Age and feeding habits of Caml grenadier Macrourus caml in Cosmonauts Sea

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Abstract

Macrourus caml is a main by-catch in the Southern Ocean fishery, and a main prey species of Antarctic toothfish, which plays an important role in Southern Ocean ecosystem. In this study, age estimation and stomach content analysis were conducted by using samples collected from the Cosmonauts Sea in 2021. The main objectives of this study were to estimate the age and diet of grenadier *Macrourus caml* and to explore the feeding habits of *Macrourus caml*. Stomach content analysis showed that *Macrourus caml* mainly fed on Malacostraca and Sagittoidea, especially the Malacostraca accounting for over 50%. The feeding habits of male and female *Macrourus caml* were similar. Before maturation, *Macrourus caml* mainly fed on Malacostraca and Cnidaria. After maturation, *Macrourus caml* mainly fed on algae, Malacostraca and Sagittoidea. The feeding bias decreased and the feeding range became wider with aging. The results would provide reference for exploring the nutritional status of Antarctic benthic fish in the ecosystem.

1 Introduction

The Grenadier, *Macrourus caml*, a benthopelagic fish belonging to the family Macrouridae, is one of the five clades among the southern hemisphere *Macrourus* (Smith et al. 2011; McMillan et al. 2012), widely distributed in Antarctic (McMillan et al. 2014). Compared with *Macrourus whitsoni, Macrourus caml* has slightly smaller and more subterminal mouth which suggests a more benthic diet (McMillan et al. 2012). *Macrourus caml* mainly inhabits in the water from about 350 to 1660m depth, and they are mostly captured by bottom trawls or bottom longlines (Daniel et al. 1990). *Macrourus caml* is a main by-catch in the toothfish fishery in Ross Sea, and also a major prey species of Antarctic toothfish (Fenaughty et al. 2003; Stevens et al. 2012; Pinkerton et al. 2013). Marriott et al. (2006) found that Macrourids, including *Macrourus caml*, comprised a substantial by-catch of the fishery, accounting for about 10% of the total landing from 1998 to 2004 in Ross Sea. Despite the abundance of *Macrourus caml*, it is not exploited by commercial fisheries within the majority of its range (Bradley et al. 2022). In spite of captured as by-catch, this species is clearly of commercial importance, and is in need of monitoring, management and exploitation. However, little is known about its biology and ecology.

The estimation of age and growth is a critical parameters of fisheries population biology (Van Beveren et al. 2014), and are essential for stock assessment and species management (Ju et al. 2019; Liao et al. 2022). From 2000 to 2006, the age and growth research of grenadier in Ross Sea was carried out by Marriott et al. (2003, 2006). But in Cosmonauts Sea, there is a lack of research. The main methods for fish age identification include calcified tissue identification, length frequency and radioactive element identification (Xie et al. 2021). Peterson successfully use length frequency to estimate age of fish in 1891, and then this method is used by researchers (Jalbani et al. 2014). But it has many defects such as susceptible to different generations, water environment, fishing gears, etc., so it is mostly as an auxiliary method for age identification (Xie et al. 2021). In recent years, radioactive element identification method is employed by some researchers, for instance, Barnett et al. (2020) defined life history of northern Gulf of Mexico Warsaw grouper *Hyporthodus nigritus* through otolith radiocarbon analysis and Sanchez el al. conducted bomb radiocarbon age validations of Warsaw Grouper and Snowy Grouper from the Gulf of Mexico (2019). Although it’s successfully applied, it is difficult to operate and the accuracy of the results obtained by this method remains to be verified. Calcified tissue identification is the most popular method and calcified tissues include scales, otoliths and other calcified tissues (fin spines, vertebrae, etc.). The scale age of *Dissostichus eleginoides* from South Georgia island determined by Cassia (1998) and *Eleginops Maclovinus* from Chile determined by Brickle (2005) are consistent with their real age. Due to the extreme climate in polar regions, most Antarctic fishes grow slowly and have a long age span, so scale identification has a large error (Zhu et al., 2017). Other calcified tissues are rarely used in the age identification of Antarctic fishes due to their difficulty in obtaining and low accuracy. At present, otolith was mostly used to identify the age of Antarctic fishes because otolith growth is more stable, less susceptible to external interference and relatively easy to obtain. Marriott et al. carried out age estimation and maturity of *Macrourus whitsoni* from the Ross Sea through otolith (2006).

Fish feeding provides necessary nutrition and energy for their growth and reproduction (Xue et al. 2004; Wang et al. 2021). The study of feeding ecology is one of the important methods to judge the growth status of fish and understand the migration, distribution, interspecies relationship and resource variation of fish, which is one of the basic contents of fishery biology research. All the diet studies on grenadier have been completed using direct observation of prey items, which is primarily dependent on the morphological characteristics of each prey species (Hynes et al. 1950; Hyslop 1980). Although most of the prey mass (90%) generally consisted of finfish and cephalopods, specific species differed among studies (Leray et al. 2015). In spite of the successful application in diet studies (Saraswati et al. 2019), morphological analysis has potential limitations when used alone. Morphological analysis is time-consuming and difficult in morphological classification. It can only determine the short-term food composition and is difficult to determine their long-term feeding characteristics (Hashimoto 1974; Mata-Sotres et al. 2016; Xi et al. 2015). Molecular identification of prey items is now being used to complement morphological analysis (Carreon-Martinez et al. 2011; Paquin et al. 2014; Symondson 2002). Molecular identification can identify the food composition of fish more quickly and accurately, and is not affected by the age, sex, growth stage and digestion degree of experimental subjects, which can make up for the shortcomings of traditional morphological identification (Xi et al. 2015).

So far, the information about *Macrourus caml* is limited in the Cosmonauts Sea. To define the biological and ecological characteristics of *Macrourus caml* in Cosmonauts Sea, it is essential to conduct further research about age and feeding habits on it in Cosmonauts Sea. The specific objectives of this study were: (i) estimate age; (ii) developmental stage of the gonads; and (iii) describe feeding habits. This study will facilitate understanding the feeding characteristics of *Macrourus caml* in Cosmonauts Sea, and provide a basis for the research of Antarctic bottom fishes, so as to promote the protection and development of potential fishery resources.

2 Material And Methods

2.1 Sampling

Samples were collected from the Cosmonauts Sea (67°00.37'S-67°01.30S, 44°15.83'E-44°29.12'E) by bottom longline in January 2021 during 37th Chinese National Antarctic Research Expedition with the icebreakers R/V *Xuelong 2.* A total of 11 fish specimens were caught in this sampling, which were identified as *Macrourus caml* by morphological analysis. The bottom longline was deployed during January 22 to January 24, with the water depth of this sampling location about 1800m. All individuals were measured for the wet weight (W, g), standard length (SL, mm), total length (TL, mm) and anal length (AL, mm), simultaneously all the sagittal otoliths were collected from the body of fish. Gonads of males and females were dissected and observed in order to macroscopically estimate the minimum size at first maturity.

2.2 Age determination

The sagittal otoliths of *Macrourus caml* were thick and irregular, which could not be clearly observed by direct observation. Therefore, it is essential to make sagittal stone section for age identification. The sagittal otoliths from the samples were cleaned and dried. The otoliths were baked at 285°C for 8 minutes, then embedded in epoxy resin and cured at 50°C for 24 hours. The resin blocks were sectioned transversely through the otolith primordia using a diamond-edged wafering blade, and polished on the cut surfaces (Marriott et al. 2003). Grind the otolith section with waterproof abrasive paper until clear bands can be observed under a microscope. The sections generally exhibited a regular pattern of translucent and opaque zones. Under transmitted light, count the zone of opacity from core to edge along the ventral growth axis to determine age of *Macrourus caml* (Fig. 1–2).

Each otolith section was individually estimated twice under conditions of unknown individual size and capture date. If the two readings are consistent, this reading is taken as the individual's final age. When not in consistent, a third reading is taken to determine the individual's age. All readings were made with no prior knowledge of the fish length, sex, weight, or any previous readings.

2.3 Sexual maturity

Gonads were collected from 11 samples, sexual maturity stages were determined with 6-staged maturity scale (Ren et al. 2020), as follows:

Stage I (immature): Gonads are very small, and cannot be distinguished by the naked eyes.

Stage II (immature): Gonads are small. Ovary is dully transparent and pinkish-whitish and spermary is grayish white or grayish brown.

Stage III (ripening): Gonads are enlarged. Ovary is filled with opaque, slightly white or yellowish ovum. Spermary is grayish brown or light red and sperm cannot be squeezed out.

Stage IV (ripening): Gonads are enlarged. Ovary is orange with large transparent ova and small white ova. Spermary is white and little sperm can be squeezed out.

Stage V (ripe): Gonads are considerably enlarged. Ripe ova are visible and large and transparent. Spermary is milky white and filled with sperm.

Stage VI (spent): Gonads are shortened, walls loose, flabby, empty, dark red with traces of ova or sperm.

2.4 Stomach content analysis

2.4.1 Morphological identification of stomach contents

Stomach and intestine samples of *Macrourus caml* cryopreserved at -80°C were taken out and made thawed at 4°C, then dissected under aseptic conditions. Stomach and intestine contents were rinsed with water on a 500μm steel sieve and recognizable prey items were identified under a stereoscopic microscope to the lowest taxon possible. As the prey was digested completely, only qualitative analysis (type identification) was carried out, and quantitative analysis such as counting and weighing was not carried out.

2.4.2 Molecular identification of stomach contents

Frozen stomach contents were taken from different locations of the stomach under aseptic conditions after thawing the fish at 4°C. Samples were stirred and put into 2ml centrifuge tubes, and then put into prepared ice boxes to ensure that the samples were at low temperature and not easy to split and deteriorate. After the tissue was broken by tissue crusher (70Hz, 600s), DNA was extracted with MasterPure™ Complete DNA and RNA Purification Kit (Cat. No.: MC85200; Lucigen, USA). PCR targeting at the 18S rDNA V8-V9 region, was then performed with Q5 Hot Start High-Fidelity 2X Master Mix kit (Cat. No.: M0494S; NEB, USA) (Table 1–2). Amplicon sequencing was performed on an Illumina Novaseq6000 sequencer, using 2×250 bp paired-end sequencing in accordance with the manufacturer's instructions, at Novogene (Beijing, China).

We investigate the microbial diversity at the genus level. In order to identify the prey items of *Macrourus caml*, after adapter/index sequences were trimmed from the obtained raw reads using fastp v0.23.1, reads with low quality and short-read length were discarded using QIIME2 software ver. 2021.2, and then the DADA2 in QIIME2 was used to get the feature tables and sequences. The obtained paired-end contigs were clustered at 99.6% identity and assigned operational taxonomic units (OTUs) using QIIME2 software. Species annotation is based on the Silva database (release 138). The specie and genus were assigned for OTUs with more than 98% and 90–98% sequence identity respectively. The OTUs classified as *Macrourus caml* were removed in the further analysis.

Table 1
PCR primers used in gene cloning and expression

Primer names	Primer sequences(5'-3')
18SV8V9F	ATAACAGGTCTGTGATGCCCT
18SV8V9R	CCTTCYGCAGGTTCACCTAC

Table 2
The reaction system of PCR amplification

Reagent name	Volume/μL
Q5 Hot Start High-Fidelity 2X Master Mix	25
Forward primer	1
Reverse primer	1
DNA	1
Nuclease free water	22
Total volume	50

3 Results

3.1 Size and age

Within the samples of *Macrourus caml*, 6 individuals were male and 5 individuals were female. The wet weight of *Macrourus caml* ranged from 220g to 900g. The overall mean wet weight of this species was 520.91g. The standard length of *Macrourus caml* ranged from 348mm to 585mm, and the overall mean standard length was 463.27mm (Table 3). The ages value for *Macrourus caml* ranged from 9 to 19 years (Table 4).

Table 3
Biological measurements of *Macrourus caml*. M and F respectively refer to male and female individuals.

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Gender	M	F	M	F	M	F	F	M	M	F	M
Maturity				3		4	4	3	3	5	
Weight/g	450	480	270	630	220	620	670	500	520	900	470
Wet weight/g	370	400		580	200	510	550	430	427	750	420
Standard Length/mm	455	485	385	490	348	485	470	460	473	585	460
Total length/mm	460	490	390	500	350	490	475	465	478	590	470
Anal length/mm	150	160	140	180	125	175	175	165	170	190	160

Table 4
Results of the age identification of *Macrourus caml*

Sample	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Age	13	13	10		9	15	12	12	10	19	10
Gender	M	F	M	F	M	F	F	M	M	F	M

3.2 Feeding habits

3.2.1 Morphological identification of stomach contents

According to the morphological analysis, the main prey of *Macrourus caml* was Malacostraca and Polychaeta. Algae, Cephalopods, fish and some benthic organisms were also found in stomachs and intestines (Table 5). As the prey was digested completely in this study, it was difficult to identify to the species level and obtain the weight and proportion of each sample. The index of relative importance (IRI) in the traditional morphological analysis of stomach contents can't be calculated.

Table 5

Prey items of *Macrourus caml* determined by morphological analyses; '**+**' represents occurrence of the prey types.

Prey types	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Malacostraca		+	+	+	+	+	+	+	+	+	+
Algae				+		+	+				
Cephalopoda				+						+	
Fish							+	+			
Polychaeta		+	+	+	+			+	+		+
Other benthos									+		

3.2.2 Molecular analysis of stomach contents

According to molecular analysis, a total of 5 types of prey were identified (Table 6). *Macrourus caml* mainly fed on Malacostraca, Sagittoidea, Cnidaria and Algea. The proportion of their contigs were 57.8%, 27.2%, 9.1% and 5.7% respectively. Among them, Malacostraca accounted for the highest proportion, more than 50% (Table 7).

Table 6

List of prey items of *Macrourus caml* as determined by NGS analysis

Prey types		Contigs											
		P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	To
Malacostraca	All Malacostraca	9376	3904	1480	3428	13967	25204	9185	754	148689	23992	60040	29
	Peracarida	7	0	292	0	9	0	12	0	1502	5	998	28
	Eucarida	9369	3904	1188	3428	13958	25204	9173	754	147187	23987	58934	29
	Malacostraca(unidentified)	0	0	0	0	0	0	0	0	0	0	108	10
Algea	All Algea	5065	6469	1982	0	18	84	7088	3384	1069	3305	889	29
	Thalassiosira	0	7	907	0	0	29	0	1207	0	1124	62	33
	Chaetoceros	0	0	0	0	0	22	0	0	0	42	0	64
	Actinocyclus curvatulus	0	0	0	0	0	0	1237	0	0	0	0	12
	Navicula	5064	0	0	0	10	0	49	0	0	883	0	60
	Cryptomonadales	0	0	0	0	0	15	0	941	0	965	0	19
	Phaeocystis	0	0	0	0	0	0	0	10	0	0	0	10
	Algea(unidentified)	1	6462	1075	0	8	18	5802	1226	1069	291	827	16
Polychaeta	*Rhynchospio*	0	0	0	0	0	0	0	0	0	1324	0	13
Sagittoidea	All Phragmophora	57001	79442	122	411	105	3383	0	0	35	54	393	14
	Eukrohnia fowleri	21	30195	39	14	0	3302	0	0	0	54	0	33
	Phragmophora(unidentified)	56980	49247	83	397	105	81	0	0	35	0	393	10
Cnidaria	All Cnidaria	13302	16	3714	9	27190	22	32	1541	117	953	286	47
	Coronatae	0	0	0	0	0	0	0	0	0	2	0	2
	Siphonophorae	10492	9	3714	9	0	0	15	1535	4	0	286	16
	Cnidaria(unidentified)	2810	7	0	0	27190	22	17	6	113	951	0	31
Total		84744	89831	7298	3848	41280	28693	16305	5679	149910	29628	61608	51

Table 7
List and proportions of prey items of *Macrourus caml* as determined by NGS analysis

Prey types		**Contigs**	**%**
Malacostraca	All Malacostraca	299999	57.8202
	Peracarida	2825	0.5445
	Eucarida	297066	57.2549
	Malacostraca(unidentified)	108	0.0208
Algea	All Algea	29397	5.6658
	Thalassiosira	3336	0.6430
	Chaetoceros	64	0.0123
	Actinocyclus curvatulus	1237	0.2384
	Navicula	6006	1.1576
	Cryptomonadales	1921	0.3702
	Phaeocystis	10	0.0019
	Algea(unidentified)	16823	3.2424
Polychaeta	*Rhynchospio*	1324	0.2552
Sagittoidea	All Phragmophora	140946	27.1652
	Eukrohnia fowleri	33625	6.4807
	Phragmophora(unidentified)	107321	20.6845
Cnidaria	All Cnidaria	47182	9.0936
	Coronatae	2	0.0004
	Siphonophorae	16064	3.0961
	Cnidaria(unidentified)	31116	5.9971
Total		518848	

3.3 Feeding habits change with gender

The average of contigs of male and female individuals was calculated. The diet of both were similar, mainly feeding on Malacostraca, Algae and Sagittoidea (Fig. 3).

3.4 Prey composition changes with age

With aging, the preference for food decreased and the feeding range was larger (Fig. 4–5). Relying on the results of gonad maturity and age estimation in this study, age 12 was identified as the mature age of *Macrourus caml*. Before sexual maturity, *Macrourus caml* mainly fed on Malacostraca and Cnidaria, and their food composition is simple. In Fig. 4, P5 and P3 mainly fed on Cnidaria and P9 and P11 mainly fed on Malacostraca, and their prey were simple. After sexual maturity, *Macrourus caml* mainly fed on Algae, Malacostraca and Sagittoidea, and their food composition became more complex (Fig. 5). Take P7, P8, P1 and P10 for instance, their prey were more diversified and they did not prefer for any one prey (Fig. 4).

4 Discussion

This study provides new information to the age and feeding habits of *Macrourus caml* in the Cosmonauts Sea. These data are useful to understand the growth and nutritional status of the *Macrourus caml*, and of great value for the protection and development of fishery resources of the Southern Ocean ecosystem.

Relying on the results of gonad maturity and age estimation in this study, age 12 was identified as the mature age of *Macrourus caml.* In the Table 3 and Table 4, both P7 and P8 aged 12 were mature, while the gonadal maturity data of the three samples aged 10 were lacking, so age 12 was considered as the sexual maturity age of *Macrourus caml.*

In our study, *Macrourus caml* mainly fed on benthic animals and small plankton in summer. Pinkerton et al. found that grenadiers (including *Macrourus caml and Macrourus whitsoni*) in the Ross Sea of the Southern Ocean mainly fed on Amphipoda (IRI was 42.8), Euphausiids (IRI: 23.2), Copepoda (IRI: 12.7), Fishes (IRI: 6.6), Mysidacea (IRI: 5.9), Isopoda (IRI: 3.3), Polychaeta (IRI: 1.6) etc. Compared with previous studies, the feeding habits of *Macrourus caml* have not changed significantly, and they still mainly fed on Crustaceans. However, no Amphipods were identified in the stomach contents of samples collected from the Cosmonauts Sea in our study. This may be because *Macrourus spp.* is a general term which can't represent a particular species, the species and distribution of biological resources in the Cosmonauts Sea are different from those in Rose Sea, the sample size in this study is small and the molecular identification used in this study has some defects, resulting in some prey types could not be identified.

We found that the main prey consumed by the two sexes were the same, but there were differences in the proportions of each prey type consumed. This suggests that both sexes have a marked preference for the specific prey, which is based on energetic demands, prey's vulnerability to capture and time spent to find and handle prey. This point has been reported in other species of the genus *Sphyrna lewini* (Torres-Rojas et al. 2010), *Lutjanus guttatus* (Valle-Lopez et al. 2021), *Lutjanus peru* (Perez-Rojo et al. 2022). For example, Valle-Lopez et al. found that although the main prey consumed by two sexes of *Lutjanus guttatus* were the same, females consumed a greater proportion of *Sardinops sagax*, whereas males ate a greater proportion of *Harengula thrissina*. In addition, with aging, *Macrourus caml* feeding bias decreased and range became wider. During different sexual maturity stages, the main prey consumed were different. Before maturation, *Macrourus caml* mainly fed on Malacostraca and Cnidaria, and their food composition is simple. After maturation, *Macrourus caml* mainly fed on Algae, Malacostraca and Sagittoidea, and their food composition became more complex. This may be because the predation ability and the nutrition needed for growth and development are different at different stages. Garcia-Rosales et al. also found the volume of stomach content in females varied significantly through the three stages of the reproductive cycle, vitellogenesis, embryonic development, and post-reproductive period (2019), and this result provide evidence to our finding. In the Fig. 5, P2 and P6 had obvious preference for one prey, which seems to conflict with the conclusion above. Limited number of samples may result in the accidental phenomenon. And each individual grows up in different environment so the occurrence of this phenomenon is reasonable, but it didn't affect the overall trend.

Here, two methods were used to identify stomach contents. Morphological analysis is popular because it is simple and accurate, but it also has potential limitations such as time-consuming and difficult in morphological classification (Hashimoto 1974; Mata-Sotres et al. 2016; Xi et al. 2015). As the prey was digested completely, only relying on the morphological classification we can hardly attain relatively accurate result. So, the molecular identification is also taken to carry on the analysis. Molecular method is not affected by the age, sex, growth stage and digestion extent of experimental subjects, which can make up for the shortcomings of traditional morphological identification (Xi et al. 2015). Even with the combination of the two methods, we found that some prey species couldn't be identified and quantified further. Low quantity of stomach contents, defects of 18s RNA amplicon sequencing and small sample size may lead to this. Although the results are not perfect, they are enough to get the conclusion above and useful to carry on further studies on grenadiers and other fishery resources in Southern Ocean.

Declarations

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Author contributions

SX analyzed the data and wrote the manuscript. PS designed the study. CZ, SX and XX conducted the experiments. JL, SM and WZ collected the samples. YT helped revise the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest and that all applicable institutional, national, or international guidelines for the use and care of animals were strictly followed in the present study

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Figures

Figure 1

Sagittal otolith section of a *Macrourus caml*aged 19 years old

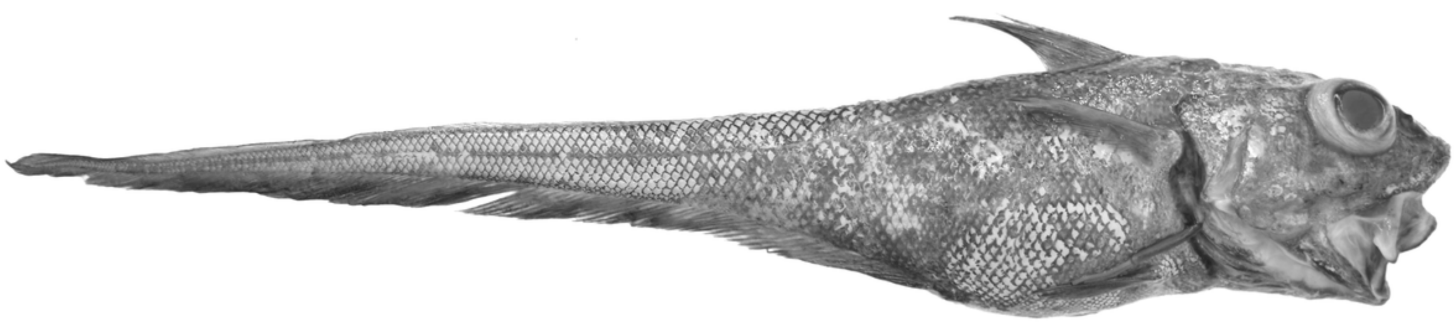

Figure 2

Sample image of *Macrourus caml*

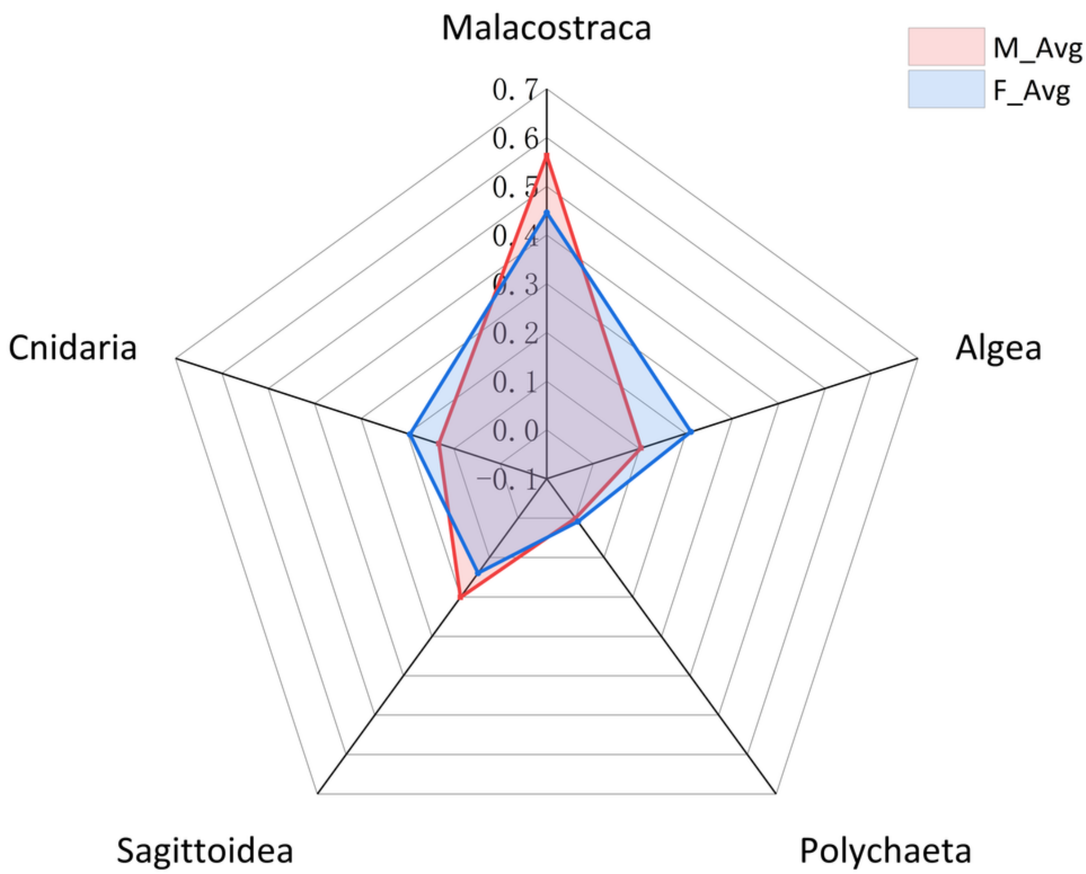

Figure 3

Distribution of prey species of male and female samples. M_Avg and F_Avg represent the mean proportion of contigs of prey of male and female *Macrourus caml*.

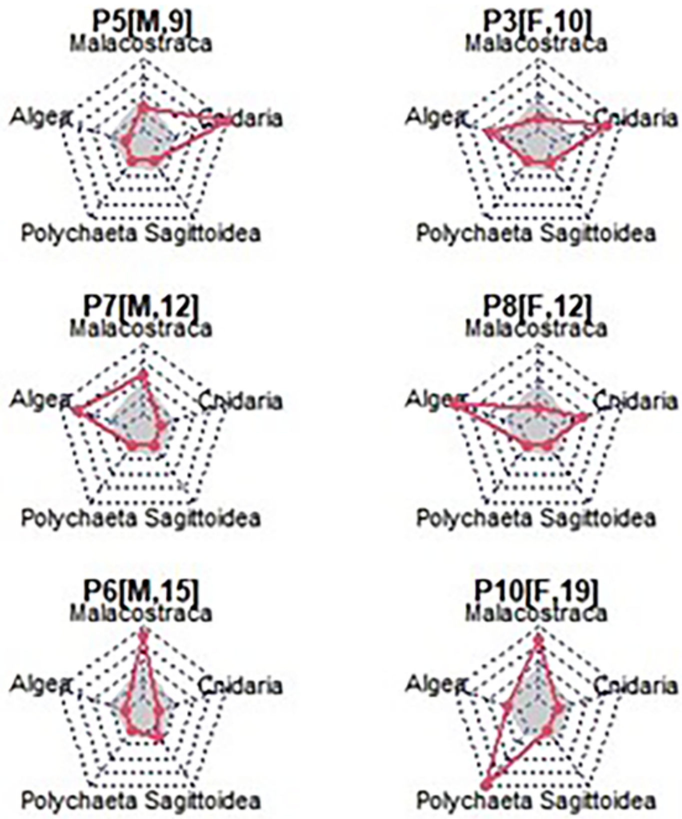

Figure 4

Distribution of the values of prey species of each sample. In P [a, b], P refers to sample name ((M: male; F: female); a refers to gender; b refers to age. P5, P3, P9, P11 are immature individuals and P7, P8, P1, P2, P6, P10 are mature individuals.

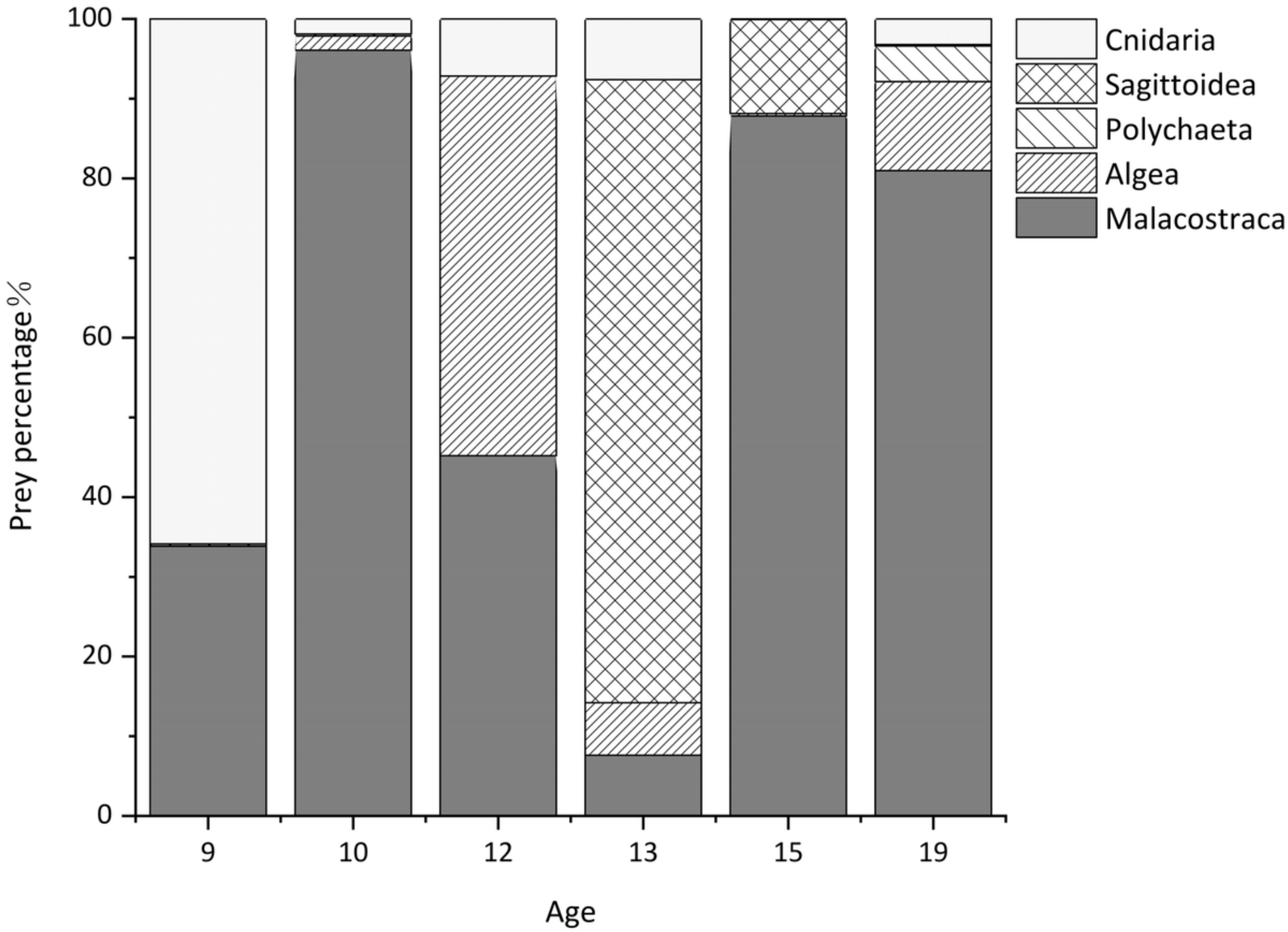

Figure 5

Changes of prey proportion with age of *Macrourus caml* in Cosmonauts Sea