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Trophic Structure and Diet of Predatory Teleost Fishes in a Tropical Demersal Shelf Ecosystem

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Predatory fishes are a major component of many tropical fisheries, although little is known about their diet and trophic structure, which can hinder effective management. We used stable isotopes $\delta^{15}N$ and $\delta^{13}C$ in conjunction with dietary prey items of five fishes (*Lutjanus sebae*, *Lethrinus punctulatus*, *Epinephelus areolatus*, *Epinephelus multinotatus*, and *Plectropomus maculatus*) to describe the diet and trophic structure across this assemblage. A total of 153 isotope and 87 stomach content samples were collected at two locations that were ≈30 km apart, over two sampling trips, separated by three months. There was clear separation of species' mean $\delta^{15}N$ and $\delta^{13}C$ values in isotopic space; the highest mean $\delta^{15}N$ was exhibited by *E. multinotatus* (13.50 ± 0.11 SE) and the lowest was *L. punctulatus* (11.05 ± 0.13). These two species had the lowest overlap of isotopic niche space, whereas the highest overlap occurred between *L. sebae* and *P. maculatus*. $\delta^{15}N$ increased with fish body size for all species. However, body size was not significantly related to $\delta^{13}C$ values for any species. There was a notable shift in both $\delta^{15}N$ and $\delta^{13}C$ between sampling trips, with $\delta^{13}C$ being more depleted in the second trip. There was also a difference in $\delta^{13}C$ between locations for all species, suggesting localised foraging. A multiple tissue comparison for *L. sebae* indicated positive relationships for both $\delta^{15}N$ and $\delta^{13}C$ between dorsal fin and muscle tissue. Mean $\delta^{15}N$ values were the same for both fin (12.1 ± 0.10 SE) and muscle tissue (12.1 ± 0.09 SE), although $\delta^{13}C$ was more enriched in fin (-15.6 ± 0.14 SE) compared to muscle tissue (-17.3 ± 0.11 SE). The most common dietary items across species were teleosts and crustaceans, which was consistent with isotope data indicating a reliance on a demersal food web ($\delta^{13}C$ values ranging from -18 to -15‰). The results from our study indicate a dynamic spatio-temporal trophic structure and diet for commercially important demersal species and highlight the benefits of a multi-facetted sampling approach.

Keywords: stable isotopes, niche overlap, mesopredators, gastrointestinal tract analysis, red emperor, niche space

INTRODUCTION

Large predators influence food web structure through consumptive and non-consumptive effects (Estes et al., 2011). However, the nature and strength of predator-prey interactions depends on a variety of factors, such as habitat type and structure, prey abundance and diversity, as well as selectivity by predators (e.g. Paine, 1992; Hixon and Beets, 1993). In the marine environment, there are a range of factors governing success of predation events that include removal or destruction of habitat through natural disturbance events (Syms and Jones, 2000), fishing pressure that selectively removes large predators first (Pauly et al., 1998), and the perceived threat of predation, leading to modification of prey behaviours (Lester et al., 2020). Fish predator-prey interactions are largely governed by size and gape, with most species being limited by the size of prey they can swallow, which can be > 50% of their own body size (Scharf et al., 1997). The diet of larger species of shark, for example, is less restricted than large teleosts as they are equipped with serrated dentition that facilitates the removal of parts of animals larger than themselves and they also generally have wider gapes than their teleost competitors (e.g. Lucifora et al., 2006; Braccini, 2008). The role of predation by piscivores on reef environments represents a top-down driver of fish community structure (Graham et al., 2003; Boaden and Kingsford, 2015; Hixon, 2015), which may indirectly influence the benthic environment (Madin et al., 2019). However, the importance and strength of top-down effects has been debated due to the complexities of ecosystem dynamics and poorly understood exploitation histories (Ruppert et al., 2013; Rizzari et al., 2015; Casey et al., 2017).

Establishing baselines of the trophic structure or establishing natural (i.e. in the absence of human impacts) trophic links of predatory marine fish communities is complicated due to these species being among the first to be harvested by commercial, recreational, and subsistence fisheries (Jennings and Polunin, 1997; Pauly et al., 1998; Pinheiro and Joyeux, 2015; Frisch et al., 2016), due to their high value and catchability. Furthermore, much of the work to date has focussed on describing predatory fish community structure in shallow waters around reefs (e.g. Frisch et al., 2014; Boaden and Kingsford, 2015; Bierwagen et al., 2019; Speed et al., 2019), although recent studies have also included mesophotic depths (> 50 m) (e.g. Lindfield et al., 2016; Asher et al., 2017).

Specific information on the diet of predatory fishes targeted by commercial fishing and their prey is often based on stomach content studies and in many instances limited to localised areas of a fishery (e.g. Salini et al., 1994; Overholtz et al., 2000). Even though the diet of most target species can be classified broadly as carnivorous, there is known intraspecific plasticity in feeding among regions (Farmer and Wilson, 2011), which complicates management at both the species and community level. Having a sound understanding of diet and trophic linkages is critical to developing ecosystem models and assessing how they respond to fishing pressure (Bascompte et al., 2005; Farmer and Wilson, 2011). Long-term studies that document ontogenetic or seasonal shifts in diet or dietary sources are rare (although see Holt et al., 2019). However, this level of information is essential to understand potential impacts that may occur across the lifecycle of a species, which is currently lacking for many seascapes (Abrantes et al., 2015). Therefore, our current understanding of the feeding ecology of large predatory fish species is limited to a restricted range of species in certain areas, which ultimately hinders effective management and conservation efforts (Farmer and Wilson, 2011).

The most common method of obtaining dietary information for fishes (and thus trophic role) is through examination of stomach contents and or intestinal tracts (Hyslop, 1980). Estimates of contributions or relative importance of dietary items using this approach range from counts of the number and percentage of prey items identified, to the gravimetric and volumetric calculation per prey item (See reviews by Hyslop, 1980; Baker et al., 2014; Amundsen and Sánchez-Hernández, 2019). This method is usually lethal and only provides an immediate snapshot of the most recently consumed items (Takahashi et al., 2020). Furthermore, identification of partially digested items can be problematic (Baker et al., 2014), and in many cases (>30% of the time) piscivore stomachs are empty due to a variety of reasons, such as intermittent feeding and stomach eversion due to swim bladder expansion during capture (See review by Vignon and Dierking, 2011; Vinson and Angradi, 2011).

Alternative methods for assessing dietary information and trophic role of fishes include molecular approaches such as analysis of stable isotopes of the consumer tissue (Post, 2002), fatty acid profiles (e.g. Bierwagen et al., 2019) and more recently, DNA identification of prey items obtained from stomach content metabarcoding (e.g. Casey et al., 2019; Takahashi et al., 2020). Of these, the analysis of stable isotopes is the most common approach used in dietary and trophic relationship studies. Stable isotope ratios of nitrogen N^{15}:N^{14} '$\delta^{15}N$' and carbon C^{13}:C^{12} '$\delta^{13}C$' in consumer tissues are often used in ecological studies to infer dietary niche, with $\delta^{15}N$ enrichment providing an indication of an organism's trophic level (Perkins et al., 2014). In comparison, $\delta^{13}C$ can provide geographic and trophic information on nutrient residence because of the predictable variation in $\delta^{13}C$ values with latitude and among different primary production types (e.g., coastal, benthic, and planktonic) (Bird et al., 2018). Stable isotopes can be used to estimate trophic positions and body length relationships (Speed et al., 2012; Frisch et al., 2014), establish spatial foraging patterns and migrations (Ménard et al., 2007; Ferreira et al., 2017), monitor dietary shifts between seasons, life stages, or life styles (e.g. benthic vs pelagic) (Nakamura and Sato, 2014; Park et al., 2020), and can be modelled to estimate prey source contributions (Frisch et al., 2014). White muscle is the most commonly used tissue for analysis of assimilated isotopic source material (usually $\delta^{13}C$ and $\delta^{15}N$), which provides a resolution or turnover rate ('isotopic half-life') of several months for large predatory fish (e.g. Nelson et al., 2011). More recently, fish fin tissue has been trialled as a non-lethal method, with generally a good correlation between isotope ratios and muscle tissue, although turnover rates can vary compared to muscle tissue (Sanderson et al., 2009; Willis et al., 2013; Galván et al., 2015; Matley et al., 2016). While the assessment of assimilated stable isotopes in predators can provide a range of inferred information, they lack the fine taxonomic resolution that can be achieved with stomach content analysis or use of DNA. Some of the limitations of direct sampling of fish stomachs or indirect molecular techniques, such as stable isotope analysis, can be avoided by using these approaches together in the same study (Matley et al., 2018; Nielsen et al., 2018). This has the potential to offer greater insights compared to the use of any of these methods independently and can resolve trophic interactions at scales necessary for ecosystem-based management (Levin and Lubchenco, 2008).

Here, we used stomach and gastrointestinal tract content in conjunction with stable isotope analyses to describe the trophic structure of predatory fishes of high importance to commercial fisheries in north-western Australia. An important aspect of our study was the ability to sample a community of commercially important predatory fishes in a large (77km x 40 km) fishery management zone on a tropical shelf that had been protected from commercial fishing for more than a decade. This allowed us to examine the trophic roles of these species in an environment where the impacts of removal by fisheries on trophic structure was minimised. We focused on five common species of snapper (Lutjanidae), emperors (Lethrinidae), and grouper (Epinephelidae) that together constitute >25% of the North Coast Demersal Resource, with an annual value of $12 million AUD (Newman et al., 2018b). Our study examined the following questions: a) How is the predatory fish community structure reflected in terms of stable isotopes δ^{15}N and δ^{13}C, and is there evidence of niche overlap among species?; b) How do isotope signatures vary between sampling periods – is there a seasonal shift?; c) Is there evidence of an increase in δ^{15}N with the body length of fish, implying that larger individuals feed higher in the food web?; d) Does fin tissue act as a suitable non-lethal proxy for muscle tissue for a key fishery target, the red emperor (*Lutjanus sebae*)?; and e) Is the isotopic composition of fish reflected in their diet obtained from stomach and gastrointestinal tract content analysis?

METHODS

Study Area

Samples were collected in the Area 3 Management Zone of the Pilbara Fish Trawl Fishery, located approximately 90 km north of Point Samson, off the Pilbara coast in Western Australia (**Figure 1**). The area encompasses ≈2,500 km^2 of sloping seabed

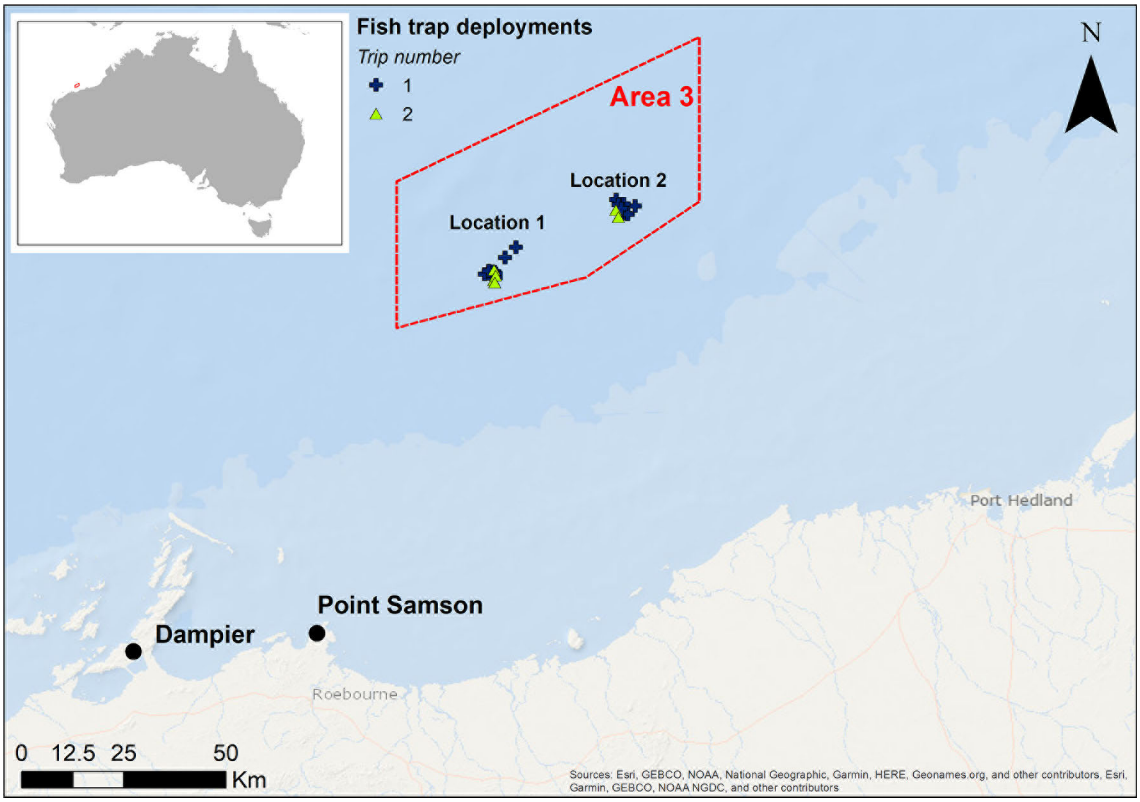


FIGURE 1 | Map of study area showing sampling locations in the Area 3 Management Zone (red box) of the Pilbara Fish Trawl Fishery, which has been a Targeted Fishery Closure since 1998. Sampling was undertaken across two trips in May/June and August/September 2018. This map was created using ArcGIS® software by Esri.

from 50 m water depth in the south to 80 m in the north, an area that has been a Targeted Fishery Closure (TFC) since 1998 and, due to the distance from shore, experiences negligible recreational fishing (Langlois et al., 2021; Meekan et al., 2021). Area 3 is situated within the middle of the fishery to maximise spillover potential to the areas surrounding it (Langlois et al., 2021). Results from a previous study indicated that the seabed was largely a homogenous environment of sand covering a hard substrate (possibly limestone), which was interspersed with small patches of < 2% sessile benthic invertebrates (predominantly sponges, and soft corals) (Meekan et al., 2021). Fish samples were collected from sites within two focal locations in Area 3, in depths between 57 and 65 m. Individual collection sites were chosen based on multibeam echosounder surveys conducted to characterise the local seafloor that indicated suitable benthic habitat for demersal fish (Mccauley et al., 2021; Meekan et al., 2021). The two locations were separated by approximately 30 km.

Target Species

The species of interest comprised: *Lutjanus sebae* (red emperor), *Lethrinus punctulatus* (bluespotted emperor), *Epinephelus areolatus* (yellowspotted rockcod grouper), *Epinephelus multinotatus* (rankin cod), and *Plectropomus maculatus* (bar-cheek coral trout). These species are known to occur across a variety of habitats, including coral and rocky reefs, rubble, seagrass beds (*L. punctulatus*), and deep offshore areas (*E. multinotatus*), down to maximum depths of between 100 and 200 m (Rome and Newman, 2010). The maximum size these fish grow to ranges from small size classes 40-50 cm (*L. punctulatus and E. areolatus*) up to medium to large size classes 90-100 cm (*P. maculatus*, *L. sebae*, and *E. multinotatus*) (Rome and Newman, 2010). None of these species have had detailed studies of dietary information to date, although the larger of the species (*E. multinotatus and P. maculatus*) are thought to be predominantly piscivorous, whereas the smaller to medium sizes (*L. sebae, E. areolatus* and *L. punctulatus*) appear to have mixed diets, including teleosts and various invertebrates (Salini et al., 1994; Farmer and Wilson, 2011; Matley et al., 2018). Three of these species (*L. punctulatus*, *L. sebae*, and *E. multinotatus*) are indicator species for monitoring and assessment of the North Coast Demersal Scalefish Resource (Newman et al., 2018b). There was a large focus on *L. sebae* in our study, given its broad distribution across the region and high-value to both commercial and recreational fishers (Newman et al., 2018a).

Collection Methods

All field work was done from the Australian Institute of Marine Science research vessel RV Solander, which is 35 m steel hull ship, purpose-built for marine science activities. Fish traps were chosen over other capture techniques such as hook and line, as they provided the advantage that catches could be size selective, non-target specimens could be released alive and shark depredation events minimised. Traps were made from steel mesh (50 mm width) and were rectangular with rounded corners measuring 600 mm in height, 1500 mm in length and 1200 mm in width (Newman et al., 2012). The vertical entrance to the trap was 600 × 200 mm in width, tapering to 600 × 100 mm internally (Newman et al., 2012). Australian sardines (*Sardinops* spp.) were used to bait traps. Traps were soaked for less than three hours, and deployments were staggered during daylight hours with two traps deployed at a time and a gap of two hours prior to the next trap deployment. On recovery, traps were raised slowly over a 20- to 30-minute period to allow for swim bladder gas reabsorption and were carefully lowered on deck *via* the stern A-frame to minimize scale damage. Fish were transported from traps to a holding tank for visual inspection. Samples were collected over two trips in May/June (end of Austral autumn/beginning of Austral winter) and August/September 2018 (end of Austral winter/beginning of Austral spring) using industry standard fish traps described above (Newman et al., 2011; Harvey et al., 2012).

Each fish was measured and photographed, and a dorsal fin ray removed and stored in a liquid nitrogen shipper (MVE CryoShipper) until they were subsequently transferred to a -80°C freezer and stored until processing could be done on land.

Species of interest were euthanized by placement into a concentrated solution of Aqui-S anaesthetic and then snap-frozen to -20°C for subsequent sampling of stomach content and analyses of stable isotopes from fish dorsal muscle tissue. Stomach contents were only retained on the second sampling trip for all species apart from *L. sebae*, which had stomach samples collected on both trips.

Stable Isotope Analysis

Less than 1 g of white dorsal muscle tissue was removed from a subsample (20 individuals) of whole *L. sebae* caught in the first sampling trip (May/June 2018), to compare with $\delta^{13}C$ and $\delta^{15}N$ samples obtained from the dorsal fin rays of the corresponding individuals. Skin was not included with the sample. Once removed, they were stored in a -80°C freezer along with the fin clips. Both fin (included dorsal fin spine and membrane) and muscle tissue samples were analysed for $\delta^{15}N$ and $\delta^{13}C$ with a continuous flow system, which consisted of a Delta V Plus mass spectrometer connected with a Thermo Flush 1112 *via* Conflo IV (Thermo-Finnigan/Germany). All $\delta^{13}C$ values are given in per mil [‰, VPDB] according to delta notation, whereas $\delta^{15}N$ values are given in per mil [‰, Air] according to delta notation (Skrzypek, 2013). Multi-point normalisation was used in order to reduce raw values to the international scale (Skrzypek, 2013) and was based on standards provided by IAEA: $\delta^{13}C$ - NBS22, USGS24, IAEA600, USGS40, IAEA603; and for $\delta^{15}N$ - N1, N2, USGS40, USGS32 and laboratory standards. Values of international standards for carbon ($\delta^{13}C$) were based on Coplen et al. (2006). Stable isotope lab analyses were undertaken at the West Australian Biogeochemistry Centre. The external error of analyses was calculated as one standard deviation of the mean values and was determined to be 0.10 ‰ for $\delta^{15}N$ and $\delta^{13}C$.

Stomach and Gastrointestinal Tract Content Analysis

Fish stomachs and gastrointestinal tracts (GIT) were thawed for several hours prior to analysis in the lab. Each sample was placed in a tray and examined using a Leica dissecting microscope (https://www.leica-microsystems.com/) with a ProSciTech digital camera mounted to the eyepiece. Stomach and GIT content were examined, and results combined, although any bait from traps found in stomachs was recorded and removed from any analyses. All items were photographed under the microscope as a permanent record. Each item was counted and categorised into the lowest taxonomic level possible. Frequency of occurrence (%F) or 'presence-absence method' was used, which is the number of stomachs containing particular prey items as a percentage of all stomachs sampled (Hyslop, 1980). This method was used because it has been found to provide a robust and interpretable measure of diet composition (Baker et al., 2014; Amundsen and Sánchez-Hernández, 2019). All items from the stomach and GIT were examined and grouped into one of four broad categories of food items: Crustacea, Teleostei, Mollusca, Other. Other included a range of spines, bones, and hard objects that potentially were parts of species of Radiolaria, Foraminifera, or Echinoidea. Unidentifiable, soft, amorphic organic material (AOM) was also noted. In addition to examination of contents from the stomach and GIT, the presence of bait used in traps was noted if present in the stomach, as well as whether stomachs were empty. A vacuity index was calculated as the number of empty stomachs/total number of the stomachs examined) × 100.

Statistical Analyses

Excessive lipid content in tissues relative to proteins and carbohydrates can bias stable isotope analyses and is recommended to be normalised for aquatic animals when the C:N ratio is > 3.5 (Post et al., 2007). Therefore, where our C:N ratio of a fish was > 3.5 we applied a normalisation of $\delta^{13}C$ using the method of Post et al. (2007): $\delta^{13}C$ —3.32 + 0.99 × C:N ratio (See also Abrantes et al., 2012; Frisch et al., 2014). Using the adjusted $\delta^{13}C$ values, the stable isotopes $\delta^{13}C$ and $\delta^{15}N$ were plotted against one another to position each fish species in relative isotopic space. This was repeated for means calculated for each sampling trip, as trips were three months apart and isotopes in fins would have likely undergone turnover during this time period, with previous studies finding half-lives of $\delta^{13}C$ and $\delta^{15}N$ in fin tissue to range between 9 and 37 days for a range of species (Suzuki et al., 2005; German and Miles, 2010; Heady and Moore, 2013; Matley et al., 2016). We applied linear models to test whether there were any significant differences between isotopes collected between sampling trips and locations.

The isotopic niche occupied by each species was calculated using the total area of convex hulls that encompassed all individuals (Vaudo and Heithaus, 2011; Frisch et al., 2014). The area and percentage of overlap niche region (N_R) was calculated through Monte Carlo bootstrapping 10,000 samples from the normal distribution for each fish. The mean value of the trophic niche and trophic niche overlap was calculated from the bootstrapped 10,000 samples for each species. The isotopic niche overlap of each species was calculated using the *overlap* function in the nicheROVER package in R (Swanson et al., 2015; R-Core Team, 2021). Isotopic niche space size was estimated by calculating the posterior distribution of niche size per species using the *niche.size* function in the nicheROVER package (Swanson et al., 2015).

The relationships between $\delta^{13}C$ and $\delta^{15}N$ with the fork length of each fish species was determined to assess whether there was any evidence of ontogenetic shifts in diet. This analysis was undertaken using a linear regression. The relationship between stable isotopes $\delta^{13}C$ and $\delta^{15}N$ taken from dorsal muscle tissue and fin samples was also used to assess differences in enrichment using linear regression.

To determine whether there had been any inadvertent sampling bias of fish body length between the first and second trip, we calculated Kernel Density Estimates (KDE) based on length frequency distributions for each species. These KDEs per trip were then compared to one another using a Kolmogorov–Smirnov (KS) test, which compared the size and shape of length-frequency distributions (trip 1 vs trip 2) (Langlois et al., 2012).

RESULTS

Predatory Fish Community Structure of $\delta^{13}C$ and $\delta^{15}N$ Isotopes

In total, we analysed 153 fin clip samples across five species of predatory fishes for $\delta^{13}C$ and $\delta^{15}N$ composition (**Table 1**), with an additional 20 muscle tissue samples analysed for corresponding individuals of *L. sebae*. The species with the highest mean $\delta^{15}N$ was *E. multinotatus* (13.50 ± 0.11 SE) and the species with the lowest was *L. punctulatus* (11.05 ± 0.13) (**Figure 2A**). The greatest range in $\delta^{15}N$ was observed in *L. punctulatus* (3.1‰) and the lowest was in *E. areolatus* (1.1‰). The results from a linear model indicated that there was a significant effect of sampling trip on $\delta^{15}N$ ($P = <0.10$) (**Figure 2B**), although the effect of location was non-significant ($P = 0.14$) (**Supplementary Figure 1**).

Patterns in $\delta^{13}C$ were opposite to those of $\delta^{15}N$, with highest mean $\delta^{13}C$ in *L. punctulatus* (-14.71 ± 0.14) and the lowest in *E.*

TABLE 1 | Summary of fin clip samples of predatory fishes used for stable isotope analyses.

Species	Samples (n)	Mean FL cm (± Se)	Mean $\delta^{13}C$	Mean $\delta^{15}N$
E. areolatus	29	32.56 (5.17)	-17.57 (0.19)	12.02 (0.06)
E. multinotatus	30	61.56 (12.41)	-18.11 (0.23)	13.50 (0.11)
L. punctulatus	40	29.76 (3.12)	-15.23 (0.17)	11.05 (0.13)
L. sebae (fin)	40	45.36 (13.62)	-16.45 (0.16)	12.43 (0.10)
L. sebae (muscle)	20	44.77 (19.19)	-17.29 (0.11)	12.06 (0.09)
P. maculatus	14	56.29 (13.29)	-17.05 (0.34)	12.93 (0.20)
Total	**153**	NA	NA	NA

An additional 20 dorsal muscle tissue samples were also collected from L. sebae to compare with corresponding fin samples from the same individuals. Standard errors are in parentheses. NA, Not Applicable.

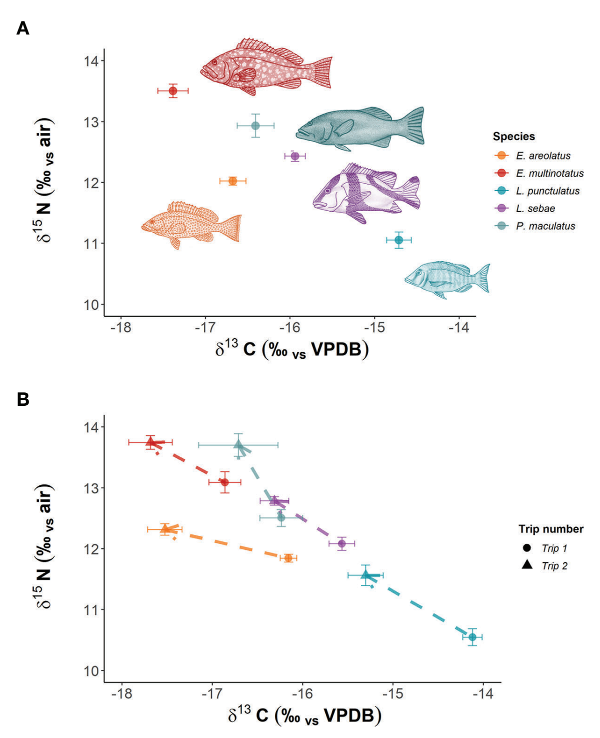


FIGURE 2 | Mean isotopic signatures of predatory fish species in NW Australia. Panel **(A)** shows overall mean values for both trips combined, while Panel **(B)** shows means per trip. Dashed lines with arrows indicate direction of trend between trips.

multinotatus (-17.38 ± 0.17). δ^{13}C ranged between 3.04 and 4.77‰ across species. The effect of sampling trip on δ^{13}C was significant (P = <0.001) (**Figure 2B**), as was the effect of location (P = 0.001) (Supp. **Figure 1**).

There was considerable overlap of isotopic niches across all species observed, with *E. areolatus* having the most restricted isotopic niche space (**Figure 3**). The greatest level of overlap of 95% niche region size occurred between *L. sebae* and *P. maculatus* 89.41% (**Table 2** and **Supplementary Figure 2**), whereas the lowest occurred between *E. multinotatus* and *L. punctulatus* 10.58%, followed by *E. multinotatus* and *E. areolatus* 11.69%. Isotopic niche size estimates were similar for *E. multinotatus* (10.86 ± 2.05 Se), *L. punctulatus* (10.00 ± 1.60 Se), and *P. maculatus* (9.44 ± 2.60 Se) (**Supplementary Figure 3**). Estimates of isotopic niche size were however considerably smaller for *L. sebae* (6.64 ± 1.05 Se) and *E. areolatus* (4.95 ± 0.93 Se).

Kernel densities of species body lengths of all species did not differ between sampling trips (**Figure 4**). There was a positive relationship with δ^{15}N (p =<0.001, R^2 = 0.64) and body length for each species of fish, with the strongest relationships for *L. sebae* and *P. maculatus* at R^2values of 0.37 and 0.32, respectively (**Figure 5**). No significant relationship between δ^{13}C and body length was observed for individual species (**Supplementary Figure 4**).

The comparison of δ^{13}C and δ^{15}N obtained from multiple tissue samples (dorsal fin and dorsal muscle) for *L. sebae* indicated significant positive relationships for both δ^{15}N (P = <0.001, R^2 = 0.7) and δ^{13}C (P = 0.03, R^2 = 0.18) (**Figure 6**). Mean isotopes for δ^{15}N were the same in both fin and muscle tissue (12.1 ± 0.10 SE and 12.1 ± 0.09 SE respectively). However, mean δ^{13}C was higher in fin tissue compared to muscle tissue (-15.6 ± 0.14 SE and -17.3 ± 0.11 SE respectively), (Paired t = 13.087, df = 19, p = <0.001). Only δ^{15}N significantly increased (p = <0.001) with fish body length for both fin and muscle tissue (fin R^2 = 0.73 muscle R^2 = 0.76) (**Supplementary Figure 5**).

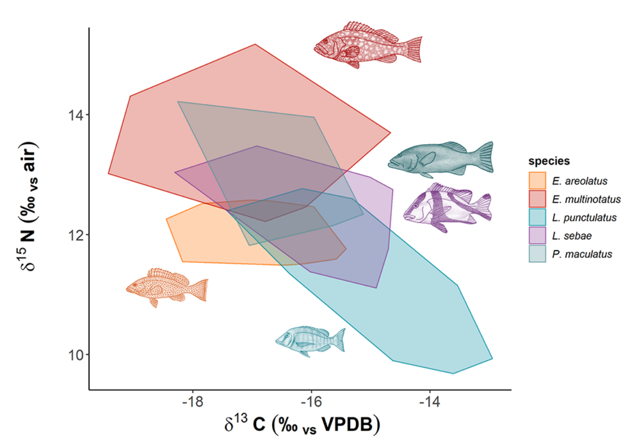


FIGURE 3 | Isotopic niche space of predatory fish species. Each minimum convex polygon encompasses all δ^{13}C and δ^{15}N values of individuals for each species.

Dietary Information From Gastrointestinal Tract Contents

Of the stomachs extracted, approximately 60% were empty and 26% contained fish remains consistent with the bait used in traps (**Table 3**). The analysis of items identified from the gastrointestinal tract (GIT) highlighted the most dominant prey item for each of the five species was unidentified fish remains (e.g., scales, fish bones and spines) with (F%) ranging from 9.1 – 60 (**Table 3**). The larger species (*E. multinotatus* and *P. maculatus*) had high proportions of amorphic organic matter (AOM) in either the stomach or GIT (52% and 60% respectively).

DISCUSSION

There was evidence of separation of the five predatory species in isotopic space, suggesting differences in dietary sources and trophic structure, which was partially confirmed by items present in the stomach and GIT for some species. The range of δ^{15}N‰ between the smallest species in our study (*L. punctulatus*) and the largest (*E. multinotatus*) was within the range of trophic discrimination factors (TDF) that indicate likely predation within this group of predators (e.g. Wyatt et al., 2010; Matley et al., 2016; Canseco et al., 2021). Indeed, *E. multinotatus* was observed to predate on both *L. punctulatus* and *E. areolatus* while in the traps during this study (pers. obs.). It is possible however, that other factors not explored in our

TABLE 2 | Mean probabilistic niche overlap metrics (%) for 95% niche region size for predatory fish.

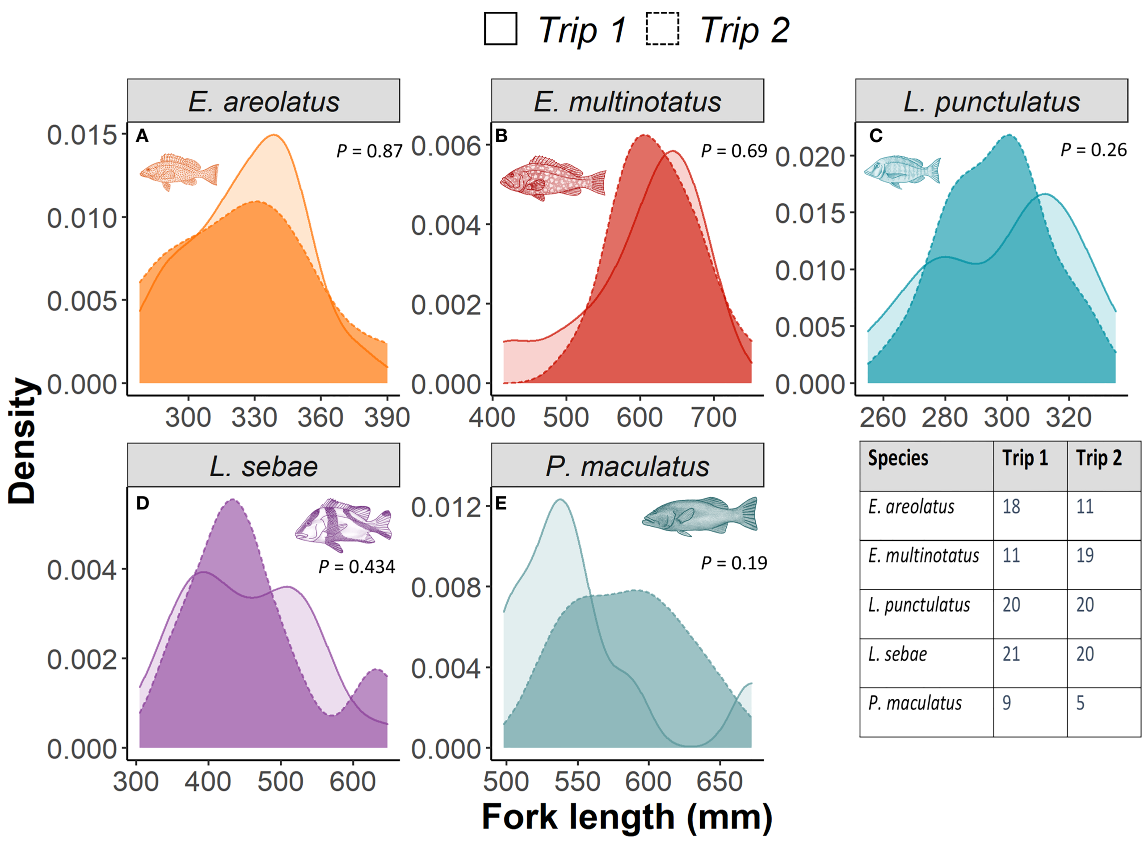
Species A	Species B ***E. areolatus***	***E. multinotatus***	***L. punctulatus***	***L. sebae***	***P. maculatus***
E. areolatus	NA	38.17	54.41	69.73	73.55
E. multinotatus	11.69	NA	8.89	37.54	69.97
L. punctulatus	27.65	6.52	NA	39.87	30.02
L. sebae	54.41	53.14	73.96	NA	89.41
P. maculatus	33.03	76.02	42.53	74.78	NA

NA, Not Applicable.

study may have influenced TDFs of these predatory fish species, such as water temperature, tissue types, growth rates, and prey type (Canseco et al., 2021).

The low values of δ^{13}C (-18 to -15‰) for all species were indicative of a food chain driven by benthic productivity (France, 1995), as has been observed for mesophotic fish communities in the East/Japan Sea (e.g. Park et al., 2020). Similarly, studies that have assessed δ^{13}C values of primary producers, consumers, and particulate organic matter in shallow coastal areas of North-Western Australia provide reference ranges for planktonic sources between -24 to -19‰ and benthic producers predominantly ranging between -16 and -7‰ (Vergès et al., 2011; Vanderklift et al., 2016). A reliance on benthic production has also been previously observed for large predatory fish species (reef sharks) in shallower, coastal waters of North-western Australia (e.g., Speed et al., 2012). Similarly, studies focussing on emperors and groupers from other regions such as the Seychelles also found comparable δ^{13}C values for *E. multinotatus* and *L. sebae* to our study (Sardenne et al., 2017) and one study of *P. maculatus* on the Great Barrier Reef estimated that 89% of its δ^{13}C was derived from benthic production (Frisch et al., 2014).

Both δ^{13}C and δ^{15}N differed for each species between sampling periods, suggesting that there were seasonal differences or potentially a dietary shift over the three-month period between sample collection. Unfortunately, only limited numbers of stomachs were collected in the first trip (primarily *L. sebae*), so a comparison of GIT contents between sampling



Species	Trip 1	Trip 2
E. areolatus	18	11
E. multinotatus	11	19
L. punctulatus	20	20
L. sebae	21	20
P. maculatus	9	5

FIGURE 4 | A comparison of kernel density distributions for fork length of each fish species between trips. P values represent KS test result for significance. The table inset provides number of individuals measured for each trip.

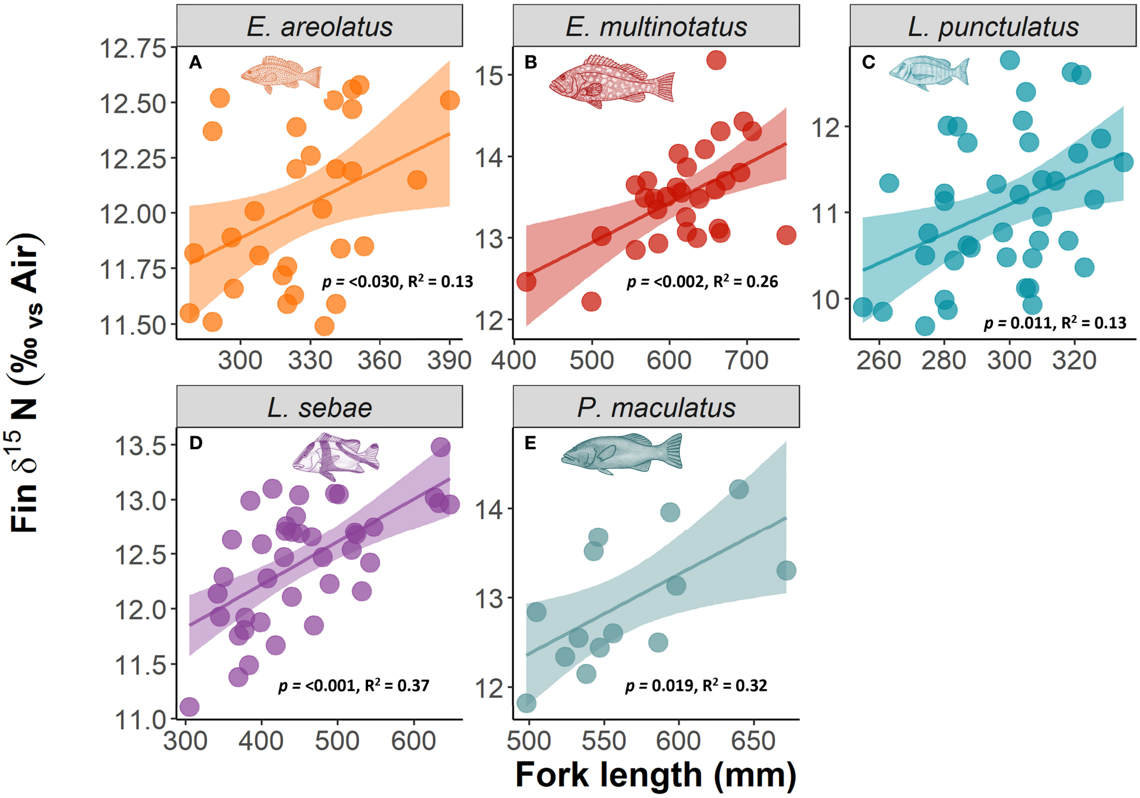


FIGURE 5 | The relationship between $\delta^{15}N$ and body length of fish. Significant relationships in bold text.

periods was not possible for most species. However, *Lutjanus sebae* caught at Location 1 during the first trip when $\delta^{15}N$ was lower, had a predominance of crustacean prey items in guts, whereas teleosts were a much greater part of the diet in the second trip, when $\delta^{15}N$ in diets was higher. This may indicate a shift in diet between seasons for this species or the changes in availability of prey. Alternatively, there might also be strong seasonal differences in the source of primary productivity in these food webs, as occurs in other fisheries (Abrantes et al., 2015). Indeed, seasonal enhancement of chlorophyll *a* concentrations at Ningaloo Reef in North-western Australia have been shown to be driven by an acceleration of the Leeuwin Current (Rousseaux et al., 2012), and fluxes in particulate organic matter composition across a reef have been observed through wind-driven upwelling (Wyatt et al., 2013). Unfortunately, a detailed assessment of productivity was beyond the scope of the current project. Seasonal differences in stable isotope ratios can also be related to a range of other variables, such as changes in light, temperature, salinity, fluctuations in food availability, or variation in feeding due to the life stage of consumer, sex, or physiology (Hyndes et al., 2013). Given the restricted range of length distributions of the species we sampled (adult fishes), life stage is unlikely to have been a major factor contributing to the observed differences in our study. Furthermore, a considerable change in growth rate between sampling periods (separated by three months) for adult fish was deemed unlikely to have been a major influencing factor on stable isotope values of these species. Indeed, a controlled feeding trial of medium to large sized coral groupers (*Plectropomus leopardus*) found that most ^{15}N incorporation was driven by metabolism, rather than growth (Matley et al., 2016).

The $\delta^{13}C$ ‰ for most species also varied between locations, which suggests that these demersal species are limited in their foraging range (**Supplementary Figure 1**). Restricted movement of *L. sebae* was confirmed in a concurrent telemetry study, which showed no dispersal of tagged individuals between the two study locations over a six-month period (Meekan et al., 2021). Other telemetry studies of tropical demersal fishes have also found limited movement, home ranges, and activity spaces of tagged individuals (e.g. Zeller, 1997; Taylor and Mills, 2013; Currey et al., 2014). Despite this tendency for restricted and repeated fidelity to small areas, longer migrations have also been observed, possibly due to reproduction, as is the case for *Lethrinus nebulosus* (e.g. Babcock et al., 2017a). The individuals in our study were primarily adults, however, juveniles of *L. punctulatus* (< 20 cm TL) are known to spend their early life in inshore macroalgal habitat before migrating offshore (Babcock et al., 2017b), where presumably they also undergo changes in diet. This species had the highest enrichment of $\delta^{13}C$, which may be a residual signature from inshore feeding, although there was no

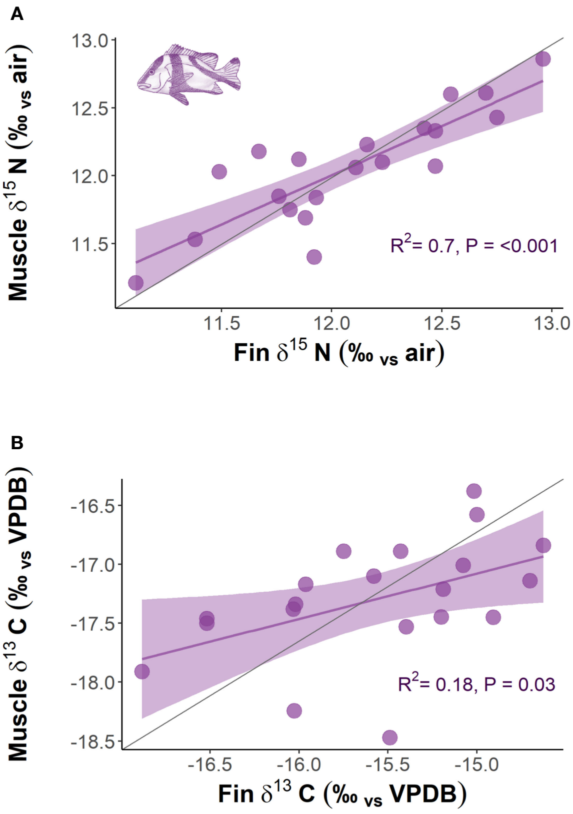


FIGURE 6 | The relationship between **(A)** $\delta^{15}N$ of dorsal muscle with fin tissue and **(B)** $\delta^{13}C$ of dorsal muscle with fin tissue for *L. sebae*. The grey line represents the 1:1 slope.

evidence of smaller individuals having higher $\delta^{13}C$ values than larger individuals (**Supplementary Figure 4**) as might be expected if this was a result of ontogenetic changes in habitats. High overlap of both $\delta^{13}C$ and $\delta^{15}N$ between locations for *L. punctulatus* suggests that they may have a less specialised, and less location-specific diet compared to the other species, which was also reflected in their stomach and GIT content (i.e., all groups of prey were represented).

Isotopic niche overlap was high (>89%) between *L. sebae* and *P. maculatus*, suggesting they are targeting prey within similar trophic levels (**Supplementary Figure 2**). Unfortunately, we were unable to confirm this finding due to *P. maculatus* having mostly empty stomach and GIT contents. However, *L. sebae* did have a high proportion of teleosts in the gut samples (60%), confirming their reliance on other species of fish as a source of nutrients, as has been observed previously (Salini et al., 1994). Similarly, diet studies on coral reefs have also confirmed that *P. maculatus* are primarily piscivorous (Kingsford, 1992; St John, 1999) and tend to feed on benthic and midwater prey, compared to other species within the genus *Plectropomus*, which feed on planktonic prey (Matley et al., 2018). The largest (*E. multinotatus*) and smallest (*L. punctulatus*) species observed in our study had minimal niche overlap (≈9%), which was unsurprising given the difference in body size (Max TL 100 cm and 38.4 cm respectively) (Bray and Gomon, 2021; Newman et al., 2021), although there is currently little information available for the diet of either of these species (Farmer and Wilson, 2011). Dietary information from stomach and GIT content analysis was limited for *E. multinotatus*, with 20 of 21 gut samples being empty. This is likely due to a lifestyle of infrequent feeding, which is thought to be common in large piscivores (Vinson and Angradi, 2011), but may be a consequence of stomach eversion due to barotrauma when fish are raised from depth. Indeed, both stomach vacuity (being empty due to infrequent feeding) and regurgitation have been reported in coral reef grouper and snapper by other studies (Vignon and Dierking, 2011).

It is important to note here that while we observed minimal overlap in isotopic niche space for some species, this may not necessarily equate directly to trophic niche differences. Indeed, trophic niche can be considered synonymous to isotopic niche only when it is primarily driven by consumer-resource interactions (Yeakel et al., 2016), which may indeed be the case with our study, although without spatially and temporally relevant environmental measurements that may affect assimilated isotope values, it is difficult to assess. Isotopic niche extent has been shown to be dependent on sampling scale (Reddin et al., 2018), with fine-scale sampling having lower environmental heterogeneity. Given the limited spatial and temporal scale of sampling in our study, environmental heterogeneity is unlikely to have had a major influence on isotope values, which provides some confidence in comparisons of isotopic and trophic niche spaces.

Frequency distributions of body length were consistent across both sampling periods for all species, although there was a slight,

TABLE 3 | Summary of stomach and gastrointestinal tract contents of predatory fishes.

	Stomach and gastrointestinal tract contents (%F)					Stomach contents	
Species	**Count (n)**	**Crustacea**	**Teleostei**	**Mollusca**	**Other**	**Bait**	**Vacuity Index**
E. areolatus	11	9.1	9.1	0.0	27.3	0	81.8%
E. multinotatus	21	0.0	33.3	0.0	0.0	0	95.2%
L. punctulatus	25	60.0	40.0	28.0	32.0	64%	8%
L. sebae	25	60	60	12	24	28%	36%
P. maculatus	5	0.0	0.0	0.0	0.0	0	100%

Data are presented in frequency of occurrence (%F). The category 'Bait' relates to the number of individuals where bait was present in the stomach, whereas 'Empty' indicates the number of individuals that had empty stomachs.

albeit non-significant difference for *P. maculatus*, likely as a result of a low sample size. The size range for all species primarily included mature individuals, which are targeted by the commercial trap and line fishery in the region. Therefore, any differences in isotopic signatures observed between trips was unlikely to be a result of differing distributions or life history stage between trips. Body length was found to have a positive relationship with δ^{15}N, which likely reflects increased size (e.g. St John, 1999) or trophic level (e.g. Costa, 2009; Dalponti et al., 2018) of prey items consumed. Despite a positive relationship between fin and muscle tissue for both δ^{13}C and δ^{15}N in *L. sebae*, there was no evidence of a difference in the enrichment of δ^{15}N between the different tissue types for this species. However, this was not true for δ^{13}C, which was significantly more enriched in fin tissue compared to muscle tissue, a pattern that has also been observed in other species of finfish (e.g. Willis et al., 2013) and sharks (e.g. Matich et al., 2010), although see Jardine et al. (2011). The closely matching values of δ^{15}N for both tissues in *L. sebae* provides some evidence that using this isotope from fin tissue clips is a good proxy for muscle tissue. This may not be the case with other species, and any tissue corrections should be tested on a species-specific basis (Willis et al., 2013).

The most common identifiable prey items based on stomach and GIT analyses were fish parts (scales and bones) followed by crustacean parts (limbs, claws, carapaces), as has also been previously identified for a species of snapper in north-west Australia, the saddle tail snapper (*Lutjanus malabaricus*) (Takahashi et al., 2020). We found that a high proportion of stomachs were empty, or contained the bait used in our fish traps. For this reason, the GIT contents were usually more informative than the stomach contents, although the drawback with this approach is that by the time prey items had progressed through the stomach, only hard components remained intact. The contents from GIT are therefore biased towards hard materials, particularly those found in fish bones and scales (e.g., collagen), and crustacean exoskeletons (e.g., chitin), and other calcium carbonate structures (e.g., mollusc shells). Several individuals, particularly from the larger species (*E. multinotatus* and *P. maculatus*), had amorphic organic matter (AOM) present in their GIT, which may have been partially digested fish remains. Future studies could adopt the use of DNA metabarcoding to help provide further resolution to identify the remains of this prey (e.g. Casey et al., 2019; Takahashi et al., 2020). Furthermore, we also advocate the collection of gut and molecular samples across all species and trips in future work, as this would provide enough data to support the potential diet shift, we observed for *L. sebae*. Additionally, collection of isotope samples from primary producers would provide $N_{base,}$ which would allow the calculation of trophic positions of predatory fish, enable mixing models to estimate the source of dietary carbon and nitrogen, and also give an indication of seasonal fluxes that could be used to help better interpret findings.

The coarse level of dietary information obtained in our study, along with community trophic structure inferred from stable isotopes are required to help form a complete picture of food-webs, which are often lacking for many important fishery species and are needed for the development of ecosystem based models to assist management (Abrantes et al., 2015). Of particular importance to modelling, is the understanding of trophic pathways and associated implications for the removal of predators from an ecosystem (Farmer and Wilson, 2011), such as the group of species of commercially important fish that were the focus of the current project. For example, some of the smaller to mid-sized fish in our study such as *L. punctulatus* and *L. sebae* had large percentages of crustaceans in their diet, while trophic discrimination factors of δ^{15}N among these predators with some of the larger sized species such as *E. multinotatus* suggested predation within this guild. Diet varies among and within species targeted by fishers and the impact of fishing on prey and predator communities will depend on the species and quantity of fish removed (Farmer and Wilson, 2011). Therefore, our findings will be of importance to current approaches to fishery management that rely on protection *via* static closures and bag and size limits of fish.

CONCLUSION

Our multi-facetted approach to defining trophic structure of a demersal predatory fish community identified differences in the isotopic niches occupied, although there was of overlap in diet for some of these species (e.g., *L. sebae* and *L. punctulatus*). The low values of δ^{13}C in fish indicated a benthic driven food-web, which fluctuated through time, either due to environmental or biological drivers. Location-specific δ^{13}C signatures in all species were indicative of animals with a restricted foraging range, which is consistent with our understanding of the limited movement patterns in demersal predatory fishes. A positive relationship with δ^{15}N and body length was also consistent across species and provided evidence of inter-guild predation between the larger (*E. multinotatus* and *P. maculatus*) and smaller species (e.g., *L. punctulatus* and *E. areolatus*). Similarities of δ^{15}N between muscle and fin tissue for *L. sebae* provided strong evidence for the use of fins as a non-lethal technique for sampling isotopes, although we were unable to compare multiple tissues for all species. Examination of both stomach and GIT contents for each species was informative for some species and partially confirmed patterns observed using stable isotopes, although the high proportion of empty stomachs limited comparisons. Our study highlights the complex spatial and temporal trophodynamics among predatory fish communities in a demersal shelf ecosystem and provides information that could contribute to ecosystem-based models to assist in the management of this commercially important guild.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by The University of Western Australia Animal Ethics Committee.

AUTHOR CONTRIBUTIONS

CS, MM, MB, MP, and BT collected data used in the manuscript. All authors assisted in conceptualising the idea for the manuscript. CS, MM, MB, MP, DM, BT, LT, JS, and SN made substantial contributions to drafting the manuscript, searching the literature, and interpreting results. All authors participated in drafting the final version of the manuscript and gave final approval of the version submitted and any revised versions.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.871611/full#supplementary-material

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