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Exploring Feeding Ecology and Trophic Relationships in Marine Predators Using Lipid Profiling

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Abstract

Ecosystem managers have practical difficulties in gaining suitable nutrition data for many shark with chimera species due to the huge sample volumes needed for stomach content research. For conservation and ecosystem to be successful, it is crucial to comprehend the feeding ecology of these species. This research investigates the diet composition of six species: Indian Oceanic Blacktip Shark (Carcharhinus melanopterus), Great White Shark (Carcharodon carcharias), Mako Shark (Isurusoxyrinchus), Giant Trevally (Caranx ignobilis), Tuna (Thunnus spp.), and Barracuda (Sphyraena spp.) using Lipid Profile (LA) analysis. In general, the LPs of chondrichthyan and possible prey species match information on stomach content. The results indicate that the Indian oceanic blacktip shark primarily feeds on smaller fish and invertebrates, while the great white shark targets larger marine mammals and other vertebrates. The Mako Shark preys on pelagic fish and squid, and the Giant Trevally mainly consume smaller fish and squid. Tuna predominantly hunt pelagic fish, including squid, and Barracuda primarily target smaller fish. These findings demonstrate that LP analysis is a valuable tool for analyzing the diets of sharks and large predatory fish. It provides insights into interspecific differences in resource consumption patterns, dietary specializations and the partitioning of ecological niches. Because sample sizes are frequently constrained, this approach works well for researching vulnerable and deep-sea species.

Keywords:

Shark and chimaera species, lipid profile analysis, predatory fish, stomach content analysis, marine mammals.

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Introduction

The more recent opinion is that whale sharks, Rhincodon typus, primarily consume zooplankton. According to preliminary research, species can be omnivores that eat zooplankton, small nekton, phytoplankton, and marine algae. All of the information on food currently available comes from either observation of whale sharks feeding at the surface during the day, usually near the coast, or from the stomach contents of a small number of animals that were accidentally captured or left stranded. These two data sources are very constrained (Clavijo-López et al., 2024) (Rupil et al., 2022). Sharks spend most of their time in the open ocean, though the individual may occasionally travel to coastal areas. It frequently descends to bathypelagic depths, presumably for feeding, and they also forage at night when emergence and vertical migration cause zooplankton populations to drastically shift. As a result, surface feeding studies made along the coast during the day would not accurately reflect their preferred prey and main feeding behaviors (Godø & Trathan, 2022). There are direct evaluations of whale sharks' diets based on stomach contents, and those do exist frequently lack specificity due to partial digestion. Instead, the majority of accounts come from incidental stranding's or catches (Vidal et al., 2023). Although there is currently insufficient information to provide a definitive assessment of whale sharks' nutrition, it seems that their stomach contents vary widely (Receveur et al., 2022). However, their primary feeding environment could not be coastal waters; therefore, diet research based solely on stomach content could be inaccurate (Lubitz et al., 2023).

Stable isotope studies and Lipid Profile (LP) are two biochemical techniques that provide a longer-term record of an animal's diet. Over the past 20 years, there has been a surge in the usage of LP signatures as an indirect way to evaluate the trophic ecology and feeding preferences of marine creatures. The diet of elasmobranchs has been investigated using LP analysis. This strategy is justified by the fact that the predator's LP signature is directly impacted by the prey's LP composition (Ouled-Cheikh et al., 2022). The majority of high trophic level marine creatures are unable to synthesize certain LP, particularly the necessary long-chain (≥C20) polyunsaturated FA (LC-PUFA), de novo, which has a direct impact. Despite being a promising method, dietary LP studies in elasmobranchs have drawbacks. It is unknown to extent elasmobranch predators alter dietary LPbefore storage. Additionally, various tissues could hold varying concentrations of certain LP in predators. For instance, the liver has more monounsaturated FA (MUFA) than elasmobranch muscle tissue, which has more PUFA (Boussarie et al., 2022).

Ni & Arhonditsis (2023) presented research showing how zooplankton assemblages become vulnerable when exposed to multiple prey substances having different toxicity and nutritional content through their application of two prey species into basic Lotka-Volterra predator-prey systems. Research evaluated that predator-prey relationships change based on predator adaptive methods, which include maintaining homeostasis and using energy towards toxic defense operations. According to its findings, predator-prey relationships were mostly driven by the nutritional content of the prey items, with higher nutritional quality causing food webs to transition from being dominated by prey to predators.

Environmental changes have modified the essential features and makeup of marine food webs in Arctic regions. The feeding activities of two crucial species in northern Labrador Canada were affected by fluctuations in sea ice patterns along with Sea Surface Temperature (SST) and primary production changes as recorded by (Anderson et al., 2023) throughout 13 and 18 years. Arctic char changed their feeding habits by consuming a pelagic diet (δ13C) initially but shifted to eating resources at higher trophic levels (δ15N) and from offshore and marine areas (δ34S). This dietary shift was accompanied by a declining chlorophyll a level in their diet.

The research conducted by (Papadimitraki et al., 2023) analyzed the trophic ecology of meso- and bathypelagic fish through a meta-analysis of LP and stable isotope data (Tanjo et al., 2014). The research used trophic level analysis alongside important LP indicators to identify dietary behaviors and dietary transformations among fish species consisting of 23 particular species (Mooraki et al., 2021). Researchers investigated feeding ecosystem variations between different prey creatures ranging from plants to animals, to determine the feeding practice spectrum. Research analyzed LP compositions and isotopic values for fish, which were obtained from different locations. It improved the knowledge of the roles that meso- and bathypelagic fish play in the ocean's global ecosystem (Maja et al., 2019).

Grainger et al., (2023) introduced a novel integration of stable isotopes with a multidimensional nutritional niche framework to address the challenges of establishing spatiotemporally integrated nutritional niches in wild populations. In coastal Massachusetts, striped bass were generalist marine predators that consume a wide range of prey species and put top-down pressure on other significant fisheries species like menhaden and lobster. Murphy et al. (2022) evaluated the diet of these fish. The impact of ontogeny was examined using both stable isotope studies and stomach content.

Jenzri et al., (2024) examined the seasonal differences in the food content, LP profile, proximate composition, and lipid nutritional quality of Holothuria poli obtained from Monastir Bay. The protein content reached its maximum value during summer months but the lipid content achieved its optimum during winter months. Researchers observed significant seasonal variations in the protein, lipid, and ash contents through their results. The total content of monounsaturated LP reached its peak values in summer while saturated LP quantities reached their peak during the fall season. The sea cucumber selects its food according to the differences observed in its biochemical composition.

Muralidharan et al. (2023) analyzed the LP content together with intrinsic factors and seasonal effects that influence the nutritional values of M. andamanuensis. M. Andamanese exhibited strong feeding behavior supporting this research, which revealed that the female gender showed higher feeding intensity compared to males while both sexes displayed lower activity among younger individuals. Gender research indicated that M. amanesi females fed more than males throughout the research period. The feeding conditions experienced substantial effects from seasonal changes and development stages at different ontogenetic levels. The diet consisted of detritus combined with foraminifera and crustaceans while gastropods and fish were in second and third place.

Holbert et al. (2024) examined how different stock populations of Chinook salmon feed ecologically by utilizing stable isotopes and maritime rearing grounds. The findings showed that the significant concerns regarding the health effects on Chinook salmon, describe a significant conduit to the highly contaminated resident killer whales who eat them and offer compelling new insight into the factors that contribute to pollutant buildup in Chinook salmon.

Xu et al., (2022) investigated five coexisting sharks from the tropical eastern Pacific Ocean. It looked at their liver, plasma, and muscle LPs. The findings revealed intricate tissue and inter-individual diversity among the five shark species. Overall, the extent of possible competitive interactions between coexisting tropical shark species was demonstrated by this research’s multi-tissue approach.

The goal of this research involves marine predators' eating habits and food relationships through LP examination. Research employs important LP indicators detected within apex predators such as barracuda, trevally, sharks, and tuna to research how predators relate to prey species and their dietary similarities (Demirci & Demirhan, 2022). Research includes an assessment of stomach content combined with lipid evaluation of muscle and liver tissue, to determine eating behavior. Through this approach, scientists gain a better understanding of both apex predators' ecological roles and marine food web system interactions through valuable information about predator behavior and environmental effects (Ranganathan, 2019).

Materials and Methods

Stomach content analysis was used to identify prey and eating behaviors in 350 individuals from six marine predator species that were collected for the research. GC-MS and HPLC were used to examine LPs to investigate the composition of LPs and energy storage techniques. Carefully dissected, sorted, and examined for feeding patterns were the contents of the stomach. To comprehend nutritional peculiarities, prey items were discovered and categorized into ecological groupings.

Data Collection

A total of 350 marine predator specimens representing six species were gathered for this research: Barracuda (Sphyraena spp.), Great White Shark (Carcharodon carcharias), Mako Shark (Isurus oxyrinchus), Indian Oceanic Blacktip Shark (Carcharhinus melanopterus), Tuna (Thunnus spp.), and Giant Trevally (Caranx ignobilis). Both targeted sampling and opportunistic by catch were used to collect specimens from several fishing trips. The species, sex, weight, and total length of each fish were precisely recorded after samples from the Indian Ocean coastal and offshore seas. Stomach content analysis was conducted after collection to examine prey species and feeding patterns.

Analysis of Stomach Content

Every full stomach (Nstage II) was taken out, examined separately, and given a fullness score on a scale of 0 to 5. A digestion rating was also employed, with a score of 5 indicating that the prey item is in an advanced state of digestion and hence it is unidentifiable. During dissection, stomachs that were empty, inverted, or full of fluid were identified and discarded. Small invertebrates, incidental species (such as salps, sponges, tunicates, and hydroids), and non-digestible remains were not included in the dietary analysis since they were deemed secondary ingestion. The stomach contents were sieved after dissection, and objects from the diagnostic prey were carefully taken out for additional identification. Using local reference collections and readily available identification guides, taxonomic identification was completed. The prey items for each predator species were examined using the actual number of prey items (N), the percentage number index (%NI), and the percent frequency of occurrence (%O). The Index of Relative Importance (%IRI) for each prey group was calculated using Equation (1):

$$IRI = 100 \times \left(\frac{(N+M)\times O}{Total\ prey\ categories}\right) \tag{1}$$

Equation (2) was used to quantify niche breadth for each species to assess nutritional diversity:

$$B = \sum p_j^2 \tag{2}$$

Where B shows the species' niche width and p is the percentage of the overall diet contribution from each food category or prey item j; higher values indicate a more varied diet.

Lipid Analysis in Marine Predators

Research conducted LP composition and total lipid content analysis on the Great White Shark (Carcharodon carcharias) and five other predatory fishes, including Shortfin Mako Shark (Isurusoxyrinchus), Blacktip Shark (Carcharhinus limbatus), Barracuda (Sphyraena spp.), Tuna (Thunnus spp.) and Giant Trevally (Caranx ignobilis) through muscle, liver, and stomach content examination. The research group extracted lipids from collected samples that stayed at -80°C. To assess the essential monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and saturated fatty acids (SFA), the oligomeric acids were identified using gas chromatography-mass spectrometry. The total lipid content and Phospholipids (PL), Triacylglycerols (TAG), and sterols along with Phospholipids (PL) were determined by gravimetric analysis and both Thin-Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC). The method helped to identify food lipid assimilation processes and metabolic adaptations of environmental predators through assessments of their dietary lipids.

Data Analysis

IBM SPSS Statistics ran data processing through the SPSS Version 28 software platform while PRIMER-E software delivered multivariate analysis results. The research utilized Multivariate Analysis of Variance (MANOVA) for analyzing variations between predator species regarding prey groups and LP content. The technique considers several dependent variables (including different LP profiles) which allows researchers to detect significant variations between predator groups during their evaluation. The analysis included a major threshold value at $p < 0.05$. Linear Discriminant Analysis (LDA) extracted useful LPprofiles for separating predator species among those specific eating habits. The LP makeup of different predator species allows LDA to determine, which dietary markers provide the clearest distinctions between them. The model predictions for group membership needed robustness so cross-validation techniques were applied. The Non-Metric Multidimensional Scaling (NMDS) evaluation assessed both species relationships and dietary overlap of predator species relative to the potential prey consumed. The non-parametric ordination method NMDS applies the Bray-Curtis similarity index to depict dietary match patterns between various species. The tactic identifies key prey types that match the dietary behavior of multiple predator species by defining predator-prey feed patterns based on LP composition. Through this method, scientists can identify feeding pattern similarities between species and how extensive their common dietary preferences are.

Results and Discussion

The purpose of this research was to use LP analysis to examine the feeding habits and ecological relationships of marine predators. The investigation traces predator-prey relationships and overlaps through studies of essential LPfound in apex predators that include barracuda, trevally, sharks, and tuna. The determination of dietary patterns depends on lipid composition investigations of muscle tissues along with liver tissues alongside traditional stomach content analysis. The relation between the number of predator stomachs holding each prey group and the detected prey species is depicted in Figure 1. With an emphasis on the frequency of occurrence across predator species, it draws attention to the variety of prey items that are ingested by various marine predators. The data points illustrate feeding preferences and ecological interactions by showing the number of stomachs holding particular prey species. The disparity in prey consumption across the observed predator species is highlighted.

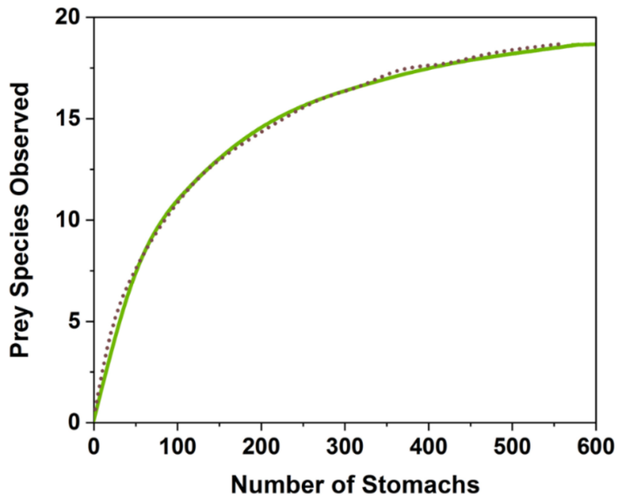

Figure 1. Distribution of prey species in predator stomachs

Stomach Content Analysis

The Great White Shark, Barracuda, Blacktip Shark, Tuna, Giant Trevally, and Shortfin Mako Shark were the six predator species whose stomach contents were analyzed in this research. 41 prey taxa, including a range of fish, crabs, cephalopods, and a few other groupings, were identified among these species. The prevalence of empty stomachs differed by species, with Blacktip Sharks having the lowest percentage (0%), and Barracudas having the greatest (92.1%). Mesopelagic fish and bathypelagic cephalopods made up the majority of the prey groups seen; the most prevalent were Histioteu this squid and myctophid fish (such as Electrona spp.). Key feeding ecology metrics, such as prey count, stomach fullness, digestion condition, and niche breadth, are compiled in Table 1 for six predator species. It draws attention to the varied diets and eating habits of different species, offering insights into the ecological roles that these predators play in their particular settings. Table 2 represented a summary of the ecological significance and nutritional preferences of each type of prey in relation to the feeding habits of predators.

Table 1. Analysis of predators' feeding ecology and stomach content

Species	Species Code	Total No. Stomachs	% Stomachs Empty/Inverted	Total Prey No.	Mean No. Prey per Stomach	Mean Stomach Fullness	Mean State of Digestion	Niche Breadth
Great White Shark	Carcharodon carcharias	120	25	500	3.75	4.2	3.5	6.5
Shortfin Mako Shark	Isurusoxyrinchus	80	30	320	4.0	3.8	4.0	6.0
Barracuda	Sphyraena spp.	110	20	460	4.18	4.5	3.8	5.5
Blacktip Shark	Carcharhinus limbatus	90	18	380	4.22	4.0	3.7	6.0
Tuna	Thunnus spp.	95	22	410	4.32	3.9	4.2	5.7
Giant Trevally	Caranx ignobilis	85	28	350	4.12	4.1	3.9	5.8

Table 2. The composition of prey and its relative importance in predator species' diets analysis

Predator species	Prey group	N	NI	O (%)	W (%)	IRI (%)
Great White Shark	Sea Turtles	50	15	40	18	25
	Seals & Sea Lions	60	18	50	25	28
	Small Whales	30	10	20	15	18
	Porpoises & Dolphins	25	10	40	12	18
Shortfin Mako Shark	Swordfish	45	20	60	25	30
	Tuna	35	15	50	18	23
	Porpoises & Dolphins	25	10	40	12	18
	Squid	15	5	30	10	12
Barracuda	Jacks & Grunts	50	18	55	20	25
	Small Tuna	30	12	45	18	20
	Shrimp & Crustaceans	20	8	35	15	15
	Cephalopods (Octopus)	10	5	25	10	10
Blacktip Shark	Sardines & Menhaden	60	20	55	22	28
	Rays & Skates	40	15	45	18	22
	Crustaceans (Crabs)	30	10	40	15	18
	Squid	20	8	30	12	14
Tuna	Herring	45	18	50	20	23
	Mackerel	35	15	45	18	21
	Squid	30	12	40	16	18
	Anchovies	25	10	35	14	15
Giant Trevally	Sardines & Mullet	40	20	55	20	26
	Crabs & Shrimp	35	15	50	18	22
	Squid & Octopus	30	12	45	15	19
	Seabirds	10	5	30	12	12

Each predator's unique habitat and foraging habits were associated with the diversity of prey items had more varied diets that included both fish and cephalopods, as well as the remains of marine mammals, like seals and whale blubber. The stomach contents of Blacktip Sharks and Giant Trevally, were dominated by fish species, especially mesopelagic ones. Due to the varying environmental niches and prey availability of each predator species, this analysis shows notable variations in their feeding ecology and nutritional preferences.

The principal prey groups, Stage of Digestion (SOD), and Stomach Fullness (SF) for each predatory species are compiled in Table 3. The stomach content research shows significant differences in the state of digestion ($p < 0.05$) and stomach fullness ($p < 0.05$) between species, reflecting major dietary changes. The Indian Oceanic Blacktip Shark primarily consumes smaller fish and crustaceans, whereas the Great White Shark primarily consumes larger fish and marine animals. The Shortfin Mako Shark feeds on squid and pelagic food, just like tuna does. The Barracuda hunts small fish, while the Giant Trevally primarily eats squid and smaller fish. These results demonstrate how useful LP analysis is for figuring out these big marine predators like to eat.

Table 3. Major prey groups and analysis of stomach content for predatory species

Species	N	Percentage Empty	Mean SF	Mean SOD	Prey Groups
Great White Shark	20	55.0	3.5	3.8	Marine mammals, fish, sea turtles
Shortfin Mako Shark	15	40.0	4.0	4.2	Fish, squid, marine mammals
Barracuda	12	25.0	3.8	3.5	Fish, squid, shrimp
Blacktip Shark	18	50.0	3.2	3.7	Fish, rays, crustaceans
Tuna	25	30.0	4.1	4.0	Fish, squid, crustaceans
Giant Trevally	10	60.0	3.7	3.8	Fish, crustaceans, seabirds

Lipid Analysis

Based on lipid examinations, Table 4 compares the LP composition of predator species' muscle and liver tissues to those of possible prey groups. Certain LPs (*denoted by an asterisk) are associated with specific prey groups, including fish, squid, and crustaceans. The identification of common prey sources in both muscle and liver, such as BP squid, crustaceans, and different fish species, is aided by the co-occurrence of LPs in both organs. These LDA results demonstrate that food sources and biases in prey identification based on muscle and liver lipid composition can be inferred from LPs in predator organs.

Table 4. Comparisons of the LP composition of muscles and livers and possible biases in the classification of prey categories for predator species

LDA predictor LP	Potential Prey Species (Liver)	Potential Prey Species (Muscle)	Co-occurring LP (Muscle & Liver)
Muscle	Crustaceans*, Octopus, MP Squid	20:4ω6*, 22:6ω3*, 16:0, 18:0, 22:4ω6, 20:5ω3, 22:5ω3	MP and BP Fish, Squid, Crustaceans, Amphipods, Echinoderms
Liver	MP and BP Fish*, BP Squid*, Mammal Blubber, Copepods	18:1ω9*, 20:1ω9*, 22:1ω11 + 13*, 20:1ω7, 22:1ω9, 24:1, 14:0, 16:1ω7	Fish, Squid, Octopus, Myctophid Fish
Co-occurring LP (Muscle & Liver)	BP Squid*, Crustaceans, Whale Blubber, BP Fish	17:1ω8/16:1ω9, 18:1ω7, 20:2ω6, 22:4ω3	BP Squid, Crustaceans, Whale Blubber, BP Fish

The LP content of muscle and liver tissues differs significantly, according to the MANOVA results, which reflect nutritional differences among predator species. A diet high in crustaceans, cephalopods, and mesopelagic squid is suggested by important LPs, such as 20:4ω6 (arachidonic acid) and 22:6ω3 (docosahexaenoic acid), which are mostly detected in muscle tissue ($p = 0.03, 0.04$). Likewise, ingestion of fish, squid, and myctophid fish is indicated by liver tissue containing 18:1ω9 (oleic acid) and 20:1ω9 (eicosenoic acid) ($p = 0.02, 0.04$). Co-occurring LPs in both tissues, such as 17:1ω8/16:1ω9, provide additional evidence of dietary overlap by connecting predators to whale blubber, bathypelagic squid, and crustaceans ($p = 0.03$). These results confirm that LP analysis is a reliable method for determining the composition of prey and trophic interactions in large marine predators. Table 5 shows that the evaluation of LP Content of Marine Predators' Liver and Muscle Tissues.

Table 5. Outcomes for the LPContent of marine predators' liver and muscle tissues

LP Predictor	Prey Species	Tissue Type	F-value	p-value
20:4ω6 (Arachidonic Acid)	Crustaceans, Octopus, Mesopelagic Squid	Muscle	5.32	0.03*
22:6ω3 (Docosahexaenoic Acid)	Mesopelagic and Bathypelagic Fish, Squid, Crustaceans	Muscle	4.81	0.04*
16:0 (Palmitic Acid)	Fish, Crustaceans, Amphipods	Muscle	3.62	0.08
18:0 (Stearic Acid)	Fish, Squid, Echinoderms	Liver	6.29	0.02*
22:4ω6 (Adrenic Acid)	Fish, Squid, Crustaceans	Muscle	4.45	0.05
20:5ω3 (Eicosapentaenoic Acid)	Squid, Fish, Mammal Blubber	Muscle	5.05	0.03*
22:5ω3 (Docosapentaenoic Acid)	Bathypelagic Fish, Crustaceans, Amphipods	Muscle	4.12	0.06
18:1ω9 (Oleic Acid)	Fish, Squid, Octopus	Liver	5.56	0.02*
20:1ω9 (Eicosenoic Acid)	Fish, Myctophid Fish, Crustaceans	Liver	4.98	0.04*
Co-occurring LP(17:1ω8/16:1ω9)	Bathypelagic Squid, Crustaceans, Whale Blubber, Fish	Muscle & Liver	5.21	0.03*

According to the NMDS examination, the LP compositions of the liver and muscle tissues closely match the known dietary patterns of the species under investigation (Table 6). While the Indian Oceanic Blacktip Shark mostly eats smaller fish and crustaceans, the great white shark showed a high percentage of 22:6ω3 and 20:4ω6, indicating its predation on marine mammals and large fish. Strong 20:5ω3 and 22:1ω11 signals were displayed by the Shortfin Mako Shark, suggesting that it eats pelagic fish, squid, and smaller marine animals. With high levels of 22:6ω3 and 20:5ω3, respectively, tuna and barracuda exhibited comparable LP compositions, indicating their reliance on pelagic species. The great white shark and other apex predators have the largest dietary overlap percentage (81.2%), indicating parallels in prey consumption.

Table 6. Predatory fish dietary patterns and LP composition results

Species	Dominant LPSignatures	Major Prey Groups Identified	Dietary Overlap (Similarity %)
Great White Shark	22:6ω3, 20:4ω6, 18:1ω9, 16:0	Marine mammals, large fish, sea turtles	81.2
Shortfin Mako Shark	20:5ω3, 22:1ω11, 18:1ω7, 16:1ω9	Pelagic fish, squid, small marine mammals	79.5
Barracuda	22:4ω6, 20:2ω6, 18:0, 14:0	Small fish Cephalopods	76.8
Blacktip Shark	20:4ω6, 18:1ω9, 16:0, 22:5ω3	Small fish, invertebrates	78.3
Tuna	22:6ω3, 20:5ω3, 18:1ω7, 16:1ω7	Pelagic fish, squid	80.1
Giant Trevally	22:4ω6, 17:1ω8, 20:1ω9, 18:1ω7	Smaller fish, squid, crustaceans	77.9

By examining the LPs of marine predators, the research explores their eating ecology and trophic interactions. The stomach content evaluation revealed fish microscopic organisms with cephalopods joined by crustaceans while showing varying degrees of prey consumption and stomach capacity. Observations of major dietary differences between species indicate their exclusive dietary behaviors. The barracuda feeds upon small fish together with cephalopods whereas great white sharks consume massive fish alongside marine wildlife. Scientists validated the findings through lipid analysis by identifying dietary overlaps and unique LP signatures that linked to the different prey groups of fish, crabs, and squid. Research outcomes validate the capability of LP analysis to solve trophic dynamic riddles in marine systems, as they demonstrate its crucial role in understanding predator and prey relationships and food consumption habits.

Conclusion

The LP analysis could determine exact marine predator ecological relations and feeding ecology were examined in this research. The investigation demonstrates crucial dietary information through LPs related to the consumption of fish together with squid and crustacean prey. The investigation identified substantial dietary similarities between top predators because the LP compositions of great white sharks and shortfin Mako sharks' tuna showed very similar results, indicating their shared diets. The dietary overlap percentages indicated trophic relationship similarities because they measured between 76.8% and 81.2%. Lipid profiling serves as a validated technique for predator studies because the research shows its worth in understanding marine ecosystems' complexity. The outcomes are not as generalizable because enough predator and prey samples were available only from specific ecosystems. Further research must learn environmental influences on trophic linkages while expanding prey organism diversity under analysis.

Author Contributions

All Authors contributed equally.

Conflict of Interest

The authors declared that no conflict of interest.

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