FAs. This is likely due to
356 the faster turnover of FAs (Kirsch et al. 1998, Beckmann et al. 2014) compared to SIs
357 (Logan & Lutcavage 2010) and the larger number and biochemical roles of FAs.
358 Seasonal differences in biota are relatively common in tropical river systems due to
359 the large outflow of freshwater during the wet season changing the sources of energy
360 and nutrients, and increasing primary and secondary production (Winemiller & Jepsen
361 1998). However, our results showed seasonal change in all SI and FA niche metrics
362 but not in the individual biochemical tracers. We observed only slight seasonal
363 changes within *Glyphis garricki* FA niche space that again may be the result of the
364 faster turnover of FAs. However, seasonal differences in $\delta^{15}N$ and $\delta^{13}C$ were also
365 marginal, suggesting that *G. garricki* are consuming similar prey over both seasons.
366
367 Distinct differences in the isotope niche space of elasmobranchs appear to be largely
368 driven by $\delta^{13}C$, with several species (*Rhizoprionodon taylori*, *C. amboinensis*, *C.*
369 *leucas* and *Pristis pristis*) characterized by values that are typically reported in marine

370 environments (-14 – -16‰; Hussey et al. 2011, Munroe et al. 2015). $\delta^{13}C$ values for
371 *P. pristis* and *C. amboinensis* were similar to previous studies (Knip et al. 2011,
372 Thorburn et al. 2014), which may have been sourced from seagrass, whilst *C. leucas*
373 and *R. taylori* had values closer to marine macroalgae (Loneragan et al. 1997). In
374 contrast, *G. garricki*, *G. glyphis* and *Himantura dalyensis* had depleted $\delta^{13}C$ values
375 suggestive of estuarine/freshwater seston signatures (-18.8 – -23.2‰; Loneragan et al.
376 1997). Notably, large standard deviations in *G. glyphis* $\delta^{13}C$ values (compared to *G.*
377 *garricki*) may be indicative of a greater variation in dietary sources consumed by *G.*
378 *glyphis* and/or our relatively small sample size in this species.
379
380 In terms of trophic position (TP), the seven elasmobranch species were broadly
381 similar, based on the spread of $\delta^{15}N$ values; with *C. leucas* having the most enriched
382 and highest mean TP (4.8±0.9). Although our analysis used juveniles, this value was
383 higher than adult TP (4.6±0.2) and sub-adult TP (4.4 ±0.3) *C. leucas* reported in
384 Mozambique (Daly et al. 2013) and Western Australia TP (~ 4.4) (Thorburn et al.
385 2014) and in the same range (~3.8 – 5.4) as those from South Africa (Hussey et al.
386 2014). This variation is likely due to the different methods of determining TP as well
387 as dietary and environmental variation (Peterson & Fry 1987). The method developed
388 by Hussey et al. (2014) provides more discrimination between predators and the
389 authors found that this method fits logically with TP calculated from prey type in
390 stomach content analysis. Moreover, the FA 18:1ω9 that is often linked to piscivory
391 and thus suggestive of a higher TP was also in relative high abundance in *C. leucas*
392 (Dalsgaard et al. 2003, Kelly & Scheibling 2012). Trophic position was not calculated
393 in the Queensland population of *R. taylori* (Munroe et al. 2015) however $\delta^{15}N$ results
394 were lower, which is possibly explained by different environmental conditions.

395 *Glyphis glyphis* appear to be consuming lower order, benthic prey as their low TP was
396 more similar to rays than sharks of this body size (Hussey et al. 2014) (~ maximum
397 size 260 cm (White et al. 2015)). *Glyphis garricki* had a higher TP than *G. glyphis*,
398 suggesting this species consumes higher order prey than its sympatric congener.
399
400 The ratio of ω3/ω6 can be used to help define a species' niche as higher ratios (>
401 ~1.5) signify a preference for marine resources whilst lower ratios (< ~1.5) indicate a
402 preference for freshwater resources (Martínez-Álvareza et al. 2005, Özogul et al.
403 2007). The elasmobranchs in the current study appeared to follow this pattern as *R.*
404 *taylori* (a marine species) had the highest ratio, whilst species collected upstream
405 tended to be lower. However, *R. taylori* was not significantly different from the other
406 species, which may have been due to their high intraspecific variation. Differences in
407 the ω3/ω6 ratio were found in teleost fish when they were acclimatizing to changing
408 saline conditions (Martínez-Álvareza et al. 2005), which may explain why *R. taylori*
409 has high variance given that they are consuming prey around the mouth of the river
410 and may experience lowered salinity. Ratios in *C. leucas* and *G. garricki* were
411 significantly different which may reflect a preference for differing salinities. This is
412 interesting, as significant differences between salinities have not previously been
413 recorded for elasmobranchs (Speers-Roesch et al. 2008), however further studies
414 would be required to confirm this in these species. The ω3/ω6 ratio may also be
415 related to growth and maturation in elasmobranchs, as a significant relationship was
416 found between TL and the ratio in *C. leucas* and *G. garricki*, which were mostly
417 juveniles. Uysal & Aksoylar (2005) found that ω3/ω6 ratios in teleost fish decreased
418 during sexual maturation.
419

Individual FAs indicate complexity in the trophic resource niches among and within species. For example, the FA profiles of *Glyphis* spp. suggest links to both marine and freshwater ecosystems due to the presence of 18:2ω6 and 20:5ω3 which are typically associated with marine algae, vascular plants and mangroves (Parrish 2013). Although *C. leucas*, shares niche space with the *Glyphis* spp., they may be utilizing more freshwater sources because *C. leucas* also had FAs in common with *H. dalyensis* such as 16:1ω7 which has been linked to mangroves and diatoms (Parrish 2013). *Himantura dalyensis* also had a low abundance of 22:6ω3 (typically associated with marine dinoflagellates (Parrish 2013)), suggesting that they utilize freshwater resources. Additionally, the relatively high abundance of 20:4ω6 conforms with their morphology and benthic feeding preference (Hall et al. 2006, Pethybridge et al. 2011). Finally, *C. leucas* had more FAs in common with the *Glyphis* spp. than *R. taylori* although *G. glyphis* had higher abundances of 20:4ω6 suggesting that there are links between marine and estuarine/coastal prey (Hall et al. 2006, McMeans et al. 2013). Thus, *C. leucas* FA profile suggests a greater intake of estuarine prey than marine, contrary to SI values. *Rhizoprionodon taylori* had higher abundances of the marine FA marker, 22:6ω3 compared to the other species, which would be expected given their marine habitat preference. The two juvenile *C. amboinensis* FA profiles were closest to *R. taylori*, and one of the dominant EFAs (20:4ω6) in *C. amboinensis* were in abundances that may be found in estuaries.

Differences in SI values and FA profiles in *C. leucas* may be a result of maternal influences, while differences in turnover between the two types of biotracers (SI, FA) are likely to be most linked to temporal differences. The significant correlation between TL and *C. leucas* $\delta^{13}C$ values, suggests that these sharks rely on different

445 food sources as they grow. Maternal signatures have been found in *C. leucas* in both
446 $\delta^{15}N$, $\delta^{13}C$ and FAs (Matich et al. 2010, Olin et al. 2011, Belicka et al. 2012),
447 however it is possible that size-related differences in $\delta^{15}N$ were not found in these
448 sharks because the size range was too small. Tissues in *C. leucas* have shown a
449 decline in isotopic values until they reach 110 – 130 cm TL (Matich et al. 2010)
450 whereas our maximum TL was 86 cm. The two *P. pristis* also had enriched SI values.
451 Considering they were juveniles and caught in the mid-section of the sampling sites
452 near some *G. garricki* who had lower $\delta^{13}C$, which may suggest a maternal influence.
453
454 As stomach contents analysis in *C. leucas* have found a broad range of species
455 including catfish (ariids), rays (batoids), and other carcharhinid sharks (Snelson et al.
456 1984, Tillett et al. 2014), a large trophic niche would be expected. This was true of SI,
457 however the size of their FA niche space was intermediate between *G. garricki* and *R.*
458 *taylori*. This pattern may indicate evidence of individual specialization as was found
459 in Florida, USA (Matich et al. 2011), diet switching (Matich & Heithaus 2014) or
460 maternal influence (Olin et al. 2011). A similar pattern was found in *R. taylori*,
461 although their FA niche size was comparatively small considering stomach content
462 analysis found a relatively broad range of species (e.g. prawns and teleost fishes)
463 (Simpfendorfer 1998). Interestingly, some *R. taylori* in Queensland occupied areas
464 near freshwater outflows during the wet season rather than moving to seagrass beds
465 with the majority of the population (Munroe et al. 2014). In light of these findings, it
466 may be that the narrow niche of *R. taylori* in the South Alligator estuary results from
467 this species targeting prey around freshwater outflows. Fatty acid niche metrics also
468 suggest that *R. taylori* consumes some riverine resources, however their prey
469 preference is largely associated with marine systems. Although FA niche metrics

470 indicate *R. taylori* and *G. garricki* share some resources, *G. garricki* mainly depend
471 on riverine resources with infrequent consumption of marine prey sources. Therefore
472 we suspect that *G. garricki* utilizes the lower South Alligator River whilst species
473 such as *R. taylori* and *C. amboinensis* take advantage of the diverse prey species in
474 the estuary.
475
476 In determining FA niche space, only five FAs could be used, which meant there was
477 an underlying assumption that these FAs represented all FAs within the species. A
478 more complete understanding of trophic niche based on FA profiles could be achieved
479 with larger sample sizes. Furthermore, a number of FAs were present in these species
480 either in relative high amounts or were the cause of separation between species that
481 are not yet associated with unique prey groups or food web source. A future area for
482 research is the identification of additional trophic markers to increase our ability to
483 distinguish food sources within and among rivers, estuaries and coastal ecosystems.
484
485 This study was the first to use SIA and FA analysis in combination to measure trophic
486 niche metrics and explore resource partitioning among an assemblage of consumers.
487 Our approach has demonstrated that elasmobranchs within the South Alligator River
488 display partitioning in trophic resource use, particularly across marine and freshwater
489 food webs that have sources related to seston, seagrass or macroalgae. Given the
490 potential for complexity in resource use, highlighted by seasonal shifts in isotopic
491 niches and the FA composition of muscle tissues, it appears likely these
492 elasmobranchs play important roles in food web connectivity in tropical aquatic
493 ecosystems. In particular, some species are utilizing food webs across the marine to
494 freshwater spectrum, which suggests that these fish provide broad and important

cross-biome trophic linkages in tropical coastal ecosystems. Differences in FA and SI niche space highlighted the advantages of combining such analyses, which are likely caused from the faster turnover of FAs, and more FA variables that may respond to different trophic resource components. Further analysis to link these FA profiles with potential prey items is needed to further increase our understanding of the role played by elasmobranchs in coastal and estuarine ecosystems. Based on our findings, it is concluded that tropical euryhaline and coastal elasmobranchs play important roles in both middle- and higher-order trophic interactions across estuarine, coastal and riverine ecosystems.

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Tables and Figures

Table 1. Number (*n*) and total length (TL) of seven euryhaline and coastal elasmobranch species from the South Alligator River, Kakadu National Park, Australia from which muscle tissue samples were taken for stable isotope (SIA) and fatty acid analysis (FAA).

Species		*n* (Wet:Dry)		Range TL (cm)		Sex ratio (M:F)	
Scientific Name	**Common Name**	**SIA**	**FAA**	**SIA**	**FAA**	**SIA**	**FAA**
Carcharhinus amboinensis	Pigeye Shark	0:2	0:1	68-68	68	1:1	0:1
Carcharhinus leucas	Bull Shark	28:6	2:20	93-72	72-139	18:2	9:1
Glyphis garricki	Northern River Shark	22:20	12:13	56-141	45-61	22:2	15:1
Glyphis glyphis	Speartooth Shark	2:3	2:6	71-120	78-136	1:4	6:2
Himantura dalyensis	Freshwater Whipray	0:2	0:2	56 -110	56-110	0:2	0:2
Pristis pristis	Largetooth Sawfish	2:0	0	87-96	-	1:1	
Rhizoprionodon taylori	Australian Sharpnose Shark	8:30	28:1	34-87	34 -87	11:3	6:2

723

724 **Table 2.** Mean values for fatty acids (FA> 0.5%), $\delta^{13}C$ and $\delta^{14}N$ and their standard
725 deviation in muscle tissue from seven elasmobranchs collected from the South
726 Alligator River, Kakadu National Park, Australia. Included are calculations for
727 trophic position (TP), total area of stable isotope convex hulls (TA), ellipse area of SI
728 (SEA_C) and major EFA niche space.

SI	*C. amboinensis*	*C. leucas*	*G. garricki*	*G. glyphis*	*H. dalyensis*	*P. pristis*	*R. taylori*
$\delta^{13}C$	-13.4	-14.9±2.3	-18.9±1.3	-18.9±1.7	-20.2	-13.4	-15.1±1.3
$\delta^{15}N$	11.7	12.95±2.8	9.3±1.9	7.6±3.6	8.9	12.6	11.1±2.5
C:N	2.6	2.8±0.2	2.8±0.2	2.7±0.1	3.2	2.7	2.8±0.4
TP	4.2	4.8±0.9	3.6±0.4	3.2±0.8	3.4	4.5	4.1±0.6
SEA_C (Wet/Dry)		18.5 (16.9/17.1)	6.9 (9.8/7.0)				10.3 (2.1/11.7)
TA (Wet/Dry)		79.1 (17.8/65.8)	28.9 (21.3/37.8)				39.1 (2.7/39.1)
FA							
18:1ω9	14.1	16.2±6.3	11.2±4.9	8.2±3.6	13.8±0.5		9.5±2.7
18:2b[v]	0.6	0.6±0.4	0.2±0.2	0.5±0.2	0.4±0.3		0.1±0.1
18:2c[v]	0.1	0.9±0.8	0.1±0.3	0.1±0.1	0.1±0.1		0.13±0.02
18:2ω6	0.6	0.8±0.9	1.8±1.1	1.8±1.1	3.8±3.7		0.9±1.0
20.2ω	0.2	2.8±2.2	0.5±0.8	0.3±0.2	0.2±0.1		0.1±0.1
20:2ω6	0.4	0.5±0.8	0.9±0.4	0.8±0.2	0.3±0.0		0.6±0.2
20:3ω9[#]	0.8	7.6±6.2	1.4±3.4	0.3±0.3	0.3±0.1		0.1±0.1
20:3ω6	0.2	0.4±0.3	0.8±0.4	0.6±0.3	0.3±0.1		0.4±0.2
22:3ω	1.7	1.01±0.7	1.7±1.8	2.6±2.4	2.9±3		1.1±1.2
20:4ω6	9.6	5.3±5.5	11.3±4.7	14.1±4.4	14.4±2.4		8.1±2.1
22:4ω6	3.5	2.1±2.1	6.4±4.2	8.0±6.5	0.2±0.2		3.0±1.4
20:5ω3	0.9	0.5±0.2	0.9±0.8	1.1±0.6	5.2±6.8		1.6±0.8
22:5ω3	0.9	1. 9±1.2	1.5±2.0	1.6±1.8	1.1±1.6		1.1±1.7
22:5ω6	1.8	1.2±0.7	2.3±1.1	2.3±1.1	1.2±0.8		2.2±0.8
22:6ω3	4.8	4.8±2.3	8.3±4.6	9.8±8.8	4.3±0.2		11.4±5.6
ω3/ω6	0.4	0.70±0.4	0.5±0.6	0.4±0.8	0.5±0.0		0.9±1.4
EFA niche		13413.7	114886.8				455.6
EFA Niche Dry /Wet			40732.8/ 64824.2				

729 EFAs (Essential fatty acids) <0.5% include: 18:2a[v], 18:4ω3, 18:3ω6, 18:3ω3, 20:4ω3/20:2, 21:5ω3,
730 21:3, 22:2a[v], and 22:2b[v].
731 # 20:3ω9 identified based on comparison with other *C. leucas* fatty acid literature; a standard was not
732 available at the time of analyses. [v] = unable to identify bonds as standard was not available at the time
733 of analyses.
734

Species	Variable	Source	DF	F-ratio	P -Value	Residual standard Error	R^2	*t*-value	Significant Tukey post hoc comparisons (p<0.05)
All	Species	$\delta^{15}N$	115	16.59	< **0.01**	2.5	0.3		*G. garricki – C. leucas* *G. glyphis – C. leucas* *R. taylori- G. garricki* *R. taylori – G. glyphis*
		$\delta^{13}C$	115	50.7	< **0.01**	1.7	0.6		*G. garricki – C. leucas* *G. glyphis – C. leucas* *R. taylori – G. garricki* *R. taylori – C. leucas* *R. taylori – G. glyphis*
		ω3/ω6	3 & 76	7.2	< **0.01**	0.6	0.22		*G. garricki – C. leucas* *G. glyphis – C. leucas*
C. leucas	Season	$\delta^{15}N$	30	0.8	0.4	2.6	0.025		
		$\delta^{13}C$	30	5.4	**0.03**	1.9	0.15		
	Season + TL	$\delta^{13}C$	29	5.1	0.01	1.8	0.26		
	Season	$\delta^{13}C$			0.7			0.4	
	TL	$\delta^{13}C$			**0.05**			-2.05	
G. garricki	Season	$\delta^{15}N$	40	0.02	0.9	1.9	0.0		
		$\delta^{13}C$	40	1.3	0.2	1.3	0.03		
R. taylori	Season	$\delta^{15}N$	36	1.4	0.2	2.5	0.04		
		$\delta^{13}C$	36	1.7	0.2	1.3	0.04		

Table 3. ANOVA and ANCOVA (shaded) of stable isotope values and the ratio of ω3/ω6 from the muscle of *Carcharhinus leucas*, *Glyphis garricki*, *G. glyphis* and *Rhizoprionodon taylori* from the South Alligator River, Kakadu National Park, Australia compared to the "wet" and "dry" season, with total length (TL) the covariate. Significant values in bold.

750 **Table 4.** Isotopic overlap (%) of stable isotopes (SI) ellipses (SEA_C) and convex hull
751 of *Carcharhinus leucas*, *Glyphis garricki* and *Rhizoprionodon taylori* from the South
752 Alligator River, Kakadu National Park, Australia. Also included are the probabilities
753 of sharks being in the major essential fatty acids (EFA) niche space of each other with
754 a confidence interval (CI) of 95%.

755

Species comparisons	% Overlap ellipse (SI)			% Overlap convex hull (SI)			EFA Ellipses
	All Seasons	Dry Season	Wet Season	All Seasons	Dry Season	Wet Season	% Probability (95% CI)*
C. leucas x G. garricki							61.7
C. leucas x R. taylori							0.4
G. garricki x C. leucas	0	19.4	0	20.8	18.3	16.8	13.2
G. garricki x R. taylori	0	0	0	12.4	6.5	0	0.6
R. taylori x C. leucas	23.7	16.4	10.9	31.3	20.6	4.0	11.2
R. taylori x G. garricki							79.9
Season comparisons							
G. garricki (Dry) *x* (Wet)		9.5			33.8		50.3
G. garricki (Wet) *x* (Dry)							35.4
C. leucas (Dry) *x* (Wet)		18.3			20.1		
R. taylori (Dry) *x* (Wet)		12.0			6.5		

756 * of 1 species being in another's niche space.

757

758

759 **Figure captions**

760 **Fig. 1** Map of the South Alligator River, Northern Territory, Australia, showing
761 capture locations of elasmobranchs. Inset shows where the river is in relation to
762 northern Australia. (Background map from Stamen Toner, and formed using QGIS
763 Development Team, (2015)).

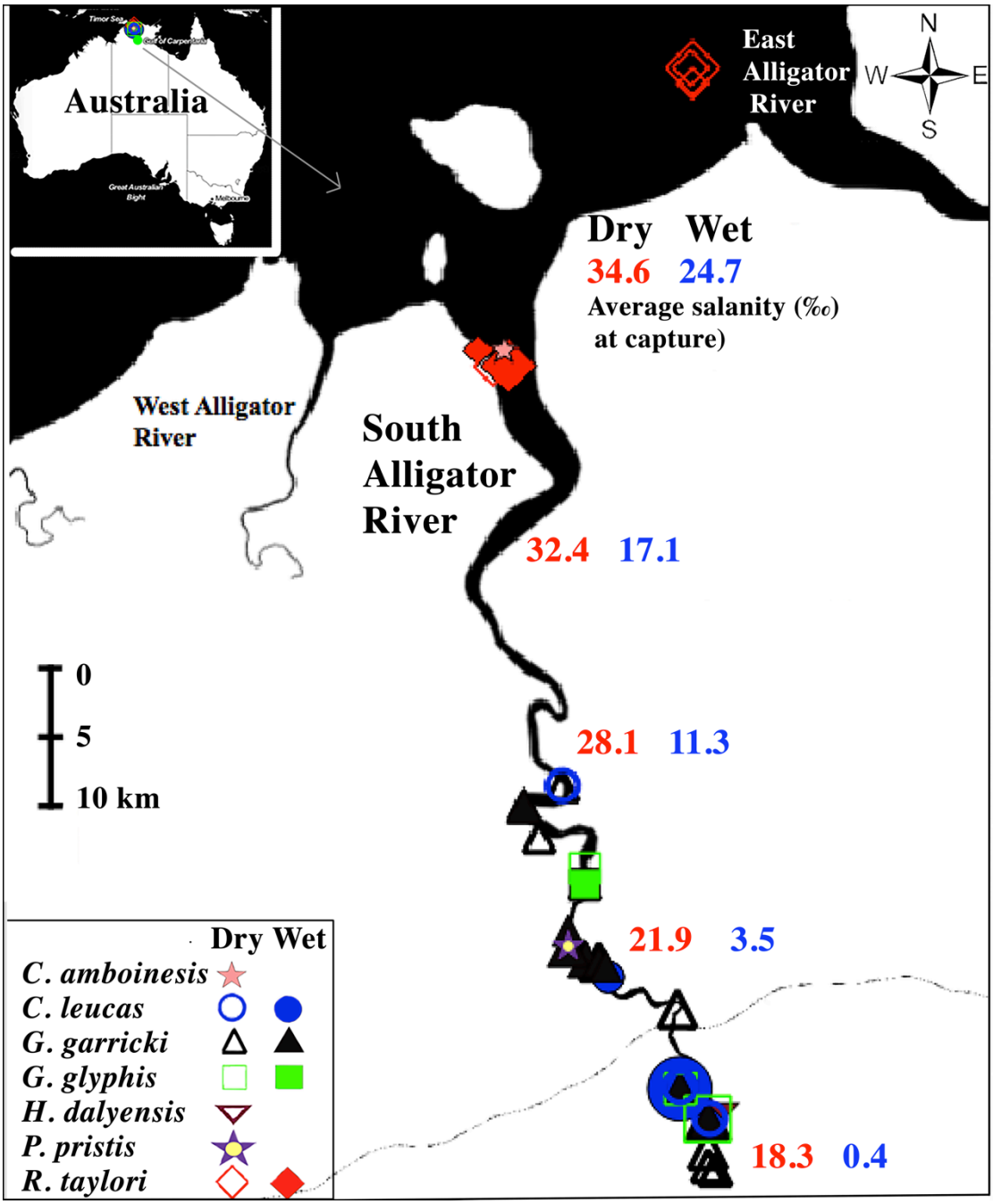
764 **Fig. 2** Biplot of mean $\delta^{13}C$ and $\delta^{15}N$ (± standard deviations) values for seven species
765 of euryhaline and coastal elasmobranchs from South Alligator River, Kakadu
766 National Park, Australia Primary producer values from the wet and dry season,
767 Embley River Estuary, Gulf of Carpentaria (Loneragan et al., 1997).

768 **Fig. 3** (a) Bivariate plot of isotopic space depicting wet vs dry niche areas within
769 standard ellipses of $\delta^{13}C$ and $\delta^{15}N$ of *Carcharhinus leucas* (blue) *Glyphis garricki*
770 (black), and *Rhizoprionodon taylori* (red) from Kakadu National Park, Australia (b)
771 Bayesian confidence intervals of isotopic niche area.

772 **Fig. 4** Principal component analysis of fatty acids in muscle tissue of *Carcharhinus*
773 *leucas*, *Glyphis garricki* and *Rhizoprionodon taylori*, *Carcharhinus amboinensis and*
774 *Himantura dalyensis* from the South Alligator River, Kakadu National Park,
775 Australia.

776 **Fig. 5** Comparisons of the posterior distributions of the probabilistic niche overlap
777 metrics (%) for a specified niche region of 95 % that an individual shark from the
778 species in the row will be found in the niche of *Carcharhinus leucas* (blue), *Glyphis*
779 *garricki* (black) (wet (black) and dry season (grey)) and *Rhizoprionodon taylori* (red)
780 of 5 major essential fatty acids (EFA). The posterior means and 95% credible
781 intervals are displayed in light blue.

Fig. 1


Australia
East Alligator River
N
W
E
S
Dry Wet
34.6 24.7
Average salanity (‰) at capture)
West Alligator River
South Alligator River
32.4 17.1
0
5
10 km
28.1 11.3
21.9 3.5
18.3 0.4
Dry Wet
C. amboinesis
C. leucas
G. garricki
G. glyphis
H. dalyensis
P. pristis
R. taylori

792 **Fig. 2**

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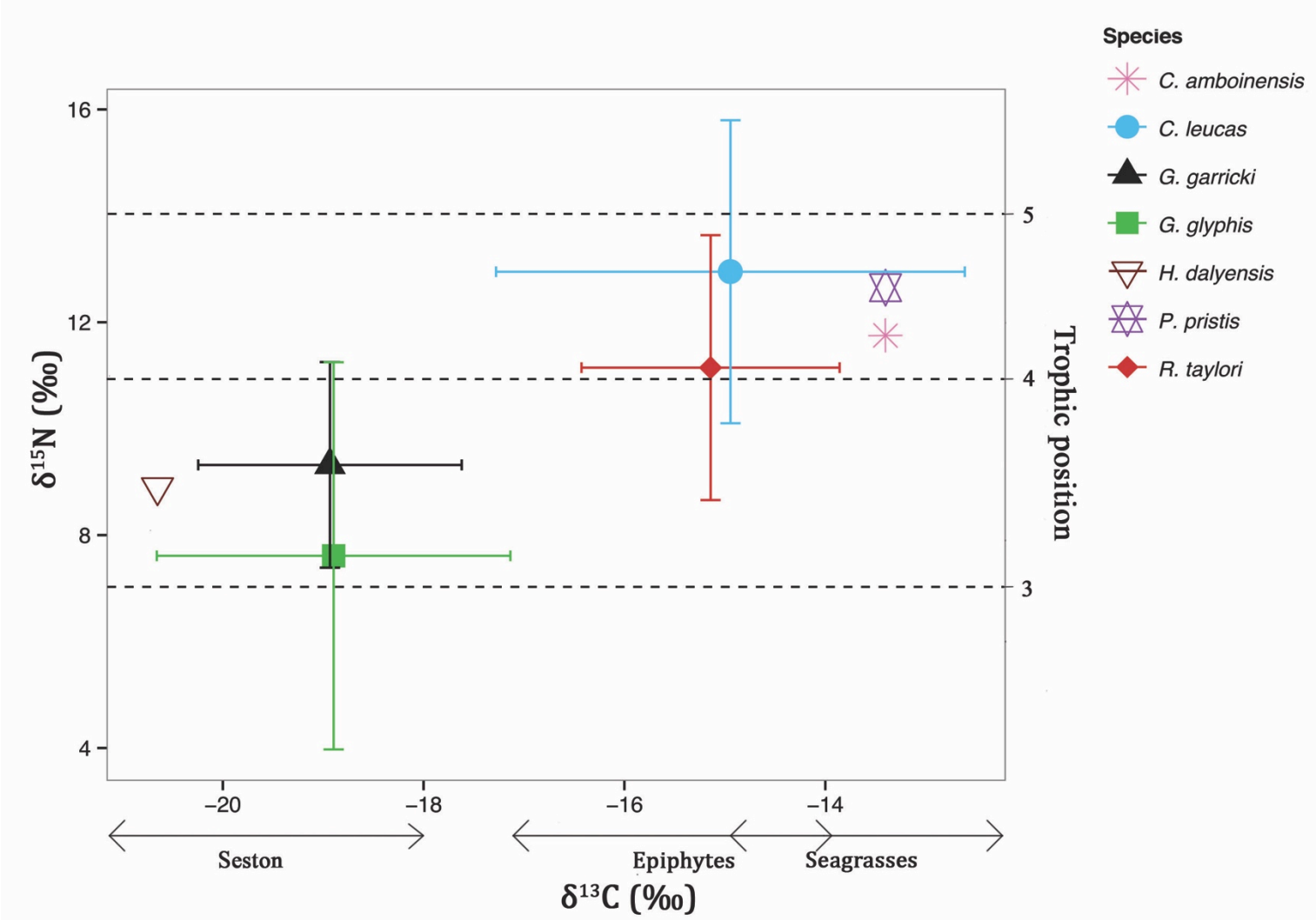

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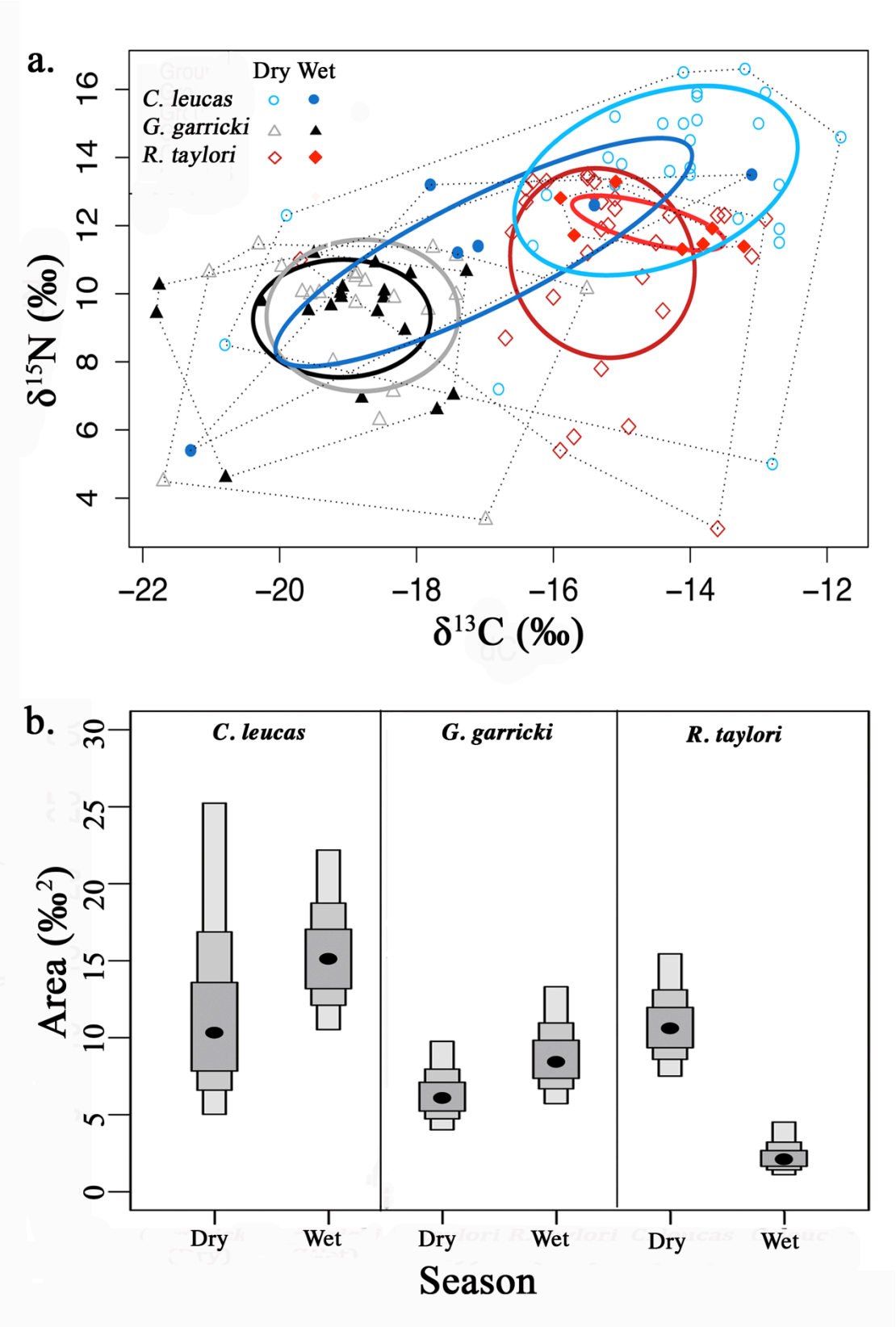
805 **Fig. 3**



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808 **Fig. 4**

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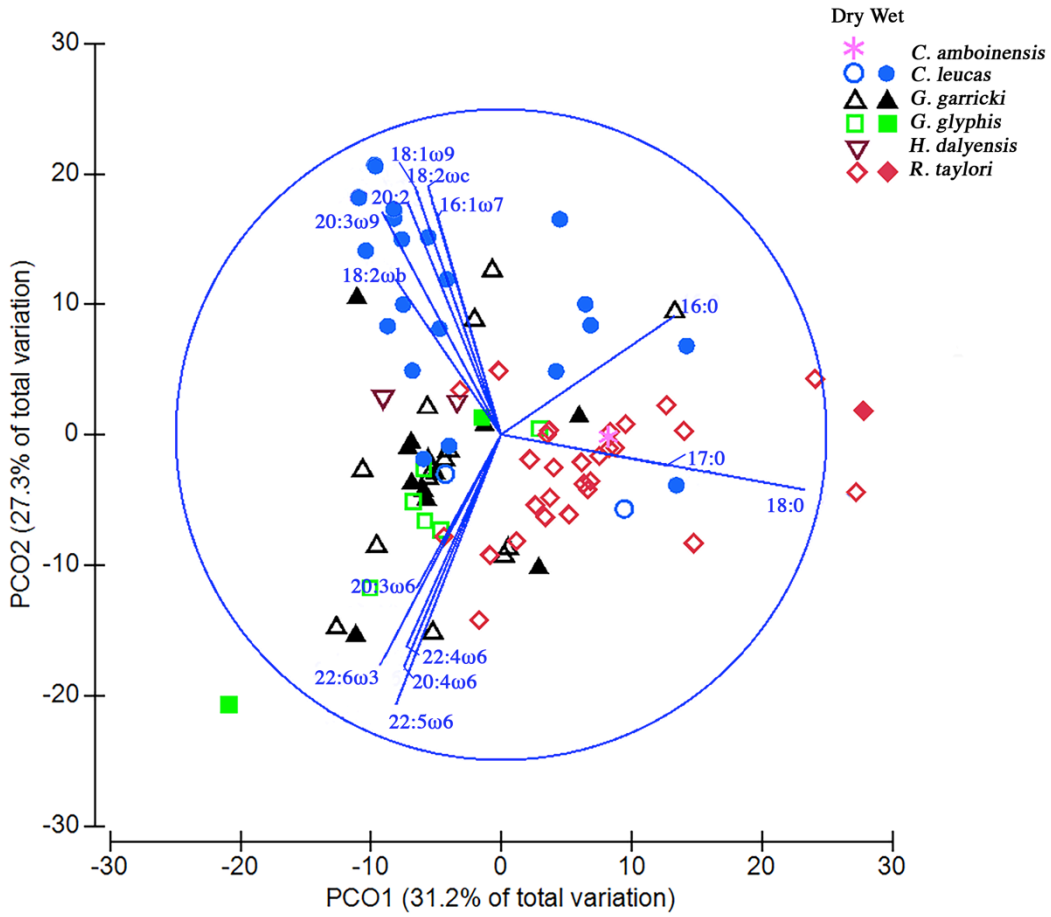
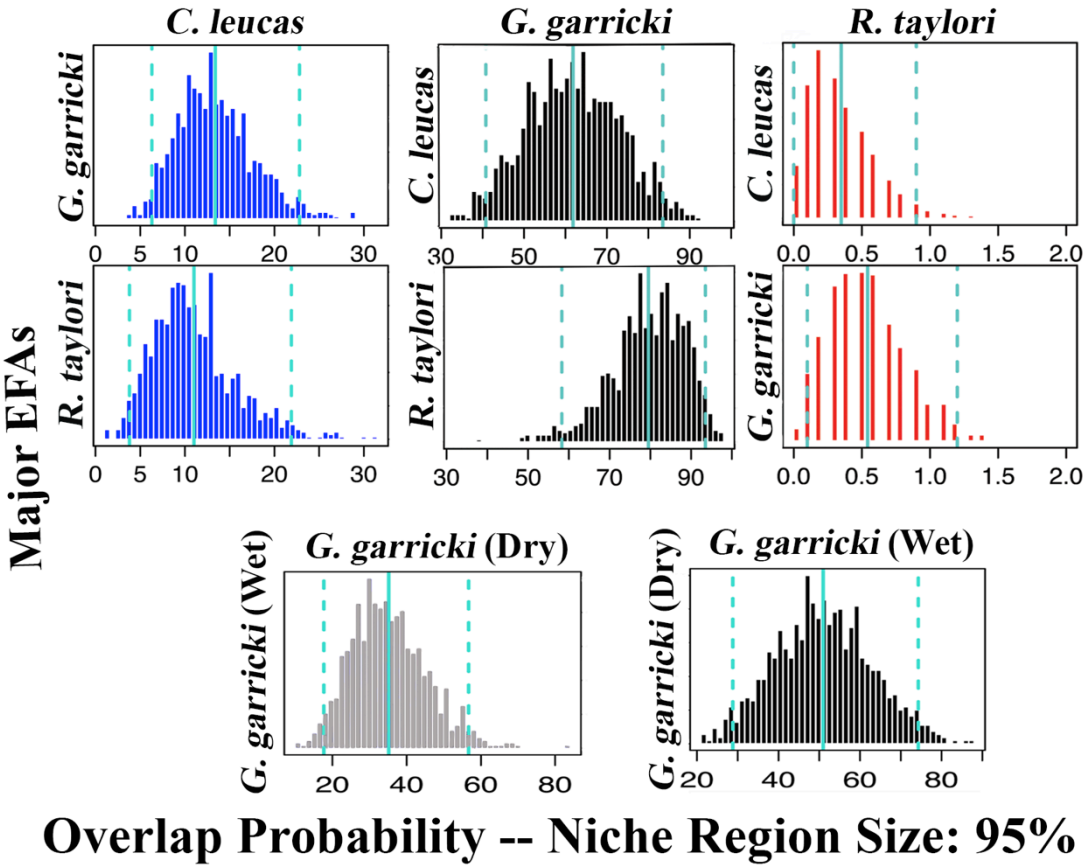


812

813 **Fig. 5**

814


C. leucas
G. garricki
R. taylori
Major EFAs
G. garricki
C. leucas
R. taylori
G. garricki (Dry)
G. garricki (Wet)
Overlap Probability -- Niche Region Size: 95%

Supplementary 1 Table S1

Mean values for all fatty acids (FA> 0.5%), and their standard deviation in muscle tissue from six elasmobranchs collected from the South Alligator River, Kakadu National Park, Australia.

FAs	*C. amboinensis*	*C. leucas*	*G. garricki*	*G. glyphis*	*H. dalyensis*	*R. taylori*
16:0	8.6	10.6±4.8	8.0±5.6	8.0±5.5	13.7±3.5	13.5±5.1
17:0	0.6	0.4±0.3	0.6±0.2	0.68±0.4	1.1±0.0	0.9±0.2
18:0	32.5	19.8±6.9	19.5±5.2	17.1±4.5	13.8±5.8	28.3±7.4
22:0	0.3	0.6±0.4	1.7±2.9	0.8±0.6	0.8±0.4	0.5±0.6
24:0	0.5	0.4±0.3	0.5±0.4	1.0±0.9	0.7±0.7	0.4±0.3
15:1	0.2	1.3±1.3	1.5±1.5	0.8±0.8	1.1±0.3	0.7±0.4
16:1ω7	0.5	1.9±1.36	0.7±0.6	0.7±0.5	1.6±0.2	0.7±0.5
17:1	1.3	1.3±0.9	1.0±0.3	2.0 ±1.7	3.8±0.7	0.5±0.2
18:1ω9	14.1	16.2±6.3	11.2±4.9	8.2±3.6	13.8±0.5	9.5±2.7
18:1ω7	6.2	4.9±2.5	5.2±1.8	4.3±2.0	4.0±1.3	7.2±2.0
17:1ω6	0.5	0.5±0.2	0.5±0.3	0.6±0.5	0.8±0.4	0.6±0.3
20:1ω9	0.8	1.0±0.4	1.0±0.5	0.8±0.7	0.3±0.3	0.7±0.3
22:1ω11	0.1	1.7±5.0	0.2±0.2	0.1±0.0	0.1±0.1	0.5±2.1
24:1ω9	0.9	0.8±0.4	0.9±0.7	1.2±0.9	0.6±0.9	0.4±0.1
18:2b^{v}	0.6	0.6±0.4	0.2±0.2	0.5±0.2	0.4±0.3	0.1±0.1
18:2c^{v}	0.1	0.9±0.8	0.1±0.3	0.1±0.1	0.1±0.1	0.13±0.02
18:2ω6	0.6	0.8±0.9	1.8±1.1	1.8±1.1	3.8±3.7	0.9±1.0
20.20	0.2	2.8±2.2	0.5±0.8	0.3±0.2	0.2±0.1	0.1±0.1
20:2ω6	0.4	0.5±0.8	0.9±0.4	0.8±0.2	0.3±0.0	0.6±0.2
20:3ω9$^{\#}$	0.8	7.6±6.2	1.4±3.4	0.3±0.3	0.3±0.1	0.1±0.1
20:3ω6	0.2	0.4±0.3	0.8±0.4	0.6±0.3	0.3±0.1	0.4±0.2
22:3	1.7	1.01±0.7	1.7±1.8	2.6±2.4	2.9±3	1.1±1.2
20:4ω6	9.6	5.3±5.5	11.3±4.7	14.1±4.4	14.4±2.4	8.1±2.1
22:4ω6	3.5	2.1±2.1	6.4±4.2	8.0±6.5	0.2±0.2	3.0±1.4
20:5ω3	0.9	0.5±0.2	0.9±0.8	1.1±0.6	5.2±6.8	1.6±0.8
22:5ω3	0.9	1. 9±1.2	1.5±2.0	1.6±1.8	1.1±1.6	1.1±1.7
22:5ω6	1.8	1.2±0.7	2.3±1.1	2.3±1.1	1.2±0.8	2.2±0.8
22:6ω3	4.8	4.8±2.3	8.3±4.6	9.8±8.8	4.3±0.2	11.4±5.6
Σ<5% FAs	0.1	5.5±0.9	5.6±0.9	6.1±1.0	5.1±0.8	3.03±0.5
i17:0	0.6	0.8±0.5	1.0±2.8	0.6±0.4	0.7±0.6	0.3±0.1
16:0FALD	0.6	0.8±0.7	1.1±1.0	1.1±1.3	0.9±0.6	0.76±0.6
18:0FALD	1.6	0.9±0.6	1.3±1.1	1.7±1.3	2.2±1.4	0.5±0.2

FAs <0.5% include 14:0 15:0, a15:0, 15:0, 14:1, 16:1ω13, 16:1ω9, 16:1ω7, 16:1ω5, 17:1ω8+a17:0, 18:1ω7, 18:1ω5, 18:1, 19:1, 20:1ω7, 20:1ω11, 20:1ω5, 22:1ω9, 22:1ω7, 24:1ω11, 24:1ω7, 16:4+16:3, 18:2a^{v}, 18:4ω3, 18:3ω6, 18:3ω3, 20:4ω3/20:2, 21:5ω3, 21:3, 22:2a^{v}, 22:2b^{v}, i16:0, 18:1FALD

822 # 20:3ω9 identified based on comparison with other *C. leucas* fatty acid literature; a standard was not
823 available at the time of analyses. [v] = unable to identify bonds as standard was not available at the time
824 of analyses. FA - Fatty acids, SAT- saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA
825 - polyunsaturated fatty acids. FALD – fatty aldehyde analyzed as dimethyl acetal.
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828 **Supplementary 2 – Fig. S1**
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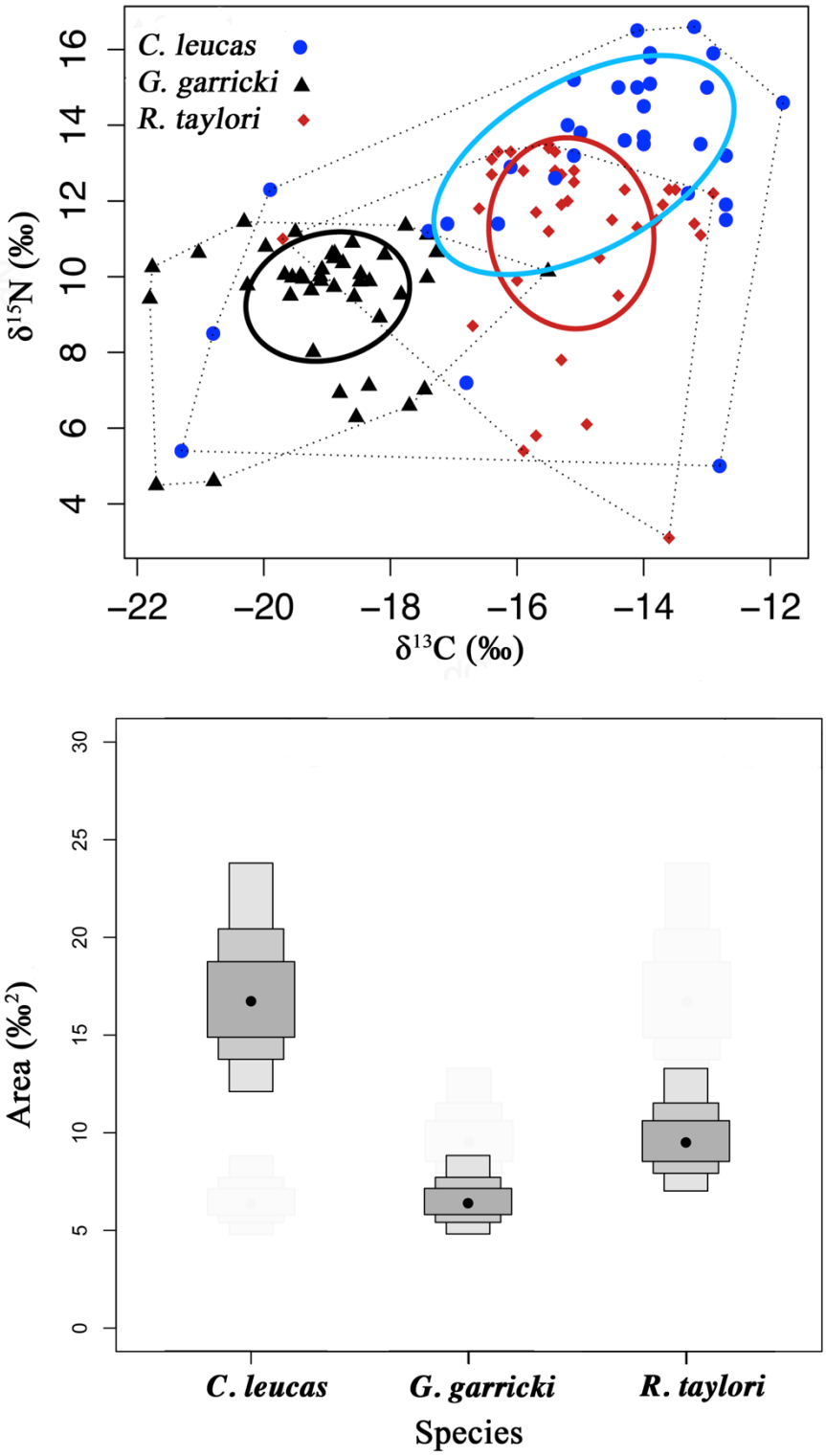

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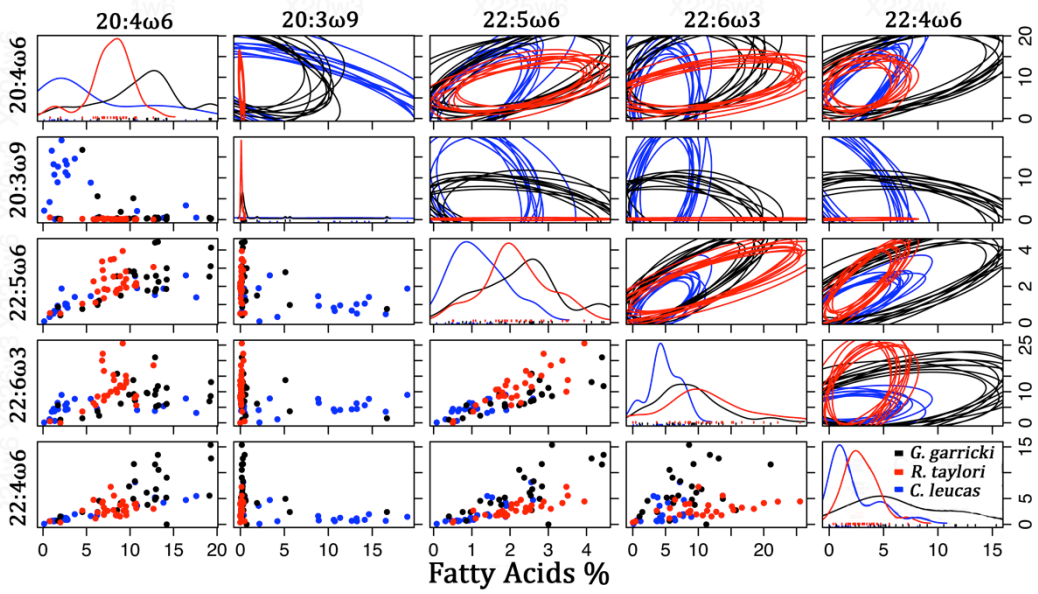
Supplementary 3 – Fig. S2

(i) Ten random elliptical projections of trophic niche region for each elasmobranch and pair of major essential fatty acids (FA) and *(ii)* *G. garricki* wet vs dry season major (elliptical plots). Also displayed are one-dimensional density plots (lines) and two-dimensional scatterplots to demonstrate normality.

a.



b.