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RESEARCH

Diet composition of the Eurasian oystercatcher (*Haematopus ostralegus osculans*) during breeding season on Yubu Island through high-throughput DNA sequencing

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Background: Prey availability plays a critical role in conserving endangered bird species, influencing their habitat use, reproductive success, and survival. Eurasian Oystercatchers (*Haematopus ostralegus*) are known to feed on diverse intertidal prey such as oysters, mussels, polychaetes, and adjust their diet in response to prey availability and habitat composition. On Yubu Island, previous studies reported shifts in oystercatcher foraging behavior and diet during the breeding season, but direct observation has limitations in accurately identifying prey at the taxonomic level. Recently, fecal DNA metabarcoding has emerged as a powerful approach for precise dietary analysis. This study aimed to identify the prey composition of Eurasian Oystercatchers on Yubu Island using this molecular method.

Results: During the breeding seasons of 2015 and 2017, a total of 207 fecal samples were collected near breeding sites. The prey items detected in the feces belonged to 14 orders and 31 genera, including decapods, bivalves, gobies, sipunculans, and isopods. Decapods were the most frequently detected, particularly species from the family Macrophthalmidae. Other major dietary components included bivalves and sipuncula. Comparison with field surveys confirmed decapods such as *Macrophthalmus abbreviatus*, *Macrophthalmus japonicus*, and *Neotrypaea japonica* as the main prey species.

Conclusions: Our results highlight that fecal DNA metabarcoding provides more accurate and detailed species-level prey identification than direct observation. Quantifying prey proportions also offered precise insights into oystercatcher dietary preferences. The high prevalence of decapods suggests that exposed mudflats were the primary foraging habitats, reinforcing previous findings that oystercatchers preferentially forage near breeding sites to maximize reproductive success. This study demonstrates the effectiveness of molecular dietary analysis for understanding prey selection and foraging strategies in shorebirds. These findings contribute to a better understanding of the breeding-season foraging ecology of the Eurasian Oystercatcher and support conservation efforts through improved knowledge of prey availability and habitat use.

Keywords: *Haematopus ostralegus osculans*, high-throughput DNA sequencing, prey items, The Eurasian oystercatchers, Yubu Island

Introduction

Prey information is crucial for conserving endangered bird species because it allows to understand the critical habitat requirements of the bird, identify potential threats to their food source, and develop effective management strategies to ensure a stable prey population, ultimately supporting the survival of the endangered species itself (Lortie et al. 2023; Tassie et al. 2011). In recent, DNA metabarcoding techniques have provided new approaches for studying animal diets, allowing the use of DNA metabarcoding to identify species composition in complex environmental samples, like animal feces, with high taxonomic accuracy and detail (Elbrecht et al. 2017; Huang et al. 2021; Taberlet et al. 2012). Fecal DNA metabarcoding has become a widely used noninvasive, accurate, and time- and cost-efficient method for diet analysis, allowing for more precise identification of dietary sources (Ando et al. 2020). In particular, within avian ecological research, fecal DNA metabarcoding has been widely applied for various purposes, including the detection of a broader range of dietary taxa compared to direct observation, understanding the flexible feeding habits of endangered bird species, and estimating the relative biomass of diets (Arazmi et al. 2025; Tang et al. 2022; Verkuil et al. 2022).

The Eurasian oystercatchers inhabiting Yubu Island belong to the East Asian subspecies (*Haematopus ostralegus osculans*), which breeds in regions such as Kamchatka, Russia, the southwestern coas of Korea, and the northeastern coastal areas of China. They are known to overwinter not only in Korea and China but also in Taiwan and Japan (Lee et al. 2018; Melville et al. 2014). It is categorized in the IUCN Red List as 'Near Threatened, NT' based on population size (< 10,000 mature individuals), and the rapid loss of habitat in the main wintering areas (BirdLife International 2019). In Korea, they are designated as Endangered Wildlife Class II and Natural Monument No. 326, receiving legal protection. Notably, the Yubu Island area in Korea is a critical habitat for the population, with over 50% of the entire East Asian population overwintering there (Melville et al. 2014), and around 300 individuals consistently staying and breeding during the breeding season, making it vital for population sustainability.

Oystercatchers are known to exploit a wide range of intertidal prey, including oysters, mussels, and polychaetes, and their diet can shift in response to prey availability and habitat composition (Gliesch et al. 2023; Schwemmer and Garthe 2011; Tjørve and Tjørve 2010). Although they are generally considered to prefer foraging along tidal boundaries with a strong preference for bivalves (DeVlas et al. 1996; Schwemmer and Garthe 2011), prey and foraging site selection can vary substantially depending on life history stage and the activity patterns of potential food sources, which are themselves strongly influenced by the tidal cycle (DeVlas et al. 1996; Hulsman et al. 1996; Low et al. 2008; Rosa et al. 2007; Schwemmer and Garthe 2011). In particular, both the stage of tidal exposure and the drainage characteristics of intertidal habitats alter the activity and visibility of key prey species, thereby shaping oystercatchers' prey choice and horizontal foraging movements across the mudflat. During the breeding season, these ecological constraints become even more critical, as the distance between nests and feeding sites (i.e., territory quality) directly affects commuting costs and chick provisioning rates, ultimately influencing reproductive success. A previous study conducted on Yubu Island also reported changes in the prey and foraging site selection of breeding Eurasian oystercatchers. Thus, providing more accurate information on prey sources is important for understanding the behavior and breeding strategies of oystercatchers.

In this study, we aimed to identify the prey consumed by Eurasian oystercatchers during the breeding season, using high-throughput DNA sequencing techniques. Additionally, we attempted to compare the consumed prey with potential prey organisms around the breeding sites to provide insights into the available food resources during the breeding period in Yubu island.

Materials and Methods

Study area

This study was conducted focusing on three uninhabited islands located approximately 2 km north of Yubu Island in Seocheon, Chungcheongnam-do (Fig. 1). Each island serves as a key breeding site for the Eurasian oystercatcher (*H. o. osculans*) that inhabits the Yubu Island area during the breeding season. In this study, each uninhabited island was classified as Site A, B, and C, accordingly (Fig. 1). Each site is located near an intertidal zone, where expansive tidal flats are exposed during low tide. The area around the islands is formed by the accumulation of sediments such as sand and mud along the coast due to sea currents. Sandy areas are particularly extensive between Site A and C, as well as in the northeastern direction of Site B. Site A, also known as Yujae-do, is a long rocky island with an area of approximately 3,000 m^2 (130 m in length and 36 m in width). The northern slope is steep, whereas the southern slope has a gentle gradient. Site B is a circular type sandy island with an area of approximately 3,600 m^2 (70 m in length and 50 m in width). The central part of the island is predominantly covered by plant communities such as *Euonymus japonicus*, *Rosa rugosa*, and *Phragmites communis*. During the breeding season, most of the breeding nests are formed in the gently sloping areas around the plant communities, with some nests also being formed directly on the sand. Site C, also known as Muk-do, a rocky island with an area of approximately 9,900 m^2 (180 m in length and 75 m in width), is the largest of the three breeding sites. The central part of the island is densely covered with plant communities of *Machilus thunbergii* and dwarf bamboo (*Sasa borealis*), and nests are commonly formed around the exposed rocky edges of the island. A total of 74 nests were recorded in the Yubu Island area in 2015 and 76 nests in 2017. In 2017, the numbers of nests at each breeding site were 22 at Site A, 37 at Site B, and 17 at Site C.

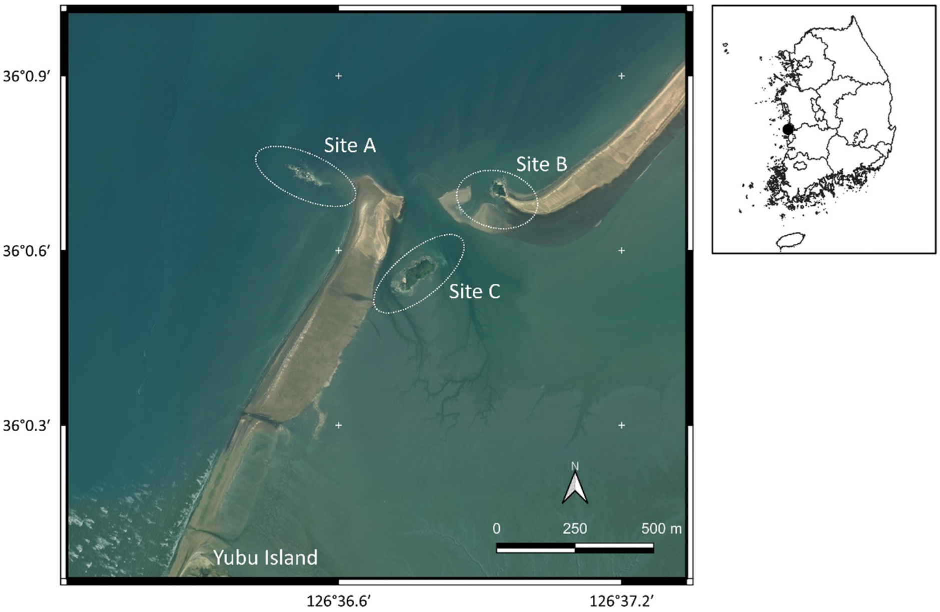

Fig. 1 Location of the study sites (A–C) around Yubu Island, Seocheon, South Korea. The dotted lines indicate the specific areas where fecal samples were collected.

Collection of fecal samples

The collection of fecal samples for detecting the food sources was conducted on foraging areas around breeding sites confirmed through visual observation during the breeding seasons of 2015 and 2017. The sampling areas were tidal flats surrounding each site, which are submerged during high tide and exposed during low tide due to tidal fluctuations. Fecal sampling was typically conducted after high tide by approaching the exposed tidal flat areas, where feeding behavior of the Eurasian oystercatcher observed, around the sites and newly identified feces were collected to minimize sample degradation and contamination. The collected fecal samples were categorized according to the breeding individuals from the nearest island to compare differences in diet based on breeding sites. Fecal samples, where it was difficult to distinguish the breeding sites, were excluded from the analysis. The collected fecal samples were individually placed in either plastic bags or 2 mL Eppendorf tubes containing 100% alcohol to minimize cross-contamination between samples, and were stored frozen at –20°C until transport to the laboratory. After transportation, the samples were immediately stored in an ultra-low temperature freezer (–80°C) until DNA extraction.

Survey of potential preys

A survey of potential prey items was carried out around the study sites in collaboration with the National Marine Biodiversity Institute of Korea to compare the identification results from fecal DNA metabarcoding with the actual occurrence of prey species. The potential prey survey was conducted four times from March to June 2018. The first three surveys were spaced approximately one month apart, while the final survey had a two-week interval. Researchers performed the surveys on foot within a walkable range around low tide at three breeding sites and their surrounding mudflats on Yubu Island to compare the detected prey of the Eurasian oystercatcher with the organisms inhabiting the area. The potential prey survey focused on large benthic invertebrates that are potential prey for the Eurasian oystercatcher. Sampling was conducted qualitatively using hands, shovels, tweezers, and sieves (mesh size 1 mm). The collected samples were preliminarily sorted by taxon in the field and transported to the laboratory while maintaining a low temperature. Some samples, for which maintaining morphological integrity was difficult, were fixed in 95% ethanol in the field. The collected samples were further identified in detail in the laboratory using microscopes. The scientific and korean names of potential prey for marine organisms were based on Marine Bio-Resource Information System of the National Marine Biodiversity Institute of Korea (https://www.mbris.kr/) and the World Register of Marine Species (WoRMS, https://www.marinespecies.org/).

Library construction of prey items and data analyses

The fecal samples stored in plastic bags were transferred to 2 mL Eppendorf tubes for DNA extraction. The 100% ethanol in the tubes was removed by centrifuging for 2 minutes to concentrate the fecal samples. Each fecal sample was homogenized by placing a 5 mm stainless steel bead in the tube and grinding it for 2 minutes at 30 Hz using a tissue grinder (MM-400; Retsch, Haan, Germany). DNA extraction was performed on fecal samples of less than 200 mg using QIAamp DNA Fast DNA Stool Kit (QIAGEN, Hilden, Germany) followed manufacturer's manual. We amplified the mitochondrial 16S ribosomal RNA subunit (rDNA) genes of potential preys using universal primer set (16S1F-degenerate: 5′-GAC GAK AAG

ACC CTA -3′ and 16S2R-degenerate: 5′- CGC TGT TAT CCC TAD RGT AAC T- 3′). The libraries for dietary analysis were prepared using the fusion primers modified in 5′ by the addition of individual-specific 7-bp MIDs (Multiplex IDentifiers) and adaptors required for the emPCR and the Ion PGM sequencing. All PCR amplifications were performed in 20 μL volumes using a Intron FastMix/Frenche™ Premix (iNtRON Biotechnology, Seoul, South Korea). Reaction mixture contained master mix, 0.25 μm of each primer, and 10 μL template DNA. PCR conditions were as follows: an initial denaturation at 94°C for 10 minutes, 40 cycles of denaturation at 94°C for 30 seconds, annealing at 54°C for 30 seconds, and elongation at 72°C for 45 seconds, and a final extension step at 72°C for 5 minutes. PCR products were checked on 1.5% or 2% agarose gels. After the reactions, PCR products were purified using the MEGAquick-spin™ Total Fragment DNA Purification Kit (iNtRON Biotechnology). The concentration of each amplification product was measured by PicoGreen based assay with microplate reader using Quant-iT™ PicoGreen™ dsDNA Reagent and Kit (Invitrogen, Carlsbad, CA, USA). After PCR pooling, libraries were quantified using the High Sensitivity DNA Chip kit on the Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). Emulsion PCRs were conducted using the Ion PGM Hi-Q OT2 kit (Life Technology, Carlsbad, CA, USA) on Ion OneTouch2 platform according to the manufacturer's instructions. The amplicon libraries were sequenced on an Ion PGM system using Ion PGM Hi-Q Sequencing kit (Life Technology) and Ion 316 Chip Kit v2 BC (Ion Torrent, Life Technologies).

Data analysis was conducted using bioinformatics program, Geneious Prime, based on "Amplicon Metagenomics" manual provided by the manufacturer. Briefly, raw sequences were trimmed based on primer sequences and filtered less than 80 bp. Reads were clustered into operational taxonomic units (OTU) by clustering the reads by similarity using a De Novo Assembler. Then, the sequences were assigned with a minimum overlap identity of no less than 97%. The taxonomy database was constructed by performing BLAST searches of individual OTUs against the NCBI nucleotide database. Sequences that were unclassified or considered less informative were filtered out. After creating a sequence classifier database from BLAST hits, the entire amplicon dataset was classified using the Sequence Classifier in Geneious Prime, based on the generated taxonomy database. Among the identified OTUs, those that were confirmed to be organisms such as Diatoms, Cryptophyta, and Euglena, which are difficult to consider as prey items, were excluded from further analysis. Additionally, to minimize the detection of prey items due to contamination, prey items detected at low proportions of less than 3% in each fecal sample were also excluded. The remaining prey items were rescaled so that the sum across all food items equaled 100%.

The proportion of prey items were calculated as both relative read abundance (RRA) and frequency of occurrence (FOO). FOO is expressed as a percent (%FOO). All mathematical expressions are as follows (Deagle et al. 2019):

$$\%\mathrm{FOO_i} = \frac{1}{\mathrm{S}}\sum_{k=1}^{S} l_{i,k} \times 100\%$$

$$\mathrm{RRA_i} = \frac{1}{\mathrm{S}}\sum_{k=1}^{S} \frac{n_{i,k}}{\sum_{i=1}^{T} n_{j,k}} \times 100\%$$

where T is the number of prey items (taxa), S is the number of samples, I is an indicator function such that $I_{i,k} = 1$ if prey item i is present in sample k, and 0 if not, and $n_{i,k}$ is the number of sequences of prey item i in sample k.

Species diversity was defined as the number of OTUs identified in each sample. Species diversity index were calculated based on the RRA of prey items. Shannon-Wiender diversity index and Simpson's Index of Diversity were calculated as follows:

$$D = 1 - \Sigma p_i^2$$

$$H' = -\Sigma\,(p_i) \times \log_e p_i$$

where p_i is the proportion of prey item i in the diet. Kruskal–Wallis rank sum test was used to compare the group means statistically. Significant differences on diet composition among species were tested using the permutational multivariate analysis of variance (PERMANOVA) based on RRA of prey items on each fecal samples at the genus level (Anderson 2001). All statistical analyses were performed using the R package (version 4.1.2).

Results

A total of 207 fecal samples were collected during the survey period (Site A: 74, Site B: 108, Site C: 25). After excluding samples predominantly amplified by non-prey sources, 55 samples (Site A: 18, Site B: 28, Site C: 9) were successfully amplified with the 16S universal primer, yielding an overall amplification success rate of approximately 26.6% of all collected samples. A total of 2,121,773 reads (mean = 38,578 ± 29,650) were obtained from 55 fecal samples.

The prey items detected in the feces were taxonomically classified into 14 different orders, with decapods, bivalves, gobies, sipunculans, and isopods being the predominant groups (Fig. 2A, Table 1, Table S1). Decapods represented the most dominant prey group across all sites, with average proportions of 62.9%, 59.64%, and 39.43% at Sites A, B, and

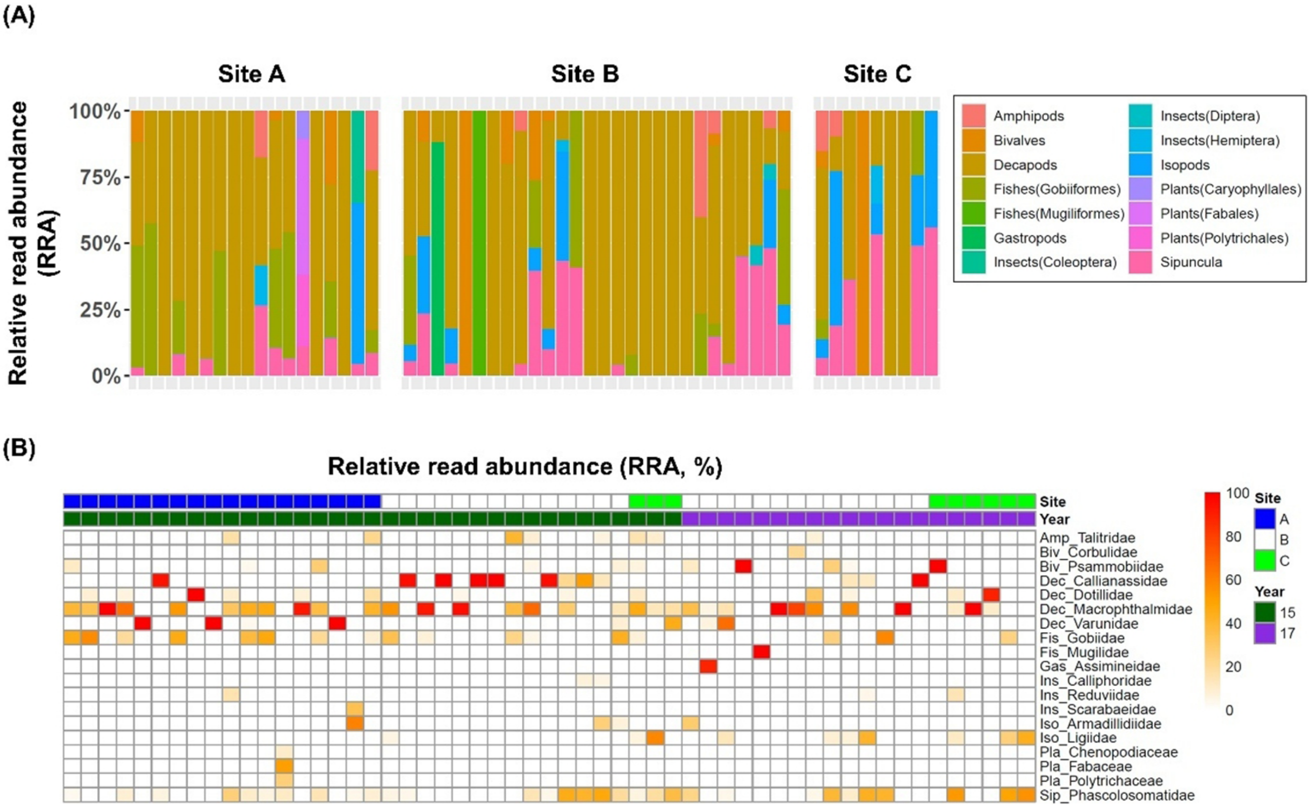

Fig. 2 Prey items detected in the feces of the Eurasian oystercatchers (*H. o. osculans*), using the high-throughput DNA sequencing approach. Relative read abundance (RRA) of prey items consumed by the Eurasian oystercatchers from each fecal sample at the order level (A) and at family level (B).

C, respectively. While the proportion of each prey item at the order level showed similar trends across all three sites, fecal samples collected from Site C exhibited relatively higher proportions of bivalves (11.78%), sipuncula (24.5%), and isopods (16.35%) compared to the other two sites. At the family level, Macrophthalmidae accounted for the highest proportion among decapods, followed by Callianassidae, Dotillidae, and Varunidae (Fig. 2B, Table 1). Psammobiidae and Corbulidae were predominant among bivalves, while Phascolosomatidae dominated among sipunculans. Among isopods, Ligiidae was the most prevalent, with Armadillidiidae also present. Other detected prey items, apart from these, generally showed detection rates of less than 5% on average.

The prey preference based on %FOO showed results similar to those based on RRA (Fig. 3, Table 1). Prey items belonging to decapods were detected with high frequency, and bivalves (Psammobiidae) and amphipods (Talitridae) also showed high detection frequencies. For isopods, the detection frequency in fecal samples collected from around site C was above 0.5, which was higher compared to the other two sites. Unlike the RRA results, gobies (Gobiidae) were detected with high frequency in FOO, indicating a discrepancy.

The species diversity indices for the three sites were as follows: site A (*H′*: 1.36, *D*: 0.689), site B (*H′*: 1.25, *D*: 0.631), and site C (*H′*: 1.32, *D*: 0.639). There was no statistically significant difference in the diversity indices among the three sites (Kruskal–Wallis rank sum test, $p = 0.665$), but, PERMANOVA analysis detected significant differences in prey composition among three sites (Pseudo-$F_{(2,\,52)} = 1.997$, $p < 0.05$).

A total of 34 potential prey species, belonging to 27 genera and 15 orders, were identified from the potential prey survey conducted around the habitat (Table 2). Comparison of potential prey sources with dietary items detected in feces indicates that *Neotrypaea japonica*, *Scopimera globosa*, *Macrophthalmus abbreviatus*, *Macrophthalmus japonicus*, and *Helice tridens* were likely preyed upon among the decapods in the habitat. In the case of bivalves, *Nuttallia japonica* is presumed to have been consumed as prey. Although six species of polychaetes were identified in the tidal flats, they were not detected in the fecal analysis. Conversely, although gastropods and isopods were detected in the fecal samples, no species from the corresponding genera were recorded in the potential prey survey.

Discussion

This study provides novel insights into the diet of Eurasian oystercatchers (*H. o. osculans*) breeding on Yubu Is-

Table 1 Prey items detected in the feces of the Eurasian oystercatchers (*H. o. osculans*)

No.	Group	Taxonomic level			RRA						%FOO		
					Site A (*n* = 18)		Site B (*n* = 28)		Site C (*n* = 9)		Site A (*n* = 18)	Site B (*n* = 28)	Site C (*n* = 9)
		Order	Family	Genus	Average	SD	Average	SD	Average	SD			
1	Amphipods	Amphipoda	Talitridae	*Talorchestia*	1.3	(3.7)	1.1	(4.5)	1.6	(3.2)	11.1	10.7	22.2
2				unc._Talitridae	1.0	(3)	1.2	(3.5)	1.2	(2.5)	11.1	14.3	22.2
3	Bivalves	Bivalvia	Corbulidae	*Corbula*	0.0	(0)	0.4	(2.4)	0.0	(0)	0.0	3.6	0.0
4				unc._Corbulidae	0.0	(0)	0.3	(1.4)	0.0	(0)	0.0	3.6	0.0
5			Psammobiidae	*Nuttallia*	2.4	(7)	4.6	(14.6)	11.3	(31.6)	16.7	21.4	22.2
6				unc._Psammobiidae	0.0	(0)	0.9	(5)	0.5	(1.5)	0.0	3.6	11.1
7	Decapods	Decapoda	Callianassidae	*Nihonotrypaea*	5.2	(22.1)	25.1	(40.5)	0.0	(0)	5.6	39.3	0.0
8			Dotillidae	*Ilyoplax*	0.2	(0.9)	0.0	(0)	0.0	(0)	5.6	0.0	0.0
9				*Scopimera*	5.8	(20.7)	1.1	(4.4)	1.2	(3.5)	11.1	7.1	11.1
10				unc._Dotillidae	2.3	(4.2)	0.6	(1.8)	11.1	(29.5)	27.8	10.7	22.2
11			Macrophthalmidae	*Macrophthalmus*	32.4	(32.1)	26.6	(33.1)	20.9	(29.5)	61.1	53.6	66.7
12				unc._Macrophthalmidae	0.0	(0)	3.3	(5.9)	1.2	(3.6)	0.0	32.1	11.1
13			Varunidae	*Helicana*	0.0	(0)	0.0	(0)	5.1	(15.3)	0.0	0.0	11.1
14				*Hemigrapsus*	16.9	(38.3)	2.3	(9.7)	0.0	(0)	22.2	10.7	0.0
15				unc._Varunidae	0.0	(0)	0.6	(3)	0.0	(0)	0.0	3.6	0.0
16	Fishes	Gobiiformes	Gobiidae	*Gymnogobius*	1.4	(3.8)	0.3	(1.5)	0.0	(0)	16.7	3.6	0.0
17				unc._Gobiidae	14.4	(19.3)	6.8	(14.8)	3.5	(8.2)	44.4	25.0	22.2
18		Mugiliformes	Mugilidae	*Liza*	0.0	(0)	3.0	(16)	0.0	(0)	0.0	3.6	0.0
19				unc._Mugilidae	0.0	(0)	0.6	(2.9)	0.0	(0)	0.0	3.6	0.0
20	Gastropods	Gastropoda	Assimineidae	unc._Assimineidae	0.0	(0)	3.1	(16.6)	0.0	(0)	0.0	3.6	0.0
21	Isopods	Isopoda	Armadillidiidae	*Armadillidium*	2.8	(11.9)	1.7	(5.4)	0.0	(0)	5.6	10.7	0.0
22				unc._Armadillidiidae	0.6	(2.3)	0.5	(1.9)	0.0	(0)	5.6	7.1	0.0
23			Ligiidae	*Ligia*	0.0	(0)	2.7	(8.2)	16.4	(21.8)	0.0	17.9	55.6
24	Insects	Coleoptera	Scarabaeidae	unc._Scarabaeidae	1.9	(8.2)	0.0	(0)	0.0	(0)	5.6	0.0	0.0
25		Diptera	Calliphoridae	*Calliphora*	0.0	(0)	0.5	(1.7)	0.0	(0)	0.0	7.1	0.0
26		Hemiptera	Reduviidae	*Panstrongylus*	0.8	(3.6)	0.2	(0.9)	1.6	(4.8)	5.6	3.6	11.1
27	Plants	Caryophyllales	Chenopodiaceae	unc._Chenopodiaceae	0.6	(2.5)	0.0	(0)	0.0	(0)	5.6	0.0	0.0
28		Fabales	Fabaceae	*Cicer*	0.9	(4)	0.0	(0)	0.0	(0)	5.6	0.0	0.0
29				unc._Fabaceae	1.9	(8.1)	0.0	(0)	0.0	(0)	5.6	0.0	0.0
30		Polytrichales	Polytrichaceae	unc._Polytrichaceae	1.5	(6.3)	0.0	(0)	0.0	(0)	5.6	0.0	0.0
31	Sipuncula	Sipunculida	Phascolosomatidae	*Phascolosoma*	5.5	(7)	12.5	(17.4)	24.5	(24.3)	55.6	53.6	66.7

RRA: relative read abundance; %FOO: frequency of occurrence; SD: standard deviation.

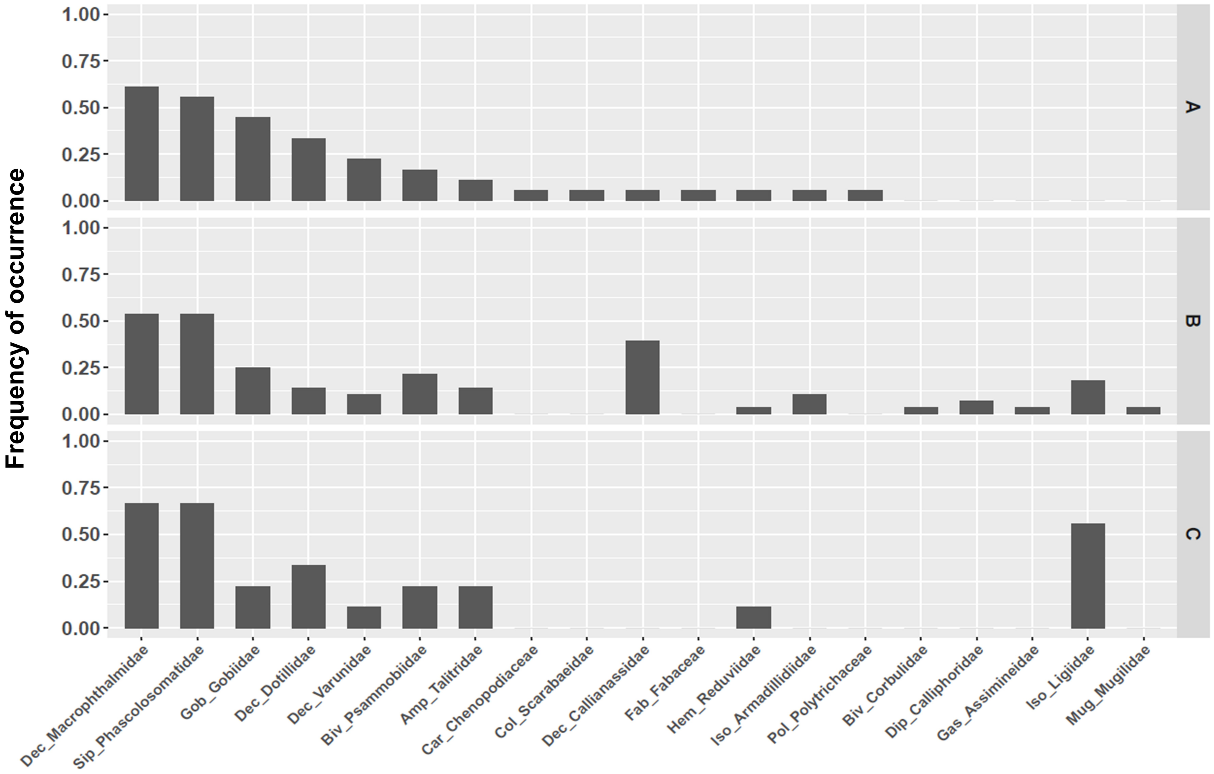

Fig. 3 Frequency of occurrence for prey items consumed by the Eurasian oystercatchers (*H. o. osculans*) at the family level.

land through high-throughput DNA metabarcoding. Previous studies on Eurasian oystercatchers inhabiting Europe and other regions have reported that they also consume a variety of marine invertebrates. Compared to previous studies relying on direct behavioral observations, our results revealed that oystercatchers breeding on Yubu Island consume a broader range of prey items, including decapods and isopods, that had not been previously reported. Notably, fecal DNA metabarcoding, as a non-invasive dietary analysis method, enabled the detection of short-term feeding patterns without interfering with breeding activities, serving as a valuable tool for understanding the foraging ecology of this species. The present study reinforces this existing knowledge with molecular evidence and contributes to a broader understanding of its ecological implications.

Fecal DNA metabarcoding-based dietary analysis can improve the accuracy and interpretative reliability of results when compared with potential prey species identified in the actual habitat. In particular, for taxa with high genetic similarity within the same taxonomic group, cross-referencing with species known to inhabit the study area enabled more precise identification of ingested prey items. Among the 34 potential prey species identified through habitat surveys, *N. japonica* and *S. globosa* (decapods), as well as *N. japonica* (bivalve), were confirmed as ingested prey items on Yubu Island based on fecal DNA metabarcoding results. This comparative approach contributes to improving the ecological interpretability of DNA-based analyses and offers a way to achieve taxonomic precision that cannot be attained through sequence similarity alone. However, the potential prey survey conducted in this study was constrained by its focus on large benthic invertebrates, resulting in limited data on smaller or cryptic taxa such as gastropods and isopods. In addition, it has limitations in comparing the variation of potential prey resources in areas adjacent to each breeding site based on tidal flat conditions even though the potential prey survey in this study was conducted around the Yubu Island breeding site. To more accurately represent the full spectrum of available prey within the habitat, future research should incorporate more comprehensive surveys. Specifically, conducting surveys in areas adjacent to each breeding site would help in the more accurate identification of species, particularly smaller invertebrate groups.

Dietary analysis using fecal DNA metabarcoding involves methodological limitations when assessing prey selectivity or preference, and these should be carefully considered in interpretation. In this study, both FOO and RRA were used in combination to estimate prey preferences. In this study, prey preferences were estimated using both %FOO and RRA, with decapods identified as a major dietary component. However, according to simulation results by Deagle et al. (2019), %FOO may overestimate rare prey items, while RRA offers more accurate dietary estimates despite moderate biases. Both metrics perform better when few prey taxa are present per sample, highlighting the need to account for potential biases in dietary metabarcoding

Table 2 Prey items identified from the potential prey survey conducted around the habitat

Phylum	Class	Order	Family	Species	Korean name
Annelida	Polychaeta	Eunicida	Oenonidae	*Arabella iricolor*	홍점갯지렁이
		Phyllodocida	Glyceridae	*Glycera nicobarica*	치로리미갑갯지렁이
				Glyceridae sp.	미갑갯지렁이류
			Nereididae	*Perinereis linea*	두토막눈썹참갯지렁이
				Nereididae sp.	참갯지렁이류
			Phyllodocidae	*Phyllodoce maculata*	네모부채발갯지렁이
Arthropoda	Thecostraca	Sessilia	Chthamalidae	*Chthamalus challengeri*	조무래기따개비
	Malacostraca	Decapoda	*Callianassidae	*Neotrypaea japonica*	쏙붙이
			Diogenidae	*Diogenes* sp.	넓적왼손집게속류
			*Dotillidae	*_Scopimera globosa_	엽낭게
			Leucosiidae	*Pyrhila pisum*	밤게
			*Macrophthalmidae	*_Macrophthalmus abbreviatus_	길게
				*_Macrophthalmus japonicus_	칠게
			Ocypodidae	*Ocypode stimpsoni*	달랑게
				Tubuca arcuata	농게
				Austruca lactea	흰발농게
			Upogebiidae	*Upogebia major*	쏙
			*Varunidae	*Helice tridens*	방게
				*_Hemigrapsus penicillatus_	풀게
Brachiopoda	Lingulata	Lingulida	Lingulidae	*Lingula anatina*	개맛
Mollusca	Bivalvia	Cardiida	*Psammobiidae	*_Nuttallia japonica_	빛조개
			Tellinidae	*Moerella hilaris*	분홍접시조개
		Ostreida	Ostreidae	*Magallana gigas*	굴
		Venerida	Veneridae	*Meretrix petechialis*	말백합
			Mactridae	*Mactra quadrangularis*	동죽
	Gastropoda	Neogastropoda	Muricidae	*Reishia clavigera*	대수리
			Nassariidae	*Nassarius festivus*	왕좁쌀무늬고둥
		Trochida	Trochidae	*Umbonium thomasi*	황해비단고둥
		Cephalaspidea	Haminoeidae	*Bullacta caurina*	민챙이
Platyhelminthes	Rhabditophora	Polycladida	Notocomplanidae	Notocomplanidae sp.	민무늬납작벌레류
Chlorophyta	Ulvophyceae	Ulvales	Ulvaceae	*Ulva compressa*	납작파래
				Ulva linza	잎파래
Ochrophyta	Phaeophyceae	Ectocarpales	Scytosiphonaceae	*Scytosiphon lomentaria*	잘록이고리매
Rhodophyta	Bangiophyceae	Bangiales	Bangiaceae	Bangiaceae sp.	김파래류

*Potential prey items belonging to the genera detected in the fecal analysis.

analyses. In addition, for prey groups such as bivalves, DNA extraction may be hindered by mucopolysaccharide secretions, and differences in digestion rates among prey taxa can bias the interpretation of prey importance based on fecal samples (Adema 2021; Chakraborty et al. 2020). Therefore, accurately assessing prey preference requires an integrative approach that incorporates ecological information, such as variation in local prey biomass and observations of foraging behavior.

Our results suggest that Eurasian oystercatchers (*H. o. osculans*) breeding on Yubu Island consumed a broader range of prey items than previously reported, highlighting the importance of decapods and isopods in addition to gastropods, bivalves and polychaetes (Lee et al. 2018, 2019). Notably, the high dietary proportion of decapods observed in this study contrasts with previous findings that identified polychaetes as the primary prey on open mudflats near breeding sites. When viewed in light of the previous studies, our results suggest that oystercatchers breeding on Yubu Island primarily consume gastropods and bivalves at the tide water line, while commonly utilizing easily accessible prey sources such as decapods and polychaetes in open mudflat areas. This indicates that despite the methodological differences between visual observation and DNA-based analysis, the oystercatchers' prey selectivity or foraging preference shows consistency in effectively utilizing available prey within specific habitat types. Previous studies on the oystercatcher (*H. ostralegus*) have indicated that individuals tend to forage closer to their nests during the breeding season. This behavior is considered a breeding strategy to maintain parental body condition while minimizing the risk of predation and conspecific disturbance to eggs and chicks (Ens et al. 1992; Schwemmer and Garthe 2011). A study on the foraging habitat use of breeding Eurasian oystercatchers (*H. o. ostralegus*) in Europe in relation to prey also reported cases of using areas near breeding sites as foraging grounds despite the relatively low biomass during the breeding season (Schwemmer et al. 2016). Similarly, a study on the breeding home range of Eurasian oystercatchers in the Sinan tidal flat region along the west coast of South Korea also demonstrated a comparable pattern, with oystercatchers concentrating their habitat use on intertidal mudflats adjacent to nesting sites (Park et al. 2025). Previous research based on behavioral observations has also suggested that Eurasian oystercatchers, which prefer to forage at the tide line, can actively adapt to their habitat depending on their life stage by foraging in open mudflat areas near breeding sites (Lee et al. 2018). The high proportion of decapods in the diet, as observed in this study, can be interpreted as a result of selective foraging behavior from a breeding strategy perspective. This strategy involves preferentially capturing easily accessible prey, such as decapods, in shallow, exposed tidal flats during low tide. Such behavior, utilizing foraging grounds in close proximity to their breeding sites, allows the oystercatchers to efficiently meet their energy requirements while simultaneously minimizing the predation risk to their eggs and chicks.

Conclusions

Dietary analysis using fecal DNA metabarcoding revealed that Eurasian oystercatchers (*H. o. osculans*) breeding on Yubu Island primarily consumed decapods, particularly *N. japonica* and *S. globosa*. In addition to these taxa, sipuncula, bivalves, and isopods were also identified as important dietary items, expanding upon previous findings based on direct observation. The application of DNA metabarcoding enabled more accurate, species-level identification of prey items and allowed for quantitative estimation of prey proportions, thereby offering improved insights into dietary preferences. The high detection frequency of decapods likely reflects the selection of exposed mudflats as primary foraging habitats, supporting previous reports that oystercatchers optimize foraging site selection near breeding sites to enhance reproductive success. These findings advance our understanding of seasonal shifts in the foraging strategies of *H. o. osculans* during the breeding period.

Supplementary Information

Supplementary information accompanies this paper at https://doi.org/10.5141/jee.25.079.

Table S1. Relative of abundance data for each samples.

Abbreviations

OTU: Operational taxonomic unit
RRA: Relative read abundance
%FOO: Frequency of occurrence
PERMANOVA: Permutational multivariate analysis of variance

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Authors' contributions

Conceptualization: S.J. and Y.S.C. Formal analysis: S.J. and I.A. Investigation: S.J., Y.S.C., S.Y.L., I.A., D.Y., H.S.K., and S.A.J. Writing—original draft preparation: S.J. Writing—review and editing: S.J., Y.S.C., and D.Y.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was conducted according to the Wildlife Protection and Management Act of the Republic of Korea and the Institutional Research Guidelines of the National Institute of Ecology (RPMT-245, 17 Feb. 2017). All academic survey permission was approved by The Cultural Heritage Administration and local government (No. 9422, 26 May, 2015 by local government and No. 2283, 5 April, 2017 by The Cultural Heritage Administration).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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