A seasonally dynamic estuarine ecosystem provides a diverse prey base for elasmobranchs

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Abstract

Tropical river and estuarine food webs sustain diverse biodiversity values and are important sources of nutrients and energy for connected aquatic and terrestrial ecosystems. High order predators, such as euryhaline elasmobranchs, play critical roles in these food webs, but a lack of detailed information on food web structure limits our ability to manage these species within their ecosystems. We analysed stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes (SI) and fatty acid (FA) biochemical tracers from putative prey species in the estuary of the South Alligator River, northern Australia. These were compared with existing data on four species of elasmobranch from the system to examine food web structure and infer dietary linkages over wet and dry seasons along an environmental gradient of sites. Layman's SI community metrics indicated that upstream food webs had the greatest trophic diversity and analyses of FAs revealed distinct prey habitat associations that changed seasonally. Mixing models of SI indicated that most *Glyphis glyphis* and *Glyphis garricki* had similar fresh water and mid–river diets whilst *Carcharhinus leucas* and *Rhizoprionodon taylori* had largely marine signatures. Multivariate analyses of FA revealed some intraspecific differences, although specialisation indices suggested the four shark species are trophic generalists. Our results show that riverine food webs can display complex spatiotemporal variations in trophic structure and that coastal and euryhaline mobile elasmobranchs forage in a range of coastal and freshwater habitats, demonstrating their influence on these food webs.

Introduction

Food webs in tropical floodplain rivers are highly connected, dominated by seasonal hydrological cycles and typically characterised by short food chains and temporally variable ecological communities (Douglas et al. 2005; Blanchette et al. 2014). Euryhaline and coastal elasmobranchs (sharks and rays) provide potentially important connections across tropical ecosystems due to their mobility and high trophic position, and are crucial in the maintenance of community structure and ecosystem function in many estuaries (Last 2002; Every et al. 2017).

Estuarine and coastal ecosystems may act as nurseries for sharks (Heupel et al. 2007), afford protection from predation and provide a diverse source of prey (Cyrus and Blaber 1992; Heupel et al. 2007). However, many of these ecosystems have been affected by habitat disturbance and fishing pressure (Gallagher et al. 2012; Dulvy et al. 2014) that have contributed to the decline of many estuarine species, including elasmobranchs (Lucifora et al. 2015). In order to conserve and manage these species, there is a need to increase our knowledge of the dietary requirements and potential trophic specialization of euryhaline elasmobranchs (Montoya et al. 2006) to better understand functional differences among species, overlaps in diet and dependencies among species and habitats (Young et al. 2015; Grubbs et al. 2016).

Previous work examining dietary composition in tropical euryhaline elasmobranchs has been largely limited to ubiquitous species such as the bull shark *Carcharhinus leucas* (Matich et al. 2011; Belicka et al. 2012; Daly et al. 2013). However, other species also comprise important components of the elasmobranch fauna of rivers and estuaries in the Indo-Pacific, but are not well studied. In northern Australia, there is a

paucity of data on the trophic ecology of coastal and euryhaline elasmobranchs, with previous studies focusing on adult to sub-adult (Tillett et al. 2014) and juvenile *C. leucas* and large tooth sawfish *Pristis pristis* (Thorburn and Rowland 2008; Thorburn et al. 2014). Some of these studies have used stomach content analysis, which provides direct dietary information, but only across a brief snapshot in time. Stomach content studies may also underestimate the contribution of soft-bodied prey or over-represent certain groups (e.g. crustaceans) due to differential rates of digestion and/or complex temporal patterns in consumption. Advances in techniques such as biochemical analysis of stable isotopes (SI) and fatty acids (FA) in body tissues have allowed for broader time scales of trophic ecology to be explored (MacNeil et al. 2005; Hussey et al. 2011; Pethybridge et al. 2011; Couturier et al. 2013; Rohner et al. 2013; Every et al. 2016).

Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have been widely used to determine niche area and overlap (Vaudo and Heithaus 2011; Every et al. 2017), food web structure (Abrantes and Sheaves 2009; Tilley et al. 2013) and community metrics across a broad range of ecosystems (Layman and Post 2005; Brind'Amour and Dubois 2013). Isotopic mixing models (Layman and Allgeier 2012; Parnell et al. 2013; Tilley et al. 2013) can be particularly useful to trace which prey or prey group (source) is likely to have been consumed by a predator (Peterson and Fry 1987). More recently, complementary FA analyses have also been used to interpret isotopic food web indices, as they provide greater specification of basal sources and can help to confirm trophic linkages (Budge et al. 2002; Iverson 2009; Kelly and Scheibling 2012). The combination of both SI and FA analyses provides a powerful means of

exploring and interpreting the trophic ecology of consumers and associated food webs (Belicka et al. 2012; McMeans et al. 2013).

The objective of the current study was to explore the structure of a tropical riverine food web in northern Australia to examine seasonal (wet versus dry season) and longitudinal patterns of trophic relationships among predator and prey species. SI and FA analyses were conducted on a suite of putative prey species and combined with published data on euryhaline (*Carcharhinus leucas, Glyphis garricki, G. glyphis*) and coastal (*Rhizoprionodon taylori*) elasmobranchs. A suite of analytical approaches were employed to assess the structure and seasonal variability of food webs at sites ranging from the estuary mouth to the upper estuarine reaches. The results of the study are discussed with regards to temporal and spatial patterns of trophic linkages between predators and their prey, and the importance of riverine ecosystem function as a driver of food webs that support high order predators in estuarine and coastal habitats.

Methods

Elasmobranch and potential prey collection

Three euryhaline elasmobranch species (*Carcharhinus leucas, Glyphis garricki, G. glyphis*) and one coastal species (*Rhizoprionodon taylori*) were collected in the South Alligator River, Australia from March 2013 to July 2014 (Table 1) as part of previous studies (Every et al. 2016; Every et al. 2017) (Fig. 1). *Rhizoprionodon taylori* were captured by baited line in the mouth of the river and *G. garricki, G. glyphis* and *C. leucas* were collected further upstream, with a combination of gill nets and baited

lines. All sharks were measured and biopsied before being released at the site of capture.

Sampling for prey occurred in the same 4 sites where sharks were collected for an earlier study (Every et al. 2017) over the wet (monsoon) (November – April) and dry (May – October) seasons. Briefly, site 1 was the furthest upstream and had a mean salinity (‰±SD) during the dry season of 21.9±5.3 and of 0.4±1.5 during the wet season, whilst at site 4 salinity was high in the dry (34.5±0.2) and lower in the wet (17.1±4.3) (Every et al. 2017) (Fig. 1). Prey were captured using a range of sampling methods: a ~5 m wide beam trawl, gill nets (mesh size ranging from 10 – 30 cm), a cast net, and custom made wire rectangular marine and opera crab pots. Prey species were also caught during gill net and line fishing for sharks. Six putative prey species (Table 1) were chosen for analysis as these: (1) appeared in sufficient numbers to be considered a significant part of the food web; (2) represented a range of trophic levels; and, (3) had been reported previously in the stomachs of study elasmobranchs (Snelson et al. 1984; Simpfendorfer 1998; Thorburn and Morgan 2004; Peverell et al. 2006). Prey species consisted of five teleost fishes and one crustacean (Table 1).

Tissue sampling & preparation

For teleost fishes, only muscle tissue was used so that larger fish could be released, which involved using a scalpel to lift scales (where present) and remove a small square of tissue from the caudal peduncle region. Smaller fish of less than 25 cm total length were euthanized in 20 L of river water using AQUI-S® (20 mg/L) (Lower Hutt, New Zealand; sensu Turchini et al. 2011; Matley et al. 2016), and then the right side of the body was filleted to obtain a sample. The invertebrate *Macrobrachium*

174 *equidens* were also euthanized in the same way before muscle was dissected from
175 within the 2nd to 4th abdominal segments, taking particular care not to include other
176 tissue (e.g. exoskeleton, gut). Elasmobranch muscle tissue was collected from
177 between the second dorsal and the caudal fin, slightly anterior and lateral to the caudal
178 peduncle using a 5 mm biopsy punch (Stiefel) (see Every et al. 2016).
179
180 Immediately after collection, all tissue was stored in liquid nitrogen at –196°C, and
181 within a week transferred to a -20˚C freezer until it was freeze-dried for analysis.
182 Preparation of samples was undertaken in the freezer to avoid tissue degeneration. All
183 tissue except muscle was removed and the muscle sample divided and weighed
184 separately for SI and FA analyses. Mean (± standard deviation (SD)) dry sample
185 weight was 1.96±0.16 mg across all prey types.
186
187 *Stable Isotope Analysis*
188 Prey muscle tissue was freeze-dried to a constant weight and then pulverized using a
189 combination of micro-scissors and a small polyethene pestle, or a coarse pestle and
190 ceramic mortar. Muscle tissue was weighed to between 400-2200 µg. Before
191 elasmobranch muscle tissue was freeze-dried it was rinsed in milli-Q water and
192 sonicated to remove excess urea as per Kim & Koch (2012). Tissue was then weighed
193 to between 400 and 1000 µg. To combust and analyze samples, a SerCon Europa EA-
194 GSL elemental analyzer and Hydra 20-22 isotope ratio mass spectrometer (Sercon
195 Ltd, UK) was used at the Australian Rivers Institute, Griffith University. Relative
196 $\delta^{13}C$ and $\delta^{15}N$ were calculated using the Peedee Belemnite Carbonate international
197 standards for $\delta^{13}C$ and Atmospheric Nitrogen with a precision of (1SD) 0.03 and
198 0.09‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively. Due to the low lipid content in the muscle of

all tissue, lipid corrections were not necessary except for threadfin catfish *N. armiger* which had a mean C:N ratio of 4.3±1.1. This ratio is over the recommended level of 3.5 which causes the $\delta^{13}C$ to be 3-4‰ to be more negative, therefore the following formula was applied (Post et al. 2007):

$$\delta^{13}C_{normalized} = \delta^{13}C_{untreated} -3:32 + 0:99 \text{ X } C:N$$

As SI analysis required a smaller amount of tissue, more individuals (cf. to FA analysis) could be examined with this method.

Fatty Acid Analysis

Prey FAs were quantitatively extracted from muscle tissue via direct transmethylation (Parrish et al. 2015). Fatty acids were liberated from the lipids within the tissue sample via solvent extraction. Tissues were freeze dried, weighed, and 3 ml of MeOH: hydrochloric acid (HCl): DCM (10:1:1) was added, vortexed and placed in heating block at 85°C for 2 hours. After cooling, 1 ml of milli-Q H_2O was added and the FA solution was extracted with 1.8 ml of 4:1 hexane:DCM solution and then vortexed for five minutes in a centrifuge to form the lipid bilayer. The upper layer was then transferred using DCM and blown down under a constant stream of N_2. The extraction process was repeated two more times before a known concentration of internal standard was added. Final concentrations of 10 mg lipid to 1.5mL DCM were made and stored in a -20°C freezer until further analysis within 7 days of extraction.

A full explanation of elasmobranch muscle tissue analysis can be found in Every et al. (2016). Briefly, lipids were quantitatively extracted using the modified Bligh & Dyer

(1959) method which is an overnight one-phase extraction process of methanol:dicloromethane (DCM);milli-Q water (2:1:0.8 by volume). Saline milli-Q water and DCM were added the next day to make the final volume 1:1:0.9. The lower phase and solvents were evaporated with a rotary evaporator and remaining lipid transported with DCM into a pre-weighed vial, blown down with nitrogen and dried to a constant mass. The final concentration in the vials was 10 mg of lipid to 1.5 ml DCM, these were then stored in the -20^{o}C freezer till further analysis. Transmethylation of elasmobranch lipids followed the same process as prey tissue.

Fatty acid composition was quantified by an Agilent Technologies 7890B gas chromatograph (GC) (Palo Alto, California USA) and an Agilent Technologies 7683B Series auto-sampler. Peaks were quantified using Agilent Technologies ChemStation software (Palo Alto, California USA), and identifications confirmed by GC-mass spectrometry (GC-MS) using a column of similar polarity to that described above and a Finnigan Thermoquest DSQ GC-MS. Fatty acid were converted to a percentage. FAs with values <0.5% were not included in statistical analysis.

Assessment of food web structure

Stable isotope data was used to calculate Layman's six metrics (Layman et al. 2007) of seasonal and spatial trophic diversity in both putative prey and shark consumer species across each site and season. The first four metrics are measures of the assemblage trophic diversity, whilst the last two measure the relative space between each other (Layman et al. 2007). These include (i) the δ^{15}N range (NR), the distance between two species with the most enriched δ^{15}N minus the most depleted δ^{15}N, where a larger range generally indicates more trophic levels. (ii) δ^{13}C range (CR), the

249 distance between two species most enriched and depleted $\delta^{13}C$, the larger the range,
250 the more basal resources are used. (iii) Total area (TA), the assemblage combined
251 isotopic niche space occupied indicating the total extent of trophic diversity. This is
252 influenced by extreme values of $\delta^{15}N$ and $\delta^{13}C$ and should be considered with these
253 values simultaneously. (iv) Centroid distance (CD), the mean Euclidean distance of
254 each species to the isotopic centroid (mean of $\delta^{15}N$ and $\delta^{13}C$ of all species in food
255 web). This is a function of species spacing and is a measure the average degree of
256 trophic diversity within a food web. (v) Mean nearest neighbour distance (MNND), is
257 a measure of the density of species packing indicated by the mean Euclidean distance
258 to all species closest neighbour in isotopic space. A large MNND indicates species
259 with more divergent trophic niches. (vi) The standard deviation of MNND (SDNND)
260 measures the evenness of species packing in isotopic space, a low SDNND indicates a
261 more even distribution (Layman et al. 2007). Metrics were calculated using the mean
262 from each species group in the R package SIAR 4.2.2, which uses Bayesian
263 approaches to account for uncertainty in the derived means of convex hulls, removes
264 potential errors and therefore increases the validity in the estimates of community
265 metrics (Jackson et al. 2011). To minimise sample size biases (Jackson et al. 2011)
266 within this analysis some species were omitted in some sites and seasons where $n < 5$
267 individuals for each species (Table 2). All sites and seasons were pooled so that those
268 species where n was < 5 in specific sites could be compared holistically.
269
270 To examine differences in the SI compositions of the putative prey taxa an analysis of
271 variance (ANOVA) was used followed by pairwise Tukey tests with Bonferroni
272 adjustments for multiple comparisons. Evaluations of Q-Q plots and residual vs fitted

graphs indicated that no data transformations were required to satisfy model assumptions.

Permutational analysis of variance (PERMANOVA) was used to explore significant differences between species EFAs in multivariate space. A homogeneity of dispersion test (PERMDISP) revealed an uneven distribution of multivariate variance ($p < 0.01$, $F_{df} = 5.40$, $_{5, 120}$). However, PERMANOVA has been found to be relatively robust to such dispersion issues (Clarke and Gorley 2006) (e.g. in our case, site or season). In these analyses the PERMANOVA (with 9999 permutations) was used to test for a significant difference between prey, prey and season, prey and capture location (sites 1, 2, 3 and 4) as factors and finally as prey, season and capture location. A pairwise test was also carried out with species and season as the factor. To assist in the interpretation of the PERMANOVA and to visualize these differences a principal coordinate analysis (PCO) was constructed using Euclidean distance resemblance matrix. Vectors were correlated to the ordination structure (at level Pearson $r > 0.1$) were provided for added clarification.

To determine which FAs may be unique to each prey species a Dufrêne- Legendre indicator species analysis (R package; labdsv (Roberts 2016)) was applied. This calculates a maximum indicator value for FAs and was based on the relative frequency and association of FAs among and within each species. This was developed to determine which species could be used as indicators for various habitats, however we have used the same calculations to determine which FAs occur more frequently in each species – therefore our species are the ‘habitat’ and the FAs are the ‘species’ according to Dufrêne & Legendre (1997). To calculate the indicator value for FAs

based on the relative frequency and association of FAs within each species we need to determine the presence / absence (P_{ij}) of FAs in a species and the abundance of FAs in the species (X_{ij}):

Where:

FA = i

Species = j

n_c = number of samples in cluster c (for cluster c in set K)

f = relative frequency

a = abundance of FAs

$d_{i,c}$ = Indictor value (IndVal)

$$f_{i,c} = \frac{\sum_{j \in c} P_{i,j}}{n_c}$$

$$a_{i,c} = \frac{(\sum_{j \in c} x_{i,j})/n_c}{\sum_{k=1}^{K}((\sum_{j \in c} x_{i,j})/n_k)}$$

$$d_{i,c} = f_{i,c} \times a_{i,c}$$

An indicator value and p – value are assigned to each FA for that particular species. The addition of the p - value, was an adaptation in the R package; labdsv (Roberts 2016) from the oringnal calculations of Dufrêne & Legendre (1997).

Isotope mixing models to investigate prey contributions to sharks

Mixing models for SI were created using the Bayesian models package MixSIAR (Moore and Semmens 2008) in R (R Core Development Team, 2014). These models use a Markov chain Monte Carlo (MCMC) resampling routine to calculate

uninformed priors based on the data given (we used 10,000 iterations). They were designed to be robust, allow multiple sources to be used and enable priors and uncertainty measures to be included (Moore and Semmens 2008). As recent work has found that more than three sources can undervalue minor dietary items (Brett 2014), prey data was grouped based on the divisions created by their $\delta^{13}C$ values. Similar $\delta^{13}C$ values such as what was found here have previously been linked to carbon sources in tropical riverine waters (including their estuaries and surrounding seagrasses) and so our putative prey species have been classified accordingly (Loneragan et al. 1997). Group 1 prey had $\delta^{13}C$ values closer to freshwater signatures and consisted of barramundi *Lates calcarifer*, rough river prawn *Macrobrachium equidens* and paper head croaker *Johnius novaeguineae*. Group 2 consisted of king threadfin salmon *Polydactylus macrochir* and threadfin catfish *Neoarius armiger* were higher in $\delta^{15}N$ than the other species and had $\delta^{13}C$ values that were in between estuarine and freshwater signatures whilst Group 3 consisted only of popeye mullet *Rhinomugil nasutus*, which had a $\delta^{13}C$ value closer to an estuarine signature. Residual errors were included in the model (Parnell et al. 2010) and uncertainties consisted of elemental concentrations based on the mass of each tissue (Parnell et al. 2010) and diet discrimination factors (DDF, the fractionation of $\delta^{15}N$ and $\delta^{13}C$ when passed through a food chain). We used $\delta^{15}N$ DDFs estimated from Bunn et al. (2013) who calculated values from a range of species in lotic environments from northern Australia and Papua New Guinea using a regression analysis and comparison of literature. We then compared the feeding behaviours to our species and used the most appropriate DDF values (see Table 2).

Fatty acid prey-predator linkages

Prey EFAs and shark EFAs (see Every et al. (2017) were compared with a main model PERMANOVA and a pairwise PERMANOVA. The fixed factor was species and a Type III (partial) sum of squares was used for both analyses. To compliment this, similarity percentages (SIMPER) based on Bray Curtis distances (Euclidean distance gives average squared distance not average similarity) was used to calculate the average similarity between the FA profiles of individuals within a species.

Individual specialisation of fatty acids

To explore the degree of individual specialisation we used the elasmobranch FA data from Every et al. (2017) (which was collected in the same time period as food web species) to calculate indices based on Roughgarden (1972). These indices are the proportion of total niche width (TNW) and within individual component (WIC) in fatty acids. These were determined using the R individual specialisation package (RInSP) (Zaccarelli et al. 2013). This test is useful when there are more than two variables; therefore, the use of two SIs is not appropriate. Values of TNW/WIC closer to 1 indicate no intraspecific differences whilst 0 suggests a high degree of individual specialization. Diet variation and individual specialization is calculated by forming a null hypothesis and then tested with Monte Carlo resampling methods, which also produces a *p* value. This multinomial sampling, randomly reallocates FA to each species. When statistically significant dietary variation exists, the observed values fall outside the range of null values. When comparing individual specialisation in different species of sharks the mean null value is used as a covariate to avoid variation from sampling effects in individual specialisation calculations (Araújo et al. 2011). All FA > 0.5% were included as there is an increase in accuracy when there are more variables associated with each individual (Bolnick et al. 2002; Zaccarelli et al. 2013).

372

373

374 **Results**

375 *Food web structure and linkages among putative prey taxa*

376 Across all sites there was an overall decrease in Layman's metrics of TA, NR, CR,
377 CD and SSD from sites 1 (river mouth) – 4 (upstream), whilst MMND stayed
378 relatively constant, apart from a slight increase of NR and MMND at site 2 during the
379 wet season (Table 3). When all sites were pooled there were distinct differences in all
380 metrics between the dry and wet seasons: TA = 22.0±2.2 and 33.7±2.7, NR = 5.0±0.4,
381 7.0±0.5 and CR = 7.8±0.38 and 9.6±0.5. Spatial differences were also apparent
382 among sites, with site 1 having higher CR, particularly during the wet season
383 (9.1±0.4) compared to the dry season (7.4±0.5). The number of trophic levels for
384 this assemblage remained quite constant across sites except for site 4, which was
385 very low (1.6±0.3). The trophic structure (MMND metric) of the assemblages
386 were largely similar, however this metric doubled from site 1 (3.3±0.3) to site 2
387 (6.0±0.7) during the wet season and was the lowest at site 1 during the dry
388 season (2.3±0.2).

389

390 Putative prey of sharks differed significantly in both $\delta^{15}N$ ($p < 0.01$, $R^2 = 14.18$, F_{df}
391 $= 4.72_{5, 143}$) and $\delta^{13}C$ ($p < 0.01$, $R^2 = 72.70$, $F_df = 76.17_{5, 143}$). Pairwise comparisons
392 were all significant ($p < 0.01$) for $\delta^{13}C$, with the exception of *N. armiger* and *P.*
393 *macrochir*. In contrast, there was a low range of mean $\delta^{15}N$ values and pairwise
394 comparisons for $\delta^{15}N$ were non-significant (Fig. 2). *Polydactylus macrochir* had
395 the highest mean $\delta^{15}N$ value followed by *L. calcarifer* and *N. armiger* both of
396 which were highly variable, indicated by their large standard deviations (SD)

that extended past *P. macrochir* (Fig. 2). Species with similar $\delta^{13}C$ consisted of *L. calcarifer* and *M. equidens* having lower $\delta^{13}C$ mean values, *J. novaeguineae* and *N. armiger* low $\delta^{13}C$ and $\delta^{15}N$, and *R. nasutus* had the highest $\delta^{13}C$ values (Table 4, Fig. 2).

Significant seasonal differences were found between the wet and dry $\delta^{13}C$ values of prey but not $\delta^{15}N$. Capture location was not significant in $\delta^{15}N$ ($p = 0.08$, $R^2 = 4.5$, $F_{df} = 2.3_{3, 145}$) but was in $\delta^{13}C$ ($p = <0.01$, $R^2 = 17.5$, $F_{df} = 10.3_{3, 145}$). Significantly different pairs were found between sites 1 and 3 ($t = 0.5$, $p < 0.01$), sites 1 and 4 ($t = 1.0$, $p < 0.01$), and sites 1 and 2 ($t = 0.7$, $p < 0.01$).

Fourteen EFAs with > 0.5 % representation within tissues appeared to separate across three broad divisions within these potential prey taxa (Table 4, Fig. 3 and see Online Resource 3 Fig. 2). One group consisted largely of *M. equidens*, the second, *P. macrochir, R. nasutus* and *J. novaeguineae* and the third *N. armiger* and *L. calcarifer*. However, it should be noted that individual *N. armiger* were dispersed over all groups whilst individual *R. nasutus* were spread amongst groups of *J. novaeguineae*, *P. macrochir* and *N. armiger.* The main EFA that separated *M. equidens* from the other prey species was 18:2ω6, whilst *L. calcarifer* were divided into two subgroups by a number of EFA; however, the most influential were 20:2ω6 and 22:4ω6. The larger group of *N. armiger* was separated principally by 20:2ω6, *J. novaeguineae* 20:5ω6 and *Polydactylus macrochir* and 22:5ω6 separated *R. nasutus*.

Significant differences in EFA profiles were found amongst prey species and there were significant interactions between species x season and species x capture location; but not between season x capture location (Table 4). Most prey species had an average similarity (from SIMPER) of over 70%, *M. equidens* had 80.8% average similarity, *J. novaeguineae* 83.5%, *L. calcarifer* 74.5%, *P. macrochir* 86.5%, *Neoarius armiger* 75.2% and *Rhinomugil nasutus* 73.2%. *Lates calcarifer* and *P. macrochir* were very similar to each other and could not be separated by their FA profile using the Dufrêne - Legendre indicator species analysis. *Johnius novaeguineae* had the most FAs (6) that resulted in their separation from the other species with *p*-values <0.05, *N. armiger* and *R. nasutus* had four, whilst *M. equidens* had three (Table 4; see Online Resource 1 and 2 for indicator values and specific FAs).

Trophic linkages between sharks and putative prey taxa

Stable isotope analysis indicated that the majority of *C. leucas* had $\delta^{13}C$ values that were higher than most prey species within the South Alligator River system with *R. nasutus* being the most notable exception (Fig. 2). However, some individuals of *C. leucas* were also isotopically similar to *P. macrochir* and *N. armiger* (Fig. 2). *Rhizoprionodon taylori* were similar in $\delta^{13}C$ values to *C. leucas* and were also similar to *R. nasutus* and *N. armiger*. Stable isotope signatures within *Glyphis* species were similar to many of the prey species, particularly *J. novaeguineae,* and *M. equidens*. The majority of *G. garricki* isotopic values were close to *M. equidens*, whilst in another group of *G. garricki*, isotopic values were similar to *L. calcarifer*.

Percentage difference of mean shark diet proportion indicated little difference between the consumption of prey during the wet and dry seasons (Table 6, Fig. 4a).

Group 2 (consisting of signatures between estuarine and freshwater) had the most difference (2.9±6.0%) and Group 1 (consisting of freshwater signatures) had the least (0.6±1.4%) (Table 6, Fig. 4a). Differences in prey consumption by shark species appeared to be more important (3.7± 2.7%) than seasonal variation (0.8±1.6%). *Carcharhinus leucas* consumed prey from Group 2 and 3 (consisting of estuarine signatures) whilst *R. taylori* showed the greater consumption of prey species from Group 3 (Table 6, Fig. 4b). *Glyphis garricki* and *G. glyphis* had the highest mean consumption from the freshwater prey group. The two *Glyphis* species consumed the most from Group 1 although *G. garricki* had the highest proportion (67.8±14.3%) compared to *G. glyphis* (Table 6, Fig 4b). Interestingly, the two *Glyphis* species consumed the lowest amount from Group 2, yet both consumed almost one third of prey from Group 3. However, *R. taylori* consumed the most of the four sharks from within Group 3.

Significant differences in EFA profiles were found among all shark and prey species ($p < 0.01$, $F_{df} = 20.26_{9}$). Pairwise tests of EFA profiles further confirmed this for all species pairs (all $p < 0.05$) except for *G. garricki* and *G. glyphis* ($p = 0.4$), which was not found to be significantly different. A PCO indicated that *Carcharhinus leucas*, *G. garricki* and *G. glyphis* all had a diverse array of EFAs (Fig. 2) and shared FAs with *P. macrochir*, *L. calcarifer*, *J. novaeguineae*, *M. equidens*, *N. armiger* and *R. nasutus.* However, there were slight interspecific differences between the sharks. *Glyphis garricki* and *G. glyphis* had high relative levels of 18:2ω6, which was not present in *C. leucas*, whilst *G. glyphis* also had high contributions of 20:5ω3. Each of these FAs were also present in *P. macrochir*, *J. novaeguineae*, *L. calcarifer*, *M. equidens*, *R. nasutus* and *N. armiger*.

Intraspecific variation in sharks

Most shark species had over 65.0% average similarity of FAs among individuals (*C. leucas* (67.4 %), *G. garricki* (68.9%), *G. glyphis* (67.7%) and *R. taylori* (77.7%)) according to similarity percentages (SIMPER). The four shark species had similar FAs WIC/TIC indices and only *C. leucas* (0.90 ($p < 0.01$)) and *G. garricki* (0.92 ($p < 0.01$)) had significant values, whereas *G. glyphis* (0.94, $p=0.28$) and *R. taylori* (0.95 $p=1$) had values that were not significant. Only *G. garricki* could be compared for seasonal differences due to the low n-value of the other shark species caught during the wet season. This comparison indicated very little change between the wet (0.92, $p < 0.01$) and dry season (0.93, $p < 0.93$).

Discussion

Spatial and seasonal differences in stable isotopes and fatty acids were found in the trophic range and diversity of putative prey of four species of sharks that utilise the South Alligator River. Whilst there were significant differences between putative prey species, some of their biochemical tracer compositions overlapped suggesting consumption of similar basal resources amongst some putative prey. *Lates calcarifer* and *N. armiger* exhibited large intraspecific variation in $\delta^{15}N$ values, indicating that individuals may be consistently feeding at different trophic levels. This suggests that these species are consuming a range of basal sources and that there is a high degree of omnivory or consumption of omnivores amongst prey species (Jepsen and Winemiller 2002). Although specific indices of specialization were not calculated for prey species, the average similarity between prey species was high and only a limited number of FAs separated prey species in the Dufrêne - Legendre indicator species

496 analysis. This may suggest that the prey community displays trophic generalism. This
497 high degree of omnivory and trophic generalism may support the general fifth
498 principal of river and wetland food webs in the wet–dry topics as outlined by Douglas
499 et al. (2005). This principal suggests that food chains are short, that species often feed
500 across a number of trophic levels, and that there is relatively low dietary specialisation
501 in tropical rivers (Douglas et al. 2005).
502
503 Elasmobranchs also exhibited similar patterns in SI and FA values and the
504 comparison of both biochemical tracers demonstrated likely dietary links between the
505 putative prey and elasmobranchs. The similarity in TNW/WIC indices and relative
506 high average similarity of FA profiles between all four shark species indicated that
507 they were generalist consumers of coastal and estuarine prey species with little
508 seasonal change. This may be a result of the diverse range of prey available in
509 estuaries and coastal areas (Douglas et al. 2005). Although there was a broad range of
510 prey collected in this study, we only selected for analysis the six species that were in
511 the greatest abundance. Being taxonomically rich but dominated by only a few
512 species may be common in tropical rivers (Douglas et al. 2005). Generalist feeding
513 was also observed in *C. leucas* based on their movement data from the Shark River
514 Estuary, Florida, USA, this study found that elasmobranch species opportunistically
515 captured prey entering the river from the flood plains (Matich and Heithaus 2014).
516 Although abundant prey was unlikely to have been missed it is possible that the
517 collective signatures of individuals from a range of species with low n-values may
518 have significant influence on the diet of elasmobranchs.
519

520 *Seasonal and spatial patterns of trophic range and dietary diversity among putative*
521 *prey taxa*

522 The influx of organic sources at certain points along the river may explain the spatial
523 differences in prey in the South Alligator River (Pusey et al. 2015). For example, site
524 1 had the greatest range of basal sources based on the CR. This site was the furthest
525 upstream and may have had a mixture of terrestrial, freshwater and some limited
526 marine basal sources, as has been found in other estuaries (Atwood et al. 2012).
527 During the wet season in the upper river (sites 1 and 2), the trophic ecology of species
528 appears to overlap more than during the dry season. This is perhaps a function of the
529 changes in abiotic factors such as salinity and changes in hydrological patterns
530 (Jardine et al. 2015; Pusey et al. 2015), which could similarly explain a slight
531 decrease in spatial trophic diversity from the upper to lower river reaches. This can
532 arise because some species do not favour the mid-reaches of the river as habitat
533 (Pusey et al. 2015) due to fluctuating conditions (e.g. salinity) caused by both
534 seasonal and tidal influences (Warfe et al. 2011; Jardine et al. 2015).
535
536 Like many tropical rivers (Winemiller and Jepsen 1998; Roach et al. 2009; Ward et al.
537 2016), season influenced the isotopic and FA composition in putative prey species
538 and thus the trophic structure of the river. However, seasonal shifts in individual FA
539 and SI biotracers were not reported previously in these elasmobranchs at this study
540 site (Every et al. 2017). This may indicate that sharks are moving to consume their
541 preferred prey or that they are consuming a variety of prey from a range of sites which
542 may make identifying seasonal change difficult. Other large predators such as the
543 estuarine crocodile *Crocodylus porous* and *L. calcarifer* across northern Australia

were also found to consume prey whose basal sources were from outside their capture location (Jardine et al. 2017).

Links between putative prey to elasmobranchs

Large variance in $\delta^{15}N$ may be attributed not only to omnivory or consumption of omnivores but may also be related to ontogenetic change as *L. calcarifer* has been found to switch from the consumption of smaller teleosts and *Macrobrachium* spp at 40 cm total length, to Ariidae and Polynemidae prey alongside an increase in consumption of Mugilid and Engraulid fishes (Davis 1985). Whilst specific dietary studies have not been conducted for *N. armiger*, dietary ontogenetic change has been reported in other *Neoarius* species (Dantas et al. 2012). Alternatively, this may be attributed to the varied diet of *N. armiger* that is reported to include teleosts, polychaetes and crustacea (Blaber et al. 1994). Due to the similarities and differences of biochemical tracers amongst the prey assemblage it appears that *P. macrochir* may feed on *J. novaeguineae,* as their EFAs overlap (high in 22:5ω6) and *P. macrochir* had higher $\delta^{15}N$ than *J. novaeguineae*. *Neoarius armiger* was the only teleost species that showed similarities to the biochemical profile of the crustacean *M. equidens*, which was very different to other putative prey species in that it was high in 18:2ω6. This difference could also be a result of differing discrimination rates between crustaceans and teleost fish (Caut et al. 2009).

Based on both biochemical tracers it appears that not all sharks consumed putative prey species where they appeared to be sympatric. *Carcharhinus leucas* was a prime example of this, with most individuals caught at site 1 not appearing to consume prey with freshwater $\delta^{13}C$ values that were caught at these same sites. Although *C. leucas*

569 had the highest mean $\delta^{13}C$ value, the mixing model indicated that they are consuming
570 the majority of prey species from Group 2 (50.6±19.4) and 3 (46.1±18.4), which
571 consisted of species with higher $\delta^{13}C$ values (more estuarine signatures) such as *N.*
572 *armiger*. Similarly, other *Neoarius* spp. have been commonly reported in the stomach
573 content analysis of populations of *C. leucas* in other estuarine ecosystems (Snelson et
574 al. 1984; Thorburn and Rowland 2008). *Carcharhinus leucas* had high $\delta^{13}C$ values,
575 which suggests that they are likely to be consuming other estuarine prey species such
576 as larger *L. calcarifer* (Heithaus et al. 2013) from these or nearby coastal locations
577 that were not caught in this study. This discrepancy in SI signatures versus capture
578 location suggests high levels of prey movement may be occurring, or that a maternal
579 signature is present within the shark consumers (Every et al. 2017). Maternal
580 signatures may occur as neonate elasmobranchs have lipid reserves in their livers,
581 which comes from the maternal food source (Olin et al. 2011). When neonates begin
582 to feed, the signatures switch back to the neonates own biochemical signature (Olin et
583 al. 2011).
584
585 Fatty acids indicated that there were dietary links between a small cluster of *C. leucas*
586 and *N. armiger*, and *L. calcarifer* and *R. nasutus*. These individuals had a size range
587 of 69.5 – 99. 5 cm, which is approximately the same size range of the entire cohort so
588 ontogenetic change is unlikely to explain these differences. Interestingly, when the
589 other sharks were included amongst the prey, *C. leucas* were very similar to *G.*
590 *garricki* and *R. taylori* FAs, which may suggest that *C. leucas* is consuming them or
591 they are consuming similar prey. Although difficult to evaluate without investigating
592 stomach contents, elasmobranchs (including other *C. leucas*) have been found in the
593 gut of adult and juvenile *C. leucas* along with a variety of teleosts fish (Snelson et al.

1984) and in Australia *C. leucas*, crocodiles, pigs and birds (Thorburn and Rowland 2008).

Stable isotope mixing models of *R. taylori* suggest that they are also consuming the majority of Group 3 prey (with more estuarine signatures) (72.7±17.5) with some from group 2 (13.4 ± 16.2) and only a very small proportion of prey from Group 1 (with more riverine signatures) (13.9. ± 1.4). The EFA profiles also support the isotope mixing model as they suggest that *R. taylori* are consuming *J. novaeguineae, P. macrochir, N. armiger* with some individuals being close to *R. nasutus.* Previous studies of *R. taylori* indicate that they consume marine species (Simpfendorfer 1998; Munroe et al. 2014), however it appears that they also consume prey that have assimilated biotracers from freshwater habitats. This is interesting, as *R. taylori* was not found to enter the river in a movement study in Queensland (Munroe et al. 2015). Some of *R. taylori* 's EFA profiles did not appear to have dietary links to any of the putative prey species and so may be consuming other marine prey similar to in the stomach content analysis conducted by (Simpfendorfer 1998). Therefore, there may be some degree of resource partitioning occurring amongst the population that was not observed here, perhaps because the sampling effort was concentrated in the estuary.

Other shark species that can tolerate riverine conditions are likely to access more riverine prey. For example, our study indicated that *G. garricki* are primarily consuming species from the freshwater prey group and had a low degree of intraspecific differences. This was supported by *G. garricki* EFA profiles, which indicated links with the freshwater and estuarine prey *L. calcarifer* and *N. armiger*

and possibly *P. macrochir* and *J. novaeguineae.* Corroborating these findings were the stomach contents of 6 individual *G. garricki* where *Neoarius* spp. and *P. macrochir* were also found (Thorburn and Morgan 2004). Although *G. glyphis* was very similar to *G. garricki*, they consumed more estuarine prey (Group 3) (33.9 ± 18.5% compared to 30.6 ± 14.0%) and less freshwater prey (61.2± 19.2% compared to 67.9 ± 14.3%). Their EFA were associated with only *L. calcarifer* and *N. armiger* and the stomach contents of seven individuals indicated *Nematalosa erebi*, the freshwater prawn *M. spinipes* and spines of catfish were also found (Peverell et al. 2006). Both of the *Glyphis* species showed a reliance on riverine resources, particularly *G. garricki* due to their apparent preference for upriver putative prey species. In contrast, *C. leucas* and *R. taylori* had strong links to the mid-river prey and very low proportion of freshwater prey according to SI mixing models. This suggests that all four shark species have important trophic connections to the riverine environment.

Conclusions

Seasonal and spatial differences in biochemical tracers within sharks and their putative prey were found in the South Alligator River with the most trophic diversity and biochemical tracer variance in the upper reaches of the estuary. This variation in dietary biochemical tracers indicates the complexity of food webs in this system and appears to be a common feature of tropical estuaries (Magnone et al. 2015). All of the sharks examined appeared to be generalist feeders, which may be due to the diverse range of putative prey species available or breadth of basal resources present in this relatively undisturbed ecosystem (Pusey et al. 2015). Further exploration is required

to explain why individual shark biotracers did not show evidence of seasonal change, yet prey species did.

Another key finding was that *C. leucas* had predominantly marine-based signatures, yet they were captured 80 km upstream. Direct investigation of the movements of sharks (e.g. via acoustic telemetry) would be informative for the interpretation of the biochemical tracer data collected in our study. Another potential way to further our knowledge of the trophic ecology of these species using FAs would be to conduct feeding trials so that the differing physiological responses to individual FAs can be calculated in dietary mixing models similar to isotopes. Nonetheless, the results of the current study demonstrate the importance of ecological processes in rivers as drivers of the food webs that support euryhaline elasmobranchs in tropical estuaries and coastal ecosystems. Recognition of the trophic connectivity that exists among rivers, estuaries and coastal waters is critical to the effective conservation and management of biodiversity in these ecosystems.

Ethical Approval

All procedures performed in this study were conducted with the approval of the Charles Darwin University Animal Ethics Committee (A12016), in conjunction with permits from NT Fisheries S17/3268 and Kakadu National Park (RK805).

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970 **Tables and Figures**

971 **Table 1.** Number (*n*) and total length of 4 sharks (Every et al., 2017) and 6 putative
972 prey species caught from the South Alligator River, Kakadu National Park, Australia
973 from which muscle tissue samples were taken for stable isotope (SIA) and fatty acid
974 analysis (FAA). Wet and dry species number, Total length (TL) (± standard deviation
975 (SD)), sex ratio and habitat are also included.

Shark Species		Sex ratio M:F	TL±SD (cm)	FAA		SIA		Habitat
Scientific Name	**Common Name**			**Wet**	**Dry**	**Wet**	**Dry**	
Carcharhinus leucas	bull shark	24:16	82.2±16.3	20	2	27	6	euryhaline
Glyphis garricki	northern river shark	22:19	94.5±24.6	12	13	22	20	euryhaline
Glyphis glyphis	speartooth shark	3:7	88.7±23.3	2	3	2	3	euryhaline
Rhizoprionodon taylori	Australian sharpnose shark	7:21	54.8±12.1	1	24	4	27	coastal
Potential Prey								
Johnius novaeguineae	paperhead croaker		7.9±3.5	8	14	11	8	estuarine
Lates calcarifer	barramundi		39.9±13.8	6	17	8	20	estuarine
Macrobrachium equidens	Rough river prawn		7.4±1.6	21	12	22	15	euryhaline
Nemapteryx armiger	threadfin catfish		27.9±5.0	10	12	12	16	estuarine /euryhaline?

Polydactylus macrochir	king threadfin salmon	44.8±18.8	8	7	8	8	euryhaline
Rhinomugil nasutus	popeye mullet	16.6±6.6	7	7	7	11	estuarine

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979 **Table 2.** Putative prey species caught in the South Alligator River, Kakadu National
980 Park, Australia and their estimated diet discrimination factor (DDF) used in the
981 mixing model, based on Bunn et al., (2013).

Species	**Feeding method**	**Diet discrimination factor (DDF)**
Macrobrachium equidens	Predatory invertebrate (March et al. 2002) based on *Macrobrachium* spp.)	1.8±1.7
Johnius novaeguineae	Omnivorous fish (predatory invertebrates/algae) (Sasaki 2001)	4.3±1.5
Lates calcarifer	Predatory fish (Davis 1985)	5.7±1.6
Polydactylus macrochir	Predatory fish (Brewer et al. 1995)	5.7±1.6
Neoarius armiger	Predatory fish (Blaber et al. 1994)	4.3±1.5
Rhinomugil nasutus	Omnivorous fish (algae / herbivores invertebrates) (Froese & Pauly 2015)(Froese and Pauly 2015)	3.9±1.4

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989 **Table 3** Laymen's metrics of the South Alligator River mid-trophic taxa and shark
990 species. Numbers of species (*n*) at each site and season are included, those with *n*
991 values < 5 were omitted from these analysis (highlighted grey). TA = Total Area, NR
992 = range of $\delta^{15}N$, CR = range of $\delta^{15}N$, CD= centroid distance, MNND =mean nearest
993 neighbour distance, SDNND = standard deviation of nearest neighbour distance.
994

Site	All		1		2		3		4	
Season	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
n										
C. leucas	6	28	6	25	0	3	0	0	0	0
G. garricki	20	22	10	6	8	14	2	2	0	0
G. glyphis	3	0	3	2	0	0	0	0	0	0
R. taylori	0	0	0	0	0	0	0	0	30	8
J. novaeguineae	14	8	9	0	2	2	3	6	0	0
L. calcarifer	20	8	11	8	5	0	4	0	0	0
M. equidens	15	22	3	8	7	0	5	11	0	3
N. armiger	16	12	7	2	3	6	6	4	0	0
P. macrochir	8	8	0	0	1	1	7	0	0	7
R. nasutus	11	7	5	0	6	0	0	7	0	0
TA	22.0±2.2	33.7±2.7	17.2±3.4	14.2±3.1	8.5±2.9	0.0±0.0	5.2±1.5	2.8±2.1	-	0.0±0.0
NR	5.0±0.4	7.0±0.5	4.7±0.7	3.9±0.4	1.9±0.6	4.3±0.6	3.5±0.7	3.8±0.5	-	2.5±0.6
CR	7.8±0.38	9.6±0.5	7.4±0.5	9.1±0.4	9.7±0.7	4.1±0.6	4.4±0.7	6.9±0.5	-	2.0±0.6
CD	2.7±0.1	3.2±0.1	2.4±0.2	3.5±0.2	3.5±0.2	3.0±0.3	2.3±0.3	2.9±0.2	-	1.6±0.3
MNND	4.6±0.1	2.0±0.2	2.3±0.2	3.3±0.3	3.1±0.3	6.0±0.7	3.1±0.4	3.8±0.3	-	3.2±0.6
SDNND	2.0±0.2	0.8±0.2	1.2±0.3	1.6±0.4	1.5±0.5	0.0±0.0	0.9±0.5	0.7±0.5	-	0.0±0.0

995 **Table 4** Mean values for essential fatty acids (EFA> 0.5%), $\delta^{13}C$ and $\delta^{15}N$ and their
996 standard deviation in muscle (SD) tissue from six potential prey species of shark
997 collected from the South Alligator River, Kakadu National Park, Australia. Included
998 are indicator FAs for each species with a p<0 .05.
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1000 ✦ an indicator fatty acid for the species with p values < 0.05 based on Dufrêne and Legendre (1997),

1001 20:3ω9* identified based on comparison with other *C. leucas* fatty acid literature; a standard was not

Species	*Polydactylus macrochir*	*Johnius novaeguineae*	*Lates calcarifer*	*Macrobrachium equidens*	*Nemapteryx armiger*	*Rhinomugil nasutus*
SI (ppm)						
$\delta^{13}C$	-17.4±1.3	-19.3±1.6	-22.9±2	-21.4±1	-21.2±2.1	-14.6±1.9
$\delta^{15}N$	8.3±1.8	8.2±1.1	8.2±1.7	8.8±1.5	9.3±3.8	6.6±1.1
C/N	2.8±0.3	2.9±0.1	3.1±0.8	2.8±0.0	4.2±1.1	2.9±0.2
EFA (%)						
18:2ω6	2.3±1.1	1.9±0.8	3.9±1.9	9.5±5.0	3.4±2.9	1.0±1.3
18:3ω3	0.6±0.3	0.2±0.1	1.2±1.1	1.1±1.2	2.0±2.7✦	0.5±0.5
20:2ω6	0.3±0.0	0.3±0.1	0.3±0.1	0.4±0.1	0.7±0.2	0.1±0.2
20:3ω6	0.2±0.2	0.5±0.6	0.6±0.2	0.2±0.1	0.4±0.2	0.4±0.2
20:3ω9*	0.1±0.1	0.6±2.2	0.0±0.0	0.2±0.4	0.0±0.0	0.0±0.0
20:4ω3/20:2	0.3±0.1	0.4±0.2	0.4±0.3	1.6±0.8✦	0.4±0.3	0.6±0.3
20:4ω6	10.6±2.3	9.8±2.5	10.6±4.7	10.3±2.8	7.1±3.9	5.7±2.0
20:5ω3	4.9±1.5	4.9±2.1	1.7±2.4	9.0±2.9 ✦	2.6±2.2	8.7±4.0
22:2a	0.5±0.1	0.6±0.3✦	0.3±0.2	0.0±0.0	0.2±0.1	0.2±0.1
22:3	2.1±0.4	1.8±0.9	1.8±1.2	0.6±0.3	2.0±0.9	3.3±3.6
22:4ω6	1.3±0.5	2.1±2.0	2±0.9	0.5±0.4	1.9±0.8	0.7±0.3
22:5ω3	0.0±0.0	0.2±0.7	0.6±1.1	0.1±0.2	0.2±0.9	3.1±4.1 ✦
22:5ω6	1.9±0.6	3.0±1.2✦	1.8±1.2	0.6±0.2	1.2±0.6	1.0±0.4
22:6ω3	13.6±3.2	16.6±4.5✦	5.6±3.1	4.7±1.4	7.1±5.4	10±4.9
EFA< 0.5%	0.0±0.0	0.2±0.1	0.2±0.1	02±0.2	0.1±0.1	0.3±0.1
SAT	4.5±0.3	4.2±0.2	4.8±0.9	4.6±1.0	5.4±1.1	4.6±0.6
MUFA	1.6±0.1	1.5±0.1	2.0±0.4	1.5±0.3	1.8±0.4	1.7±0.6
PUFA	2.3±0.2	2.6±0.2	1.8±0.5	2.3±0.4	1.7±0.6	2.1±0.7
ω3/ω6	0.7±0.2	0.8±0.5	1.8±0.8	0.7±0.2	1.0±0.4	0.6±1.0

1002 available at the time of analyses EFA (essential fatty acids) <0.5 include 18:3ω6, 18:4ω3, 18:2a, 18:2b,

18:2c, 21:5ω3, 21:3, 22:2b, SAT – saturated fatty acid, MUFA - monounsaturated fatty acids, PUFA – polyunsaturated fatty acid

Table 5: Comparison of species, season (wet and dry) and site locations (1 - 4) of essential fatty acids from the mid-taxa species (*Johnius novaeguineae*, *Lates calcarifer*, *Macrobrachium equidens*, *Nemapteryx armiger*, *Polydactylus macrochir* and *Rhinomugil nasutus*) in the South Alligator River, Kakadu, Australia using PERMANOVA. DF = degrees of freedom.

Variable	DF	Pseudo-F	P(perm)	Unique perms
Species	3	11.6	<0.01	9932

Capture location	1	6.2	<0.01	9952
Species x Season	3	2.3	<0.01	9925
Species x Capture location**	8	1.6	0.01	9892
Season x Capture location**	2	1.4	0.2	9947
Species x Season x Capture location **	2	4.4	<0.01	9944

**Not all species were included in capture location

Table 6 Stable Isotope mixing model results from four species of sharks, *Carcharhinus leucas*, *Glyphis garricki*, *G. glyphis* and *R. taylori* in the South Alligator River, Australia. Results are the percentage mean proportion of the shark consuming from each prey group and the combined results over all prey groups and the difference between seasons ± standard deviation (SD). Group 1 consists of *Lates calcarifer*, *Macrobrachium equidens*, *Johnius novaeguineae*, Group 2 *Polydactylus macrochir*, *Neoarius armiger* and Group 3 was only *Rhinomugil nasutus*.

Prey group:	**Group 1 %** ± SD	**Group 2 %** ± SD	**Group 3 %** ± SD

Shark species			
All Sharks	33.5±20.6	21.6±18.4	44.9±22.0
C. leucas	3.3±6.4	50.6±19.4	46.1±18.4
G. garricki	67.8±14.3	1.6±4.8	30.6±14.0
G. glyphis	61.2±19.2	4.9±9.9	33.9±18.5
R. taylori	13.9±1.39	13.4±16.2	72.7±17.5
Wet	2.8±3.9	47.4±12.2	49.8±10.9
Dry	3.5±5.3	44.5±18.2	52.0±16.5

Figures

Fig. 1 Map of the South Alligator River, Northern Territory, Australia showing capture locations of elasmobranch and prey taxa. Each site is separated by a yellow line. Insert shows map of Australia with a black cross indicating where the river is in relation to northern Australia. Map data: Google, TerraMetrics

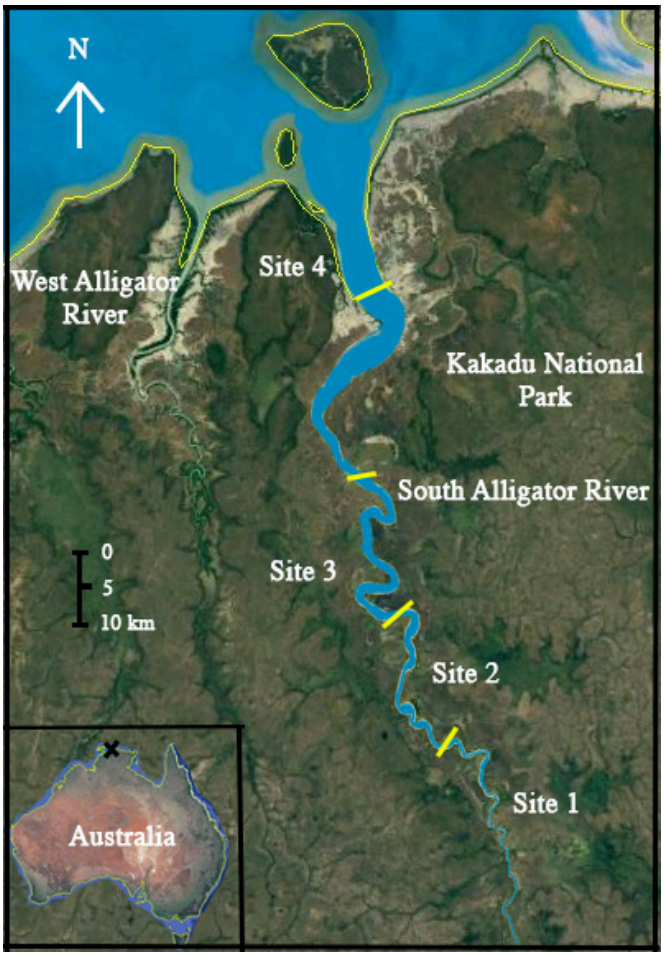
Fig. 2 Biplot of mean $\delta^{13}C$ and $\delta^{15}N$ (and standard deviation) for mid-trophic prey species (black dots) (*Johnius novaeguineae*, *Lates calcarifer*, *Macrobrachium equidens*, *Nemapteryx armiger*, *Polydactylus macrochir* and *Rhinomugil nasutus*),

overlayed the wet (coloured circles) and dry (coloured triangles) season isotope values (adjusted for trophic discrimination) in the shark consumers (*Carcharhinus leucas*, *Glyphis garricki*, *G. glyphis* and *Rhizoprionodon taylori*).

Fig. 3 Principal coordinate ordination of essential fatty acids (EFA) that were > 0.05% in percentage abundance within both prey and shark species (black circles surrounding symbol), with vector overlays indicating the most influential FAs (Pearson's r > 0.1) to explain the ordination structure.

Fig. 4 Box and whisker plots from MixSIAR of a) Seasonal difference of shark diet. b) Sharks and the proportion of the source (Prey) that makes up their diet. Prey grouped based on $\delta^{13}C$ and source of C estimated from Loneragan et al. (1997) estuarine = *Rhinomugil nasutus*, mid-river = *Polydactylus macrochir* + *Lates calcarifer* + *N. armiger*, freshwater = *Macrobrachium equidens.*

1078 **Fig. 1**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West Alligator River
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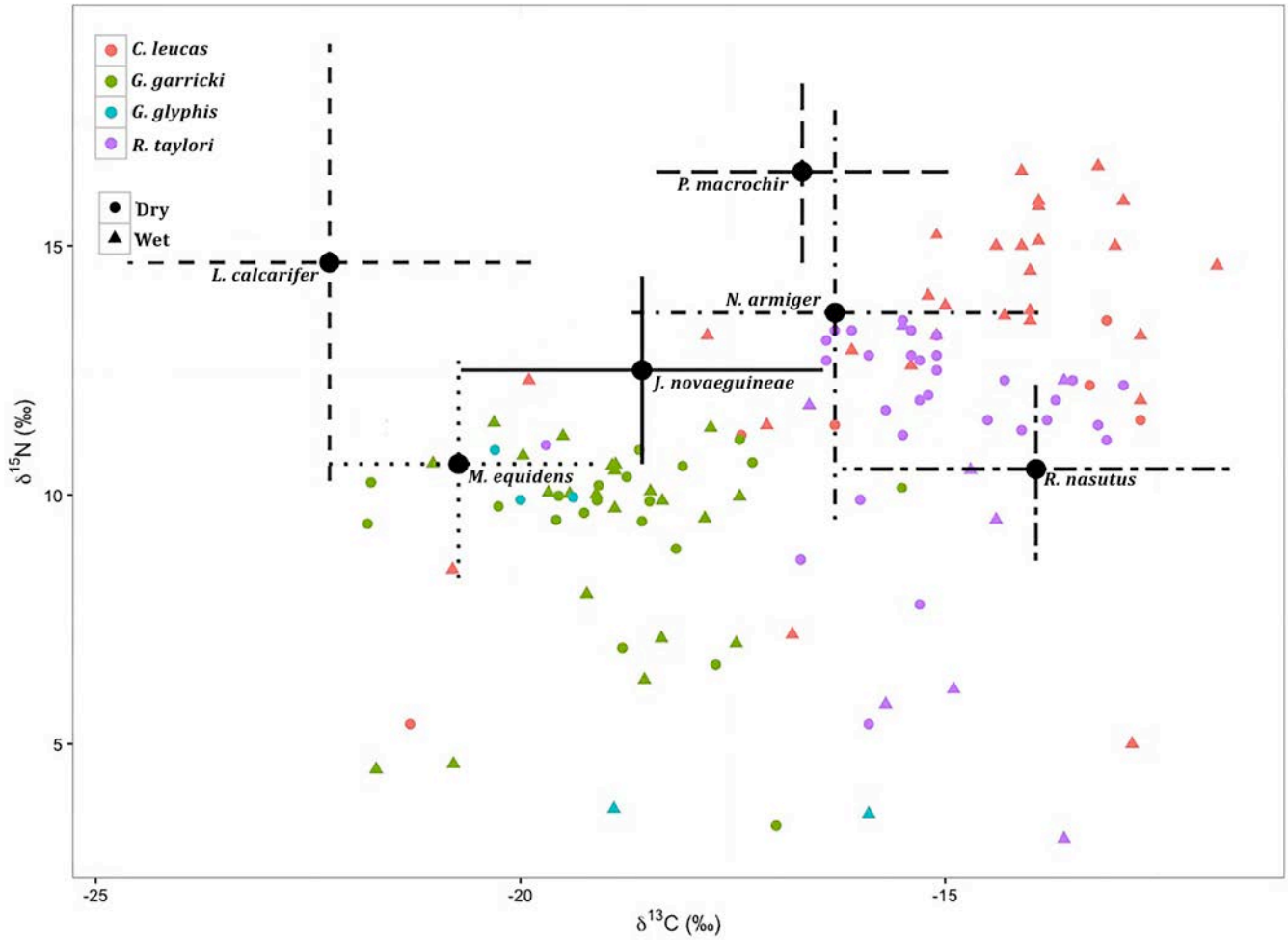

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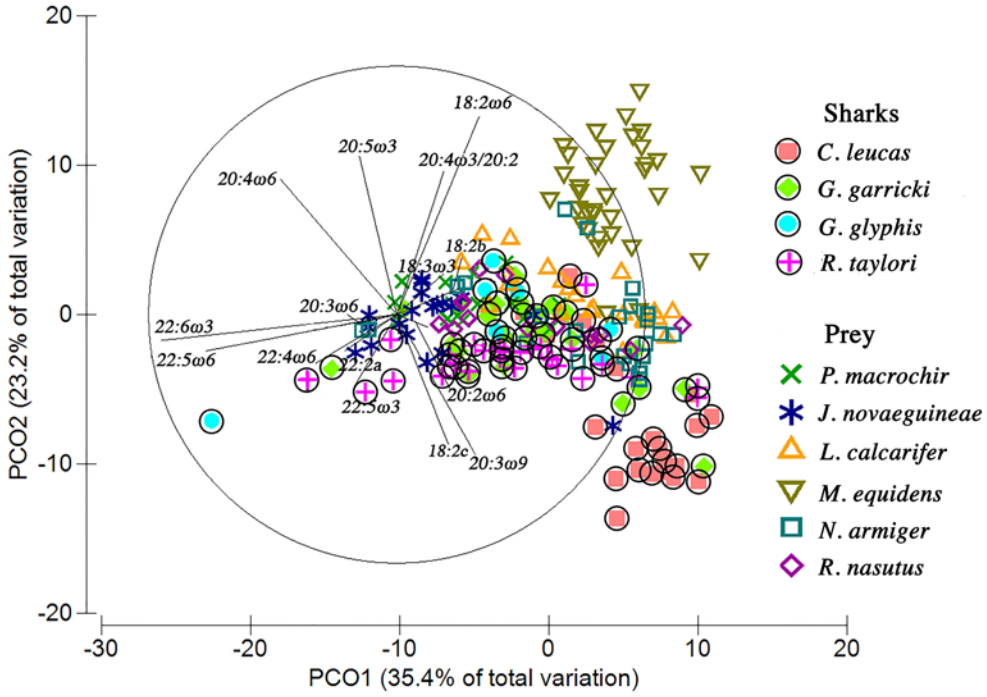

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1103 **Fig. 4**

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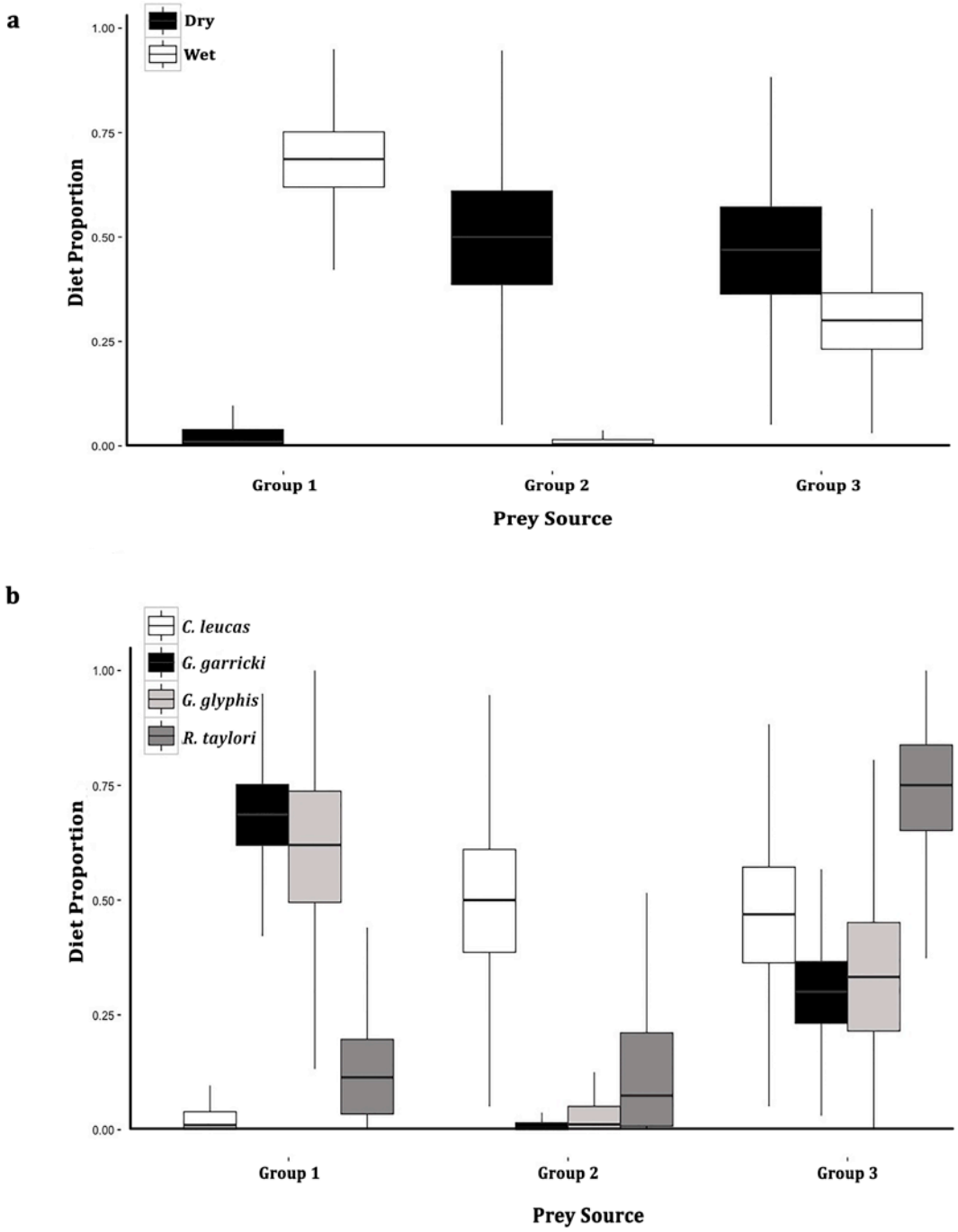

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Supplementary

Online Resource 1, Table 1 Fatty acids (FA) within prey (*Johnius novaeguineae*, *Lates calcarifer*, *Macrobrachium equidens*, *Nemapteryx armiger*, *Polydactylus macrochir* and *Rhinomugil nasutus*) and sharks species (*Carcharhinus leucas*, *Glyphis garricki*, *G. glyphis* and *Rhizoprionodon taylori*) found in the South Alligator River, Kakadu, Australia that can be used as indictors for a specific species according to a Dufrêne- Legendre indicator species analysis (Dufrêne and Legendre 1997). Note: The analysis was unable to find indicator FAs for *P. macrochir* and *L. calcarifer*.

Indicator FAs	Species	Indicator Value	P - value
20.3ω9	*C. leucas*	0.7	0.00
20.2		0.7	0.00
18.2c		0.3	0.00
18.1ω9		0.2	0.00
18.2b		0.2	0.00
20.1ω9		0.2	0.02
22.1ω11		0.4	0.05
15.1	*G. garricki*	0.2	0.01
20.2ω6		0.2	0.02
20.3ω6		0.2	0.04
22.0		0.2	0.06
16.0FALD		0.2	0.08

i17.0		0.2	0.60
20.4ω6	*G. glyphis*	0.2	0.00
18.0FALD		0.2	0.00
22.4ω6		0.2	0.00
24.1ω11		0.2	0.00
18.0	*R. taylori*	0.2	0.00
18.1ω7		0.2	0.00
24.0	*J. novaeguineae*	0.3	0.00
22.6ω3		0.2	0.00
22.5ω6		0.2	0.00
20.1ω7		0.3	0.00
24.1ω9		0.2	0.00
22.2a		0.2	0.03
18.2ω6	M. equidens	0.3	0.00
20.4ω3/20.2		0.3	0.00
20.5ω3		0.3	0.00
17.0		0.2	0.00
17.1		0.2	0.01
20.0		0.2	0.14
14.0	*N. armiger*	0.5	0.00
16.0		0.2	0.00
20.1ω11		0.2	0.00
18.3ω3		0.3	0.01
16.1ω7	*R. nasutus*	0.3	0.00

15.0	0.3	0.00
22.5ω3	0.3	0.00
17.1ω6	0.2	0.01
22.3	0.1	0.64

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Online Resource 2, Table 2 Mean values for fatty acids (FA> 0.5%) and their standard deviation in muscle (SD) tissue from six potential prey species of shark collected from the South Alligator River, Kakadu National Park, Australia. Included are significant indicator FAs for each species ($p < 0.05$).

Species	*Polydactylus macrochir*	*Johnius novaeguineae*	*Lates calcarifer*	*Macrobrachium equidens*	*Nemapteryx armiger*	*Rhinomugil nasutus*
14:0	0.3±0.4	0.0±0.0	0.2±0.2	0.2±0	2.0±2.6	0.8±1.0
15:0	0.5±0.3	0.3±0.1	0.7±0.5	0.5±0.1	0.6±0.3 ✦	1.3±1.1 ✦
16:0	20.5±2.6	16.4±2.4	19.6±4.3	20.5±7.3	25.9±7.4 ✦	22.9±5.7
17:0	1.2±0.1	1.2±0.5	1.5±0.6	1.8±0.7 ✦	1.5±0.4	0.9±0.5
18:0	12.6±1.2	12.5±1.3	14.1±5.9	12±2.8	12±1.6	9.9±2.1
20:0	0.3±0.0	0.4±0.2	0.4±0.1	1.0±0.3	0.4±0.1	0.3±0.1
22:0	0.5±0.1	0.9±0.2	1±0.8	1.0±0.4	0.4±0.1	0.3±0.1
24:0	0.4±0.0	1.6±0.5✦	0.7±0.4	0.3±0.1	0.2±0.1	0.6±0.4
15:1	0.5±0.4	0.3±0.1	0.7±0.7	0.7±0.3	0.4±0.4	0.3±0.2
16:1ω7	3.1±1.7	1.8±0.9	2.8±2.3	2.0±1.2	3.0±1.7	5.8±4.1✦
17:1	1.1±0.3	0.9±0.3	1.8±1.5	2.2±0.8 ✦	0.6±0.6	0.5±0.3
17:1ω6	0.8±0.2	0.7±0.2	1.0±0.4	0.5±0.2	0.6±0.1	1.2±1.0 ✦
18:1ω7	3.4±0.5	3.0±1.4	3.2±1.1	2.6±0.8	3.2±0.6	3.4±0.7
18:1ω9	9.7±1.2	8.5±1.9	12.8±4.5	9.8±3.2	11.8±4.2	8.2±4.5
20:1ω7	0.2±0.1	0.6±0.7✦	0.1±0.0	0.0±0.0	0.2±0.1	0.3±0.2
20:1ω9	0.5±0.3	0.3±0.1	0.3±0.2	0.1±0.0	0.7±0.3	0.2±0.3
20:1ω11	0.1±0.1	0.1±0.1	0.3±0.2	0.2±0.1	0.5±0.3✦	0.1±0.0
24:1ω9	0.5±0.4	1.4±0.5✦	0.5±0.3	0±0.0	0.3±0.2	0.7±0.8
24:1ω11	0.0±0.0	0.1±0.0	0.1±0.1	0±0.0	0.0±0.0	0.1±0.2
< 0.5%	0.0±0.0	0.0±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
16:0FALD	0.5±0.4	0.5±0.2	0.3±0.3	0.1±0.2	0.4±0.4	0.4±0.4

18:0FALD	0.4±0.1	0.4±0.2	0.4±0.4	0.4±0.6	0.3±0.2	0.4±0.5
i17:0	0.3±0.1	0.3±0.0	0.6±0.2	0.3±0.1	0.5±0.1	0.0±0.0

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1143 ✦ an indicator fatty acid for the species with p values < 0.05 based on Dufrêne and Legendre (1997),

1144 FA <0.5 includes 14:1, 15:0, 16:1ω5, 16:1ω7, 16:1ω9, 16:1ω13, 16:4+16:3, , 17:1ω8+17:0, 18:1,

1145 18:1ω5, 18:1ω7, 18:2, 18:3ω6, 18:4ω3, 19:1, 20:1ω5, 21:5ω3, 21:3, 22:2b, 22:1ω7, 22:1ω9, , 24:1ω7,

1146 18:1FALD, i15:0, i16:0 FALD - fatty aldehyde analyzed as dimethyl acetal

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1160 **Online Resource 3, Fig. 1** Principal coordinate ordination of essential fatty acids
1161 (EFA)>0.05% in percentage abundance in prey species, with vector overlays of the
1162 most influential FAs (Pearson's correlation above 0.1).
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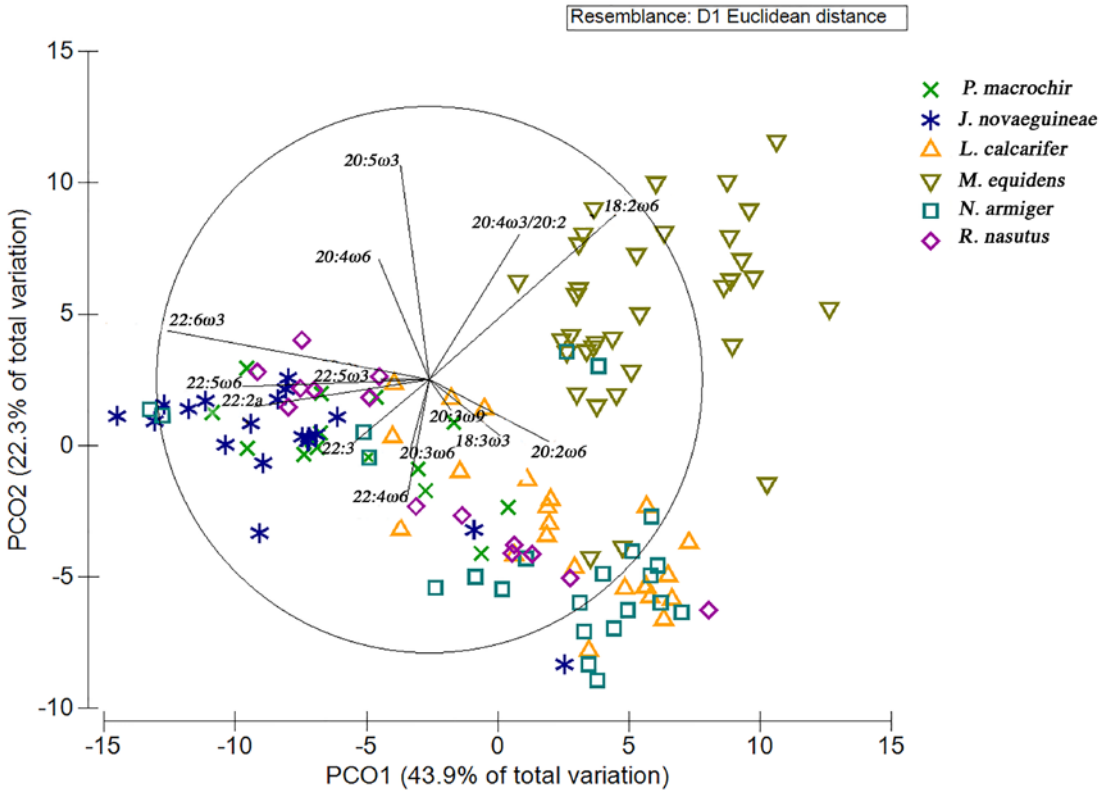

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