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*Article*

# Recovery of Macrobenthic Food Web on Rocky Shores Following the *Hebei Spirit* Oil Spill as Revealed by C and N Stable Isotopes

**Hyun Je Park [1], Changseong Kim [2] and Chang-Keun Kang [2,*]**

[1] Department of Marine Bioscience, Gangneung-Wonju National University, Gangneung 25457, Korea; phj13579@gwnu.ac.kr
[2] School of Earth Sciences & Environmental Engineering, Gwangju Institute of Science and Technology, Gwangju 61005, Korea; changseong@gist.ac.kr
* Correspondence: ckkang@gist.ac.kr; Tel.: +82-62-715-2834

**Abstract:** The impact of large-scale oil spills on organisms can lead to modifications of the food web structure. To assess the effects of the *Hebei Spirit* oil-spill accident on the trophic structure of the macrobenthic community on intertidal rocky shores along Taean Peninsula on the western coast of Republic of Korea 4 years after the *Hebei Spirit* oil spill, we analyzed carbon and nitrogen stable isotope ratios of macrobenthic consumers and their potential food sources in two heavily oil-impacted and one non-impacted sites. The results show no significant differences in isotopic ratios of feeding groups and their potential food sources between the polluted and reference sites, suggesting similar trophic structures given similar resource use by consumers. Similar isotopic niches and substantial overlap areas of feeding groups between the affected and reference sites suggest that the oil-impacted sites have re-achieved the trophic functions of the natural ecosystem. This study provides valuable information on the ecological processes of trophic recovery in coastal ecosystems impacted by oil spills.

**Keywords:** *Hebei Spirit* oil spill; stable isotopes; intertidal rocky shore; feeding group; trophic recovery; isotopic niche

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**Citation:** Park, H.J.; Kim, C.; Kang, C.-K. Recovery of Macrobenthic Food Web on Rocky Shores Following the *Hebei Spirit* Oil Spill as Revealed by C and N Stable Isotopes. *Water* **2022**, *14*, 2335. https://doi.org/10.3390/w14152335

Academic Editor: Guangyi Wang

Received: 27 June 2022
Accepted: 25 July 2022
Published: 28 July 2022

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## 1. Introduction

Coastal environments are threatened by a wide range of anthropogenic issues such as overfishing, eutrophication, chemical pollutants, and habitat destruction leading to the modification of the structure and function of coastal ecosystems [1]. Oil spills in oceans are serious environmental disasters with detrimental biological and socioeconomic effects that can adversely affect biotic communities at regional and national scales [2,3]. Large-scale oil spills can have long-term impacts on the survival and reproduction of marine organisms and on prey–predator interactions and can lead to habitat destruction, resulting in the delayed recovery of marine ecosystems because of the cascading direct/indirect effects [4–6]. Such impacts on individual species or populations can lead to the modification of the structure or dynamics of food webs via complex indirect effects through bottom–up or top–down cascades [7,8]. Accordingly, monitoring of long-term consequences of oil spills at ecosystem levels is essential for understanding responses through chronic and indirect long-term impacts, which can help assess the recovery process for coastal habitat protection and management [9,10].

The *Hebei Spirit* oil spill (HSOS) that occurred in December 2007 severely affected the entire ecosystem, as 10,900 tons of three different forms of crude oil (Iranian Heavy, United Arab Emirates Upper Zakum, and Kuwait Export) was released over 200 km along the western coast of Korea [11]. The spilled oil covered soft and rocky intertidal areas, resulting in toxic effects on living organisms, damage to the marine ecosystem, and collapse of the fisheries and mariculture industries [12]. Although the concentrations of petroleum hydrocarbons have now nearly decreased to pre-oil spill levels in most coastal areas, residual oils in sediments tend to exist for prolonged periods and are, thus, considered to have long-term impacts on various ecosystem components (especially benthic organisms) at individual and community levels [10,13]. After the HSOS, many studies have reported the status of residual oil in the environments, ecological toxicity in organisms, changes in community structure, and recovery processes through integrated ecosystem monitoring [10,13–16]. However, after the HSOS accident, despite the importance of understanding the functioning of the food web, including diverse trophic levels in the oil-impacted regions, there is little information on the trophic recovery of the benthic food web through quantitative or qualitative assessments of the resilience status of the coastal ecosystems [17].

The analysis of carbon and nitrogen stable isotope ratios can enable the tracking of trophic transfer of organic matter and can help in identifying the trophic structure of aquatic food webs by providing time-integrated information on consumers' diet [18,19]. This analysis assumes that the stable isotope ratios of a consumer's tissues reflect those of its dietary sources with predictable trophic enrichments over turnover periods by metabolic processes ($\leq$1‰ for carbon and 2‰ to 4‰ for nitrogen) [20–22]. Several studies have demonstrated that organic matter pathways and trophic structures in coastal ecosystems can be influenced by a variety of anthropogenic activities [23–25]. Therefore, monitoring isotope ratios of organic matter sources and animals as major ecosystem components may allow us to assess the ecological effects of an oil spill accident, such as the HSOS on the coastal food web and the trophic recovery of the oil-impacted region.

Here, we analyzed carbon and nitrogen stable isotope signatures of macrobenthic consumers and their potential food sources on heavily oil-impacted rocky intertidal shores approximately 4 years after the HSOS, and compared these signatures with those in the non-impacted regions (control sites). We hypothesize that if fauna and flora on the rocky shores are still impacted by residual oils, the isotopic signatures of macrobenthic consumers at the oil-impacted sites will be dissimilar to those at the control sites. Our major objective was to assess the effects of an oil-spill accident on trophic pathways of organic matter and the trophic structure (including isotopic niches of consumers) of macrobenthic communities on rocky shore intertidal habitats. A previous study on the macrobenthic food web impacted by the HSOS only focused on the soft-bottom ecosystem, which showed a similar food web structure between oil-impacted and control sites [17]. To our knowledge, this is the first study to examine the trophic recovery of a rocky shore macrobenthic food web based on the isotopic assessment of the rocky shore ecosystem impacted by the HSOS.

## 2. Materials and Methods

### *2.1. Site Description*

The Taean Peninsula is located on the western coast of the Korean peninsula and has a semidiurnal and macrotidal regime (spring tidal range of 4–6 m). The sampling sites consist of two heavily oil-impacted sites (Gurepo, 36°53′31″ N 126°11′55″ E and Padori, 36°44′14″ N 126°07′49″ E: referred to as sites G and P, respectively) and one non-impacted reference site (Yeonpo, 36°41′33″ N 126°13′18″ E: referred to as site Y), which are located on the coastal line of the peninsula (Figure 1). The concentrations of total petroleum hydrocarbon in seawater off the Taean coast were 1.5–7300 μg $L^{-1}$ (mean 732 μg $L^{-1}$) immediately after the HSOS, which decreased rapidly to 2.0–224 μg $L^{-1}$ (mean 31 μg $L^{-1}$) in 1 month in response to clean-up activities and strong tidal currents [26]. Overall, the water quality off the Taean coast—in terms of the concentration of total petroleum hydrocarbons—recovered to below the environmental standard (10 μg $L^{-1}$) within 1 y after the HSOS [12]. Ecological indices (species richness and diversity) of macrobenthic invertebrates at the oil-impacted sites increased gradually by 1 y after the HSOS, and their mean densities were similar to those at the reference site [11,27].

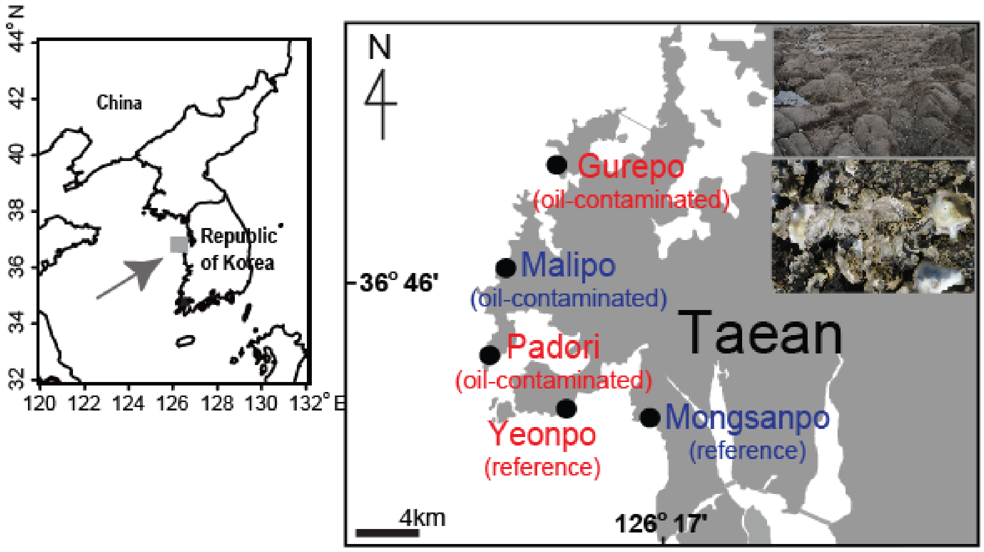


**Figure 1.** Map of the Taean peninsula on the western coast of Korea. Sampling areas (this study, red color) are located at two heavily oil-impacted sites (Gurepo and Padori) and one non-impacted reference site (Yeonpo). Blue color indicates the sampling sites (Malipo and Mongsanpo) investigated by [17].

### *2.2. Sample Collection and Processing*

Two sampling campaigns were carried out at the three sampling sites in July and November of 2013. Macrobenthic invertebrates, organic matter sources, and macroalgae were sampled at each site. The samples of macroinvertebrate consumers were collected using a 0.0625 m$^2$ (25 × 25 cm) quadrat by hand with a stainless-steel knife during low spring tides. Macroinvertebrates were sorted and kept alive overnight in filtered seawater to enable them to evacuate their gut contents at the laboratory. Individuals for macrofauna were identified at the lowest taxonomic level possible under a dissecting microscope; then, their muscle tissues were dissected, lyophilized, and homogenized to a fine powder with a ball mill (Retsch MM200 Mixer Mill, Hyland Scientific, WA, USA) for stable isotope analysis. The prepared samples were kept in a deep freezer (−50 °C) before this.

For collecting suspended particulate organic matter (SPOM), water samples were obtained at each site during flood tides using a van Dorn sampler on board and then filtered through pre-combusted (450 °C for 4 h) Whatman GF/F glass fiber filters (0.70 µm nominal pore size) after removing any zooplankton and large particles using a 200-µm mesh net. Epilithic algae (EA) were obtained from several small-sized rocks by scraping the rock surface using a fine brush. The prepared particles were prefiltered through a 200-µm sieve to remove large materials, and then EA were sampled through the pre-combusted GF/F filters. The filter samples for SPOM and EA were fumed under a 1 N HCl stream overnight to remove inorganic carbonates and then oven-dried for 48 h at 50 °C and wrapped in an aluminum foil. Two dominant macroalgae (*Ulva pertusa* and *Sargassum thunbergii*) were sampled by hand during low tides and then were scraped with a razor blade to remove epibionts and rinsed with Milli-Q water. The macroalgal samples were freeze-dried and ground to a fine powder in a ball mill as mentioned above. The treated samples of organic matter sources and macroalgae were kept in a deep freezer (−50 °C) until subsequent analysis.

### *2.3. Stable Isotope Analysis*

For stable isotope analysis, powder and filter samples were packed into tin combustion cups and foils, respectively. The capsulated samples were introduced into an elemental analyzer (Vario MICRO Cube CHNOS elemental analyzer; Elementar Analysensysteme GMbH, Hanau, Germany) to oxidize at a high temperature (1030 °C), and then the resultant $CO_2$ and $N_2$ gases were analyzed for their stable isotope ratios using a continuous flow isotope ratio-mass spectrometry (IsoPrime100; Cheadle Hulme, UK). Stable isotope data are expressed in conventional delta notation (δ, ‰) based on the relative difference between isotopic ratios of the sample and reference gases of Vienna Pee Dee Belemnite for carbon and atmospheric $N_2$ for nitrogen. Data were obtained using the following equation:

$$\delta X\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 1000, \quad (1)$$

where X is $^{13}C$ or $^{15}N$ and R values are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios. Sucrose (ANU $C_{12}H_{22}O_{11}$; NIST, Gaithersburg, MD, USA) and ammonium sulfate ($[NH_4]_2SO_4$; NIST) with established relationships to international standards were used as reference materials. Standard deviations of repeated measurements of internal urea standard were 0.13‰ and 0.20‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

### *2.4. Data Analysis*

Permutational multivariate analysis of variance (PERMANOVA) using the software PRIMER version 6 combined with the PERMANOVA + PRIMER add-on [28] was conducted to assess spatial and seasonal differences in the $\delta^{13}C$ and $\delta^{15}N$ values of organic matter sources (SPOM, EA, and macroalgae) and consumers between the oil-impacted and reference sites between July and November. For consumer species, PERMANOVA was performed on the isotopic data of feeding groups (suspension feeders, deposit feeders, grazers, omnivores, and carnivores) rather than at species levels to assess the community trophic structure impacted by the oil spill. The significance level was set to $p < 0.05$ for all statistical tests.

To test the similarity in the resource use by consumer feeding groups between the oil-impacted and reference sites, the overall variability in their isotopic niche areas (‰$^2$) was investigated using the package Stable Isotope Bayesian Ellipses in R (software R version 3.5.3) [29,30]. To compare each feeding group in the isotopic niche areas between the sites, we used the indices of total area (TA) and small sample size-corrected standard ellipse area (SEAc) with a confidence interval of 95% as quantitative indicators of the trophic diversity of consumers [29,31]. In addition, the percentage of isotopic niche overlap of each feeding group between the sites was calculated by the ratio between the overlap areas based on the SEAc representing the core isotopic niche with the 'nicheRover' package in R [32].

## 3. Results

### *3.1. Stable Isotope Ratios of Potential Food Sources*

There were no significant differences in the $\delta^{13}C$ and $\delta^{15}N$ values of all potential food sources (SPOM, EA, *U. pertusa*, and *S. thunbergii*) among sites (PERMANOVA, $p > 0.746$ for all cases) (Table 1), whereas their significant seasonal differences were observed between July and November (PERMANOVA, $p < 0.011$ for all cases). No significant effects of the interaction term (season × site) were found for all potential food sources ($p > 0.688$ for all cases). The $\delta^{13}C$ values of potential food sources ranged from −23.6 ± 0.4‰ (*S. thunbergii* at site G) to −12.8 ± 0.5‰ (*U. pertusa* at site P) in July and from −23.2 ± 0.3‰ (*S. thunbergii* at site G) to −13.5 ± 0.6‰ (*U. pertusa* at site Y) in November (Table 1). By contrast, their $\delta^{15}N$ values varied from 3.5 ± 0.4‰ (*S. thunbergii* at site G) to 7.8 ± 1.2‰ (SPOM at site P) in July and from 4.0 ± 0.3‰ (*S. thunbergii* at site P) to 6.1 ± 0.5‰ (*U. pertusa* at site P) in November.

### *3.2. Stable Isotope Ratios of Consumer Species*

The mean $\delta^{13}C$ and $\delta^{15}N$ values of macrobenthic consumers showed similar ranges among sites: −19.2 ± 0.2‰ to −12.7 ± 0.5‰, 4.7 ± 0.6‰ to 12.2 ± 0.7‰, and −19.1 ± 0.4‰ to −12.7 ± 0.3‰, and 5.1 ± 0.2‰ to 12.3 ± 0.4‰, −19.2 ± 0.3‰ to −12.7 ± 0.5‰, and 4.9 ± 0.3‰ to 12.5 ± 0.4‰ at sites G, P, and Y, respectively (Figure 2 and Table 2). Three-way PERMANOVA showed that there were no significant differences in the $\delta^{13}C$ and $\delta^{15}N$ values among sites (pseudo-$F_{2,504} = 1.05$, $p = 0.390$). By contrast, significant differences in the $\delta^{13}C$ and $\delta^{15}N$ values of macrobenthic consumers were observed between seasons (pseudo-$F_{1,504}$ = 47.61, $p$ = 0.001) and among feeding groups (pseudo-$F_{4,504}$ = 61.32, $p$ = 0.001). All interaction terms showed significant effects ($p < 0.009$ for all cases).

**Table 1.** Mean $\delta^{13}C$ and $\delta^{15}N$ values of potential food sources (SPOM, suspended particulate organic matter; Epilithic algae; *U. pertusa*, *Ulva pertusa*; *S. thunbergii*, *Sargassum thunbergii*) at the two heavily oil-impacted (Gurepo and Padori) and one non-impacted reference (Yeonpo) sites in July and November. PERMANOVA test of $\delta^{13}C$ and $\delta^{15}N$ values for each potential food source among the three sites and between the two seasons (significance at $p < 0.05$). Data represent means ± 1 SD.

| | **Gurepo** | | | | | **Padori** | | | | | **Yeonpo** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Potential Food Source** | | **$\delta^{13}C$** | | **$\delta^{15}N$** | | | **$\delta^{13}C$** | | **$\delta^{15}N$** | | | **$\delta^{13}C$** | | **$\delta^{15}N$** | |
| | ***n*** | **Mean** | **SD** | **Mean** | **SD** | ***n*** | **Mean** | **SD** | **Mean** | **SD** | ***n*** | **Mean** | **SD** | **Mean** | **SD** |
| July | | | | | | | | | | | | | | | |
| SPOM | 8 | −20.4 | 0.6 | 7.3 | 1.0 | 8 | −20.2 | 1.1 | 7.8 | 1.2 | 6 | −20.3 | 0.9 | 7.5 | 0.8 |
| Epilithic algae | 6 | −15.4 | 1.0 | 7.7 | 0.8 | 5 | −15.2 | 0.8 | 7.5 | 1.0 | 6 | −15.7 | 0.7 | 7.4 | 0.9 |
| *U. pertusa* | 6 | −12.9 | 0.5 | 6.7 | 0.4 | 6 | −12.8 | 0.5 | 6.8 | 0.4 | 6 | −13.0 | 0.6 | 6.9 | 0.4 |
| *S. thunbergii* | 6 | −23.6 | 0.4 | 3.5 | 0.4 | 6 | −23.4 | 0.5 | 3.7 | 0.3 | 6 | −23.4 | 0.6 | 3.8 | 0.5 |
| November | | | | | | | | | | | | | | | |
| SPOM | 7 | −20.8 | 0.9 | 5.4 | 0.9 | 6 | −20.6 | 0.6 | 5.7 | 0.9 | 8 | −20.9 | 1.0 | 5.8 | 0.6 |
| Epilithic algae | 6 | −14.5 | 0.6 | 5.9 | 0.6 | 6 | −14.9 | 0.7 | 5.6 | 0.6 | 6 | −14.4 | 0.6 | 5.6 | 0.6 |
| *U. pertusa* | 6 | −13.8 | 0.5 | 5.8 | 0.4 | 6 | −13.6 | 0.5 | 6.1 | 0.5 | 6 | −13.6 | 0.6 | 5.9 | 0.4 |
| *S. thunbergii* | 6 | −23.2 | 0.3 | 4.2 | 0.3 | 6 | −23.0 | 0.3 | 4.0 | 0.3 | 6 | −22.9 | 0.4 | 4.1 | 0.3 |

| PERMANOVA | Season | | Site | | Interaction | |
|---|---|---|---|---|---|---|
| | pseudo-*F* | *p*-value | pseudo-*F* | *p*-value | pseudo-*F* | *p*-value |
| SPOM | 30.52 | 0.001 | 0.47 | 0.746 | 0.01 | 0.966 |
| Epilithic algae | 40.87 | 0.001 | 0.11 | 0.911 | 0.50 | 0.695 |
| *U. pertusa* | 32.92 | 0.001 | 0.29 | 0.789 | 0.21 | 0.877 |
| *S. thunbergii* | 6.45 | 0.011 | 0.16 | 0.920 | 0.51 | 0.688 |

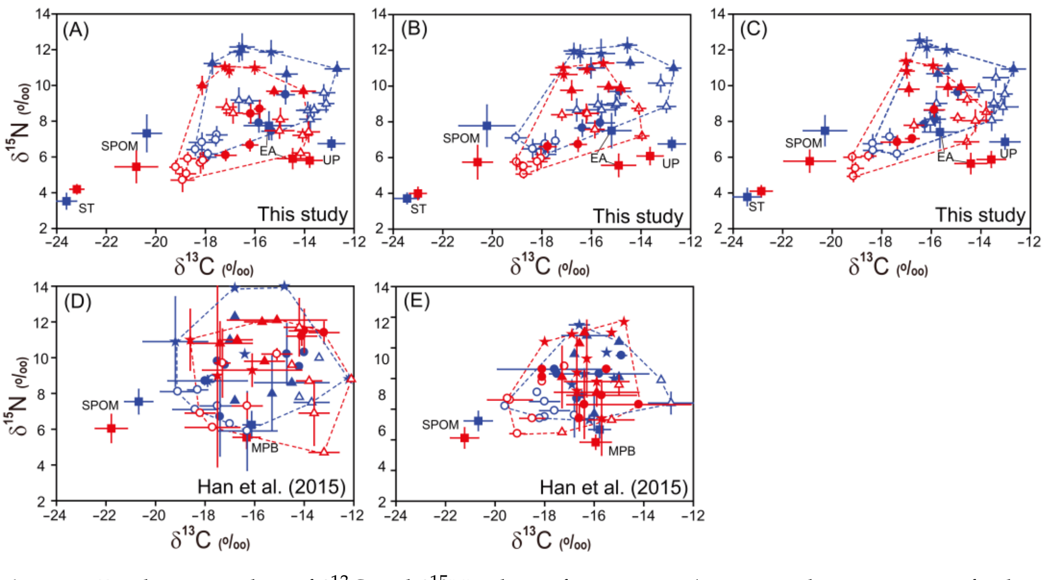


**Figure 2.** Dual isotope plots of $\delta^{13}C$ and $\delta^{15}N$ values of consumers (empty circles, suspension feeders; filled circles, deposit feeders; empty triangles, grazers; filled triangles, omnivores; stars, carnivores) at the three oil-contaminated sites ((**A**), Gurepo; (**B**), Padori; (**D**), Malipo) and two non-impacted reference sites ((**C**), Yeonpo; (**E**), Mongsanpo) in July (blue color) and November (red color) for this study and in spring and summer for [17]. Values of the potential food sources are illustrated by filled squares. SPOM, suspended particulate organic matter; EA, epilithic algae; UP, microphytobenthos; MPB *Ulva pertusa*; ST, *Sargassum thunbergii*. Values are means (‰) ± 1 standard deviation.

**Table 2.** Mean $\delta^{13}C$ and $\delta^{15}N$ values of macrobenthic consumers collected at the two heavily oil-impacted (Gurepo and Padori) and one non-impacted reference (Yeonpo) sites in July and November. Data represent means ± 1 SD. Taxa: B, bivalvia; C, crustacea; E, echinodermata; G, gastropoda; P, polychaeta; Pp, polyplacophora.

| No. | Species Name | Taxa | Gurepo | | | | | Padori | | | | | Yeonpo | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $\delta^{13}C$ | | $\delta^{15}N$ | | | $\delta^{13}C$ | | $\delta^{15}N$ | | | $\delta^{13}C$ | | $\delta^{15}N$ | |
| | | | *n* | Mean | SD | Mean | SD | *n* | Mean | SD | Mean | SD | *n* | Mean | SD | Mean | SD |
| | July | | | | | | | | | | | | | | | | |
| | Suspension feeder | | | | | | | | | | | | | | | | |
| 1 | *Balanus albicostatus* | C | 4 | −17.9 | 0.4 | 6.0 | 0.2 | | | | | | | | | | |
| 2 | *Chthamalus challengeri* | C | 4 | −17.9 | 0.3 | 6.2 | 0.3 | 4 | −17.9 | 0.4 | 6.2 | 0.3 | 4 | −17.4 | 0.3 | 6.2 | 0.3 |
| 3 | *Crassostrea gigas* | B | 4 | −17.5 | 0.4 | 7.2 | 0.5 | 4 | −17.4 | 0.2 | 6.9 | 0.6 | 4 | −17.7 | 0.3 | 7.1 | 0.5 |
| 4 | *Ditrupa arietina* | P | | | | | | 3 | −19.1 | 0.4 | 7.1 | 0.3 | | | | | |
| 5 | *Hydroides ezoensis* | P | 3 | −17.6 | 0.3 | 7.0 | 0.3 | 3 | −17.9 | 0.5 | 6.8 | 0.6 | | | | | |
| 6 | *Lasaea undulata* | B | 3 | −18.1 | 0.5 | 6.8 | 0.5 | 4 | −18.1 | 0.4 | 6.3 | 0.3 | 4 | −18.4 | 0.4 | 6.4 | 0.4 |
| 7 | *Mytilus galloprovincialis* | B | 4 | −18.4 | 0.3 | 6.4 | 0.3 | 4 | −18.4 | 0.4 | 6.5 | 0.5 | 4 | −18.4 | 0.4 | 6.8 | 0.5 |
| | Deposit feeder | | | | | | | | | | | | | | | | |
| 8 | *Barleeia angustata* | G | 4 | −15.8 | 0.4 | 7.9 | 0.5 | 4 | −15.6 | 0.6 | 7.9 | 0.4 | 4 | −15.8 | 0.4 | 8.1 | 0.4 |
| 9 | Gammarian amphipod | C | | | | | | 4 | −16.4 | 0.3 | 7.6 | 0.5 | 4 | −16.3 | 0.4 | 7.8 | 0.4 |
| 10 | *Nainereis laevigata* | P | 3 | −15.3 | 0.4 | 7.4 | 0.4 | | | | | | | | | | |
| 11 | *Parhyale* sp. | C | 4 | −14.8 | 0.3 | 9.5 | 0.5 | 4 | −14.9 | 0.5 | 9.7 | 0.4 | 4 | −14.9 | 0.3 | 9.6 | 0.4 |
| | Grazer | | | | | | | | | | | | | | | | |
| 12 | *Chlorostoma lischkei* | G | 4 | −16.2 | 0.5 | 9.1 | 0.2 | 4 | −15.6 | 0.5 | 8.7 | 0.5 | | | | | |
| 13 | *Chlorostoma turbinata* | G | 4 | −16.6 | 0.3 | 9.2 | 0.7 | 4 | −16.6 | 0.4 | 8.7 | 0.6 | | | | | |
| 14 | *Heminerita japonica* | G | | | | | | | | | | | 4 | −16.0 | 0.4 | 7.9 | 0.5 |
| 15 | *Littorina brevicula* | G | 4 | −13.9 | 0.4 | 8.6 | 0.4 | | | | | | 4 | −13.1 | 0.6 | 8.8 | 0.4 |
| 16 | *Lottia dorsuosa* | G | 4 | −13.6 | 0.6 | 8.6 | 0.3 | | | | | | 4 | −13.5 | 0.2 | 9.0 | 0.6 |
| 17 | *Lottia kogamogai* | G | | | | | | | | | | | 4 | −14.1 | 0.5 | 9.7 | 0.4 |
| 18 | *Lottia tenuisculpta* | G | 4 | −13.1 | 0.3 | 9.0 | 0.5 | 4 | −13.0 | 0.4 | 8.9 | 0.3 | 4 | −13.0 | 0.3 | 9.5 | 0.6 |
| 19 | *Monodonta neritoides* | G | | | | | | 4 | −15.0 | 0.4 | 8.9 | 0.6 | | | | | |
| 20 | *Nipponacmaea schrenckii* | G | | | | | | 4 | −15.7 | 0.4 | 9.0 | 0.6 | | | | | |
| 21 | *Nodilittorina radiata* | G | | | | | | | | | | | 4 | −15.9 | 0.4 | 8.6 | 0.3 |
| 22 | *Omphalius rusticus* | G | | | | | | 4 | −15.0 | 0.4 | 9.0 | 0.3 | | | | | |
| 23 | *Patelliida conulus* | G | 4 | −13.2 | 0.4 | 9.6 | 0.4 | | | | | | | | | | |
| 24 | *Patelloida pygmaea* | G | | | | | | | | | | | 4 | −15.8 | 0.4 | 9.0 | 0.7 |
| 25 | *Turbo coronata coreensis* | G | | | | | | 4 | −13.2 | 0.5 | 10.2 | 0.5 | 4 | −13.4 | 0.5 | 10.5 | 0.5 |
| 26 | *Acanthoschitona achates* | Pp | 4 | −13.8 | 0.4 | 8.1 | 0.8 | | | | | | 4 | −13.6 | 0.4 | 8.1 | 0.6 |
| | Omnivore | | | | | | | | | | | | | | | | |
| 27 | *Asterina pectinifera* | E | 4 | −12.7 | 0.5 | 10.9 | 0.4 | 4 | −12.7 | 0.3 | 11.0 | 0.4 | 4 | −12.7 | 0.5 | 10.9 | 0.4 |
| 28 | *Hemigrapsus penicillatus* | C | 4 | −17.7 | 0.3 | 11.2 | 0.6 | 4 | −16.0 | 0.5 | 11.0 | 0.6 | 4 | −15.7 | 0.4 | 10.7 | 0.5 |
| 29 | *Pagurus* sp. | C | 4 | −14.7 | 0.4 | 10.6 | 0.4 | 4 | −14.4 | 0.5 | 11.3 | 0.3 | 4 | −15.3 | 0.4 | 10.9 | 0.3 |
| | Carnivore | | | | | | | | | | | | | | | | |
| 30 | *Ceratostoma inornatum* | G | 4 | −15.3 | 0.5 | 11.9 | 0.6 | 4 | −15.6 | 0.5 | 11.8 | 0.8 | 4 | −15.4 | 0.5 | 12.0 | 0.3 |
| 31 | *Ceratostoma rorifluum* | G | 4 | −16.5 | 0.5 | 12.2 | 0.7 | 4 | −16.5 | 0.6 | 11.8 | 0.5 | 4 | −16.2 | 0.4 | 12.1 | 0.6 |
| 32 | *Hima fratercula hypolia* | G | | | | | | 4 | −14.6 | 0.4 | 12.3 | 0.4 | | | | | |
| 33 | *Thais clavigera* | G | 4 | −16.6 | 0.4 | 11.9 | 0.5 | 4 | −16.7 | 0.4 | 12.0 | 0.4 | 4 | −16.5 | 0.5 | 12.5 | 0.4 |
| | November | | | | | | | | | | | | | | | | |
| | Suspension feeder | | | | | | | | | | | | | | | | |
| 1 | *Balanus albicostatus* | C | 4 | −18.9 | 0.5 | 4.7 | 0.6 | | | | | | | | | | |
| 2 | *Chthamalus challengeri* | C | 4 | −18.8 | 0.4 | 5.1 | 0.3 | 4 | −18.7 | 0.3 | 5.1 | 0.2 | 4 | −19.1 | 0.2 | 4.9 | 0.3 |
| 3 | *Crassostrea gigas* | B | 4 | −18.2 | 0.2 | 5.7 | 0.6 | 4 | −18.0 | 0.3 | 6.0 | 0.5 | 4 | −18.5 | 0.3 | 6.1 | 0.3 |
| 4 | *Hydroides ezoensis* | P | 3 | −18.1 | 0.2 | 5.8 | 0.5 | 3 | −18.2 | 0.4 | 5.8 | 0.5 | | | | | |
| 5 | *Lasaea undulata* | B | 4 | −18.7 | 0.3 | 5.9 | 0.3 | 4 | −18.7 | 0.3 | 5.5 | 0.2 | 4 | −19.1 | 0.3 | 5.4 | 0.3 |
| 6 | *Mytilus galloprovincialis* | B | 4 | −19.2 | 0.2 | 5.4 | 0.4 | 4 | −19.0 | 0.3 | 5.8 | 0.4 | 4 | −19.2 | 0.3 | 6.0 | 0.3 |
| | Deposit feeder | | | | | | | | | | | | | | | | |
| 7 | *Barleeia angustata* | G | 4 | −16.2 | 0.3 | 6.7 | 0.3 | 4 | −16.5 | 0.3 | 6.7 | 0.4 | 4 | −16.8 | 0.3 | 7.0 | 0.2 |
| 8 | Gammarian amphipod | C | 4 | −17.2 | 0.4 | 6.1 | 0.3 | 4 | −17.8 | 0.3 | 6.6 | 0.3 | 4 | −17.4 | 0.6 | 6.8 | 0.4 |
| 9 | *Nainereis laevigata* | P | 3 | −16.2 | 0.3 | 8.4 | 0.5 | | | | | | | | | | |
| 10 | *Parhyale* sp. | C | 4 | −15.8 | 0.2 | 8.7 | 0.5 | 4 | −16.2 | 0.5 | 8.5 | 0.5 | 4 | −15.9 | 0.4 | 8.6 | 0.4 |

**Table 2.** *Cont.*

| No. | Species Name | Taxa | Gurepo | | | | | Padori | | | | | Yeonpo | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | δ$^{13}$C | | δ$^{15}$N | | | δ$^{13}$C | | δ$^{15}$N | | | δ$^{13}$C | | δ$^{15}$N | |
| | | | *n* | Mean | SD | Mean | SD | *n* | Mean | SD | Mean | SD | *n* | Mean | SD | Mean | SD |
| | Grazer | | | | | | | | | | | | | | | | |
| 11 | *Chlorostoma lischkei* | G | 4 | −16.9 | 0.4 | 8.5 | 0.6 | 4 | −16.2 | 0.5 | 8.5 | 0.4 | | | | | |
| 12 | *Chlorostoma turbinata* | G | 4 | −17.2 | 0.4 | 8.8 | 0.5 | 4 | −17.2 | 0.4 | 8.4 | 0.3 | | | | | |
| 13 | *Heminerita japonica* | G | | | | | | | | | | | 4 | −16.7 | 0.4 | 7.8 | 0.4 |
| 14 | *Littorina brevicula* | G | 3 | −15.0 | 0.3 | 8.1 | 0.6 | | | | | | 3 | −14.9 | 0.3 | 8.2 | 0.2 |
| 15 | *Lottia dorsuosa* | G | 4 | −14.1 | 0.4 | 7.2 | 0.4 | | | | | | 4 | −14.2 | 0.5 | 8.0 | 0.6 |
| 16 | *Lottia tenuisculpta* | G | 4 | −13.8 | 0.4 | 7.4 | 0.6 | 4 | −14.0 | 0.3 | 7.2 | 0.2 | 4 | −13.8 | 0.2 | 8.5 | 0.6 |
| 17 | *Omphalius rusticus* | G | 3 | −15.0 | 0.6 | 7.5 | 0.4 | 3 | −15.9 | 0.4 | 7.6 | 0.5 | | | | | |
| 18 | *Turbo coronata coreensis* | G | | | | | | | −14.1 | 0.3 | 8.8 | 0.3 | 4 | −14.6 | 0.4 | 9.2 | 0.3 |
| 19 | *Acanthoschitona achates* | Pp | 4 | −14.2 | 0.3 | 6.2 | 0.2 | | | | | | 4 | −14.6 | 0.5 | 6.9 | 0.4 |
| | Omnivore | | | | | | | | | | | | | | | | |
| 20 | *Asterina pectinifera* | E | 4 | −14.0 | 0.5 | 9.7 | 0.3 | 4 | −14.8 | 0.4 | 9.9 | 0.4 | 4 | −14.8 | 0.6 | 9.9 | 0.4 |
| 21 | *Hemigrapsus penicillatus* | C | 4 | −18.1 | 0.2 | 10.0 | 0.5 | 4 | −16.8 | 0.4 | 9.8 | 0.5 | 4 | −16.9 | 0.4 | 9.8 | 0.4 |
| 22 | *Pagurus* sp. | C | 4 | −15.2 | 0.3 | 9.7 | 0.2 | 4 | −15.3 | 0.4 | 10.0 | 0.3 | 4 | −15.3 | 0.5 | 9.9 | 0.5 |
| | Carnivore | | | | | | | | | | | | | | | | |
| 23 | *Ceratostoma inornatum* | G | 4 | −16.0 | 0.4 | 11.0 | 0.4 | 4 | −16.2 | 0.3 | 10.9 | 0.2 | 4 | −15.9 | 0.4 | 11.1 | 0.4 |
| 24 | *Ceratostoma rorifluum* | G | 4 | −17.0 | 0.2 | 10.8 | 0.4 | 4 | −17.1 | 0.5 | 10.6 | 0.4 | 4 | −17.0 | 0.3 | 10.8 | 0.4 |
| 25 | *Hima fratercula hypolia* | G | | | | | | 4 | −15.5 | 0.4 | 11.3 | 0.2 | | | | | |
| 26 | *Thais clavigera* | G | 4 | −17.2 | 0.4 | 11.0 | 0.3 | 4 | −17.1 | 0.3 | 11.0 | 0.2 | 4 | −17.0 | 0.3 | 11.4 | 0.5 |

PERMANOVA for comparing each feeding group revealed that isotopic values for all feeding groups differed significantly between the two seasons ($p = 0.001$ for all cases) (Table 3), whereas no significant differences among sites were observed (suspension feeders, pseudo-$F_{2,\,117} = 0.55$, $p = 0.663$; deposit feeders, pseudo-$F_{2,\,73} = 1.07$, $p = 0.355$; grazers, pseudo-$F_{2,\,160} = 1.47$, $p = 0.212$; omnivores, pseudo-$F_{2,\,71} = 0.58$, $p = 0.626$; carnivores, pseudo-$F_{2,\,79} = 0.79$, $p = 0.533$). No significant effects of the interaction term (season × site) were observed for all feeding groups ($p > 0.391$ for all cases). All feeding groups in July had higher mean δ$^{13}$C and δ$^{15}$N values than those in November. In addition, the mean δ$^{13}$C and δ$^{15}$N values of feeding groups showed similar trends among sites ranging from −19.0 ± 0.4‰ (suspension feeder at site Y in November) to −14.3 ± 1.3‰ (grazer in July), and from 5.4 ± 0.6‰ (suspension feeder at site G in November) to 12.2 ± 0.5‰ (carnivore in July), respectively.

### *3.3. Isotopic Niche Areas*

Regardless of the season, the isotopic niche areas (‰$^2$) for each feeding group exhibited similar TA and SEAc values among the three sampling sites (Table 4). By contrast, the omnivore group had different TA (3.27 to 6.38 in July and 2.34 to 5.22 in September) and SEAc values (1.89 to 3.45 in July and 1.24 to 2.53 in September) among the sites. In both July and November, suspension feeders had the lowest TA and SEAc values (2.01 and 0.80 and 1.31 and 0.66 at site Y, respectively), whereas grazers had the highest values (9.45 and 3.45 at site Y for TA and 7.29 and 3.17 at site G for SEAc, respectively). In July, all the feeding groups had substantial overlapping proportions of isotopic niche areas between the polluted and control sites, ranging from 58% for carnivores to 84% for suspension feeders between sites P and Y (Figure 3A). The isotopic overlapping proportions between the impacted and control sites in November showed relatively low values than those in July, ranging from 40% for suspension feeders to 92% for grazers between sites P and Y (Figure 3B).

**Table 3.** Mean $\delta^{13}C$ and $\delta^{15}N$ values of consumer feeding groups (suspension feeder, deposit feeder, grazer, omnivore, carnivore) at the two heavily oil-impacted (Gurepo and Padori) and one non-impacted reference (Yeonpo) sites in July and November. PERMANOVA test of $\delta^{13}C$ and $\delta^{15}N$ values for each consumer feeding group among the three sites and between the two seasons (significance at $p < 0.05$). Data represent means ± 1 SD.

| | Gurepo | | | | | Padori | | | | | Yeonpo | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Potential Food Source** | | **$\delta^{13}$C** | | **$\delta^{15}$N** | | | **$\delta^{13}$C** | | **$\delta^{15}$N** | | | **$\delta^{13}$C** | | **$\delta^{15}$N** | |
| | ***n*** | **Mean** | **SD** | **Mean** | **SD** | ***n*** | **Mean** | **SD** | **Mean** | **SD** | ***n*** | **Mean** | **SD** | **Mean** | **SD** |
| July | | | | | | | | | | | | | | | |
| Suspension feeder | 22 | −17.9 | 0.4 | 6.6 | 0.6 | 22 | −18.1 | 0.6 | 6.6 | 0.5 | 16 | −17.9 | 0.5 | 6.6 | 0.5 |
| Deposit feeder | 11 | −15.3 | 0.6 | 8.3 | 2.5 | 12 | −15.6 | 0.8 | 8.4 | 1.0 | 12 | −15.7 | 0.7 | 8.5 | 0.9 |
| Grazer | 28 | −14.4 | 1.4 | 8.9 | 0.6 | 28 | −14.9 | 1.3 | 9.0 | 0.7 | 36 | −14.3 | 1.3 | 9.0 | 0.9 |
| Omnivore | 12 | −15.0 | 2.2 | 10.9 | 0.5 | 12 | −14.4 | 1.5 | 11.1 | 0.4 | 12 | −14.6 | 1.5 | 10.8 | 0.4 |
| Carnivore | 12 | −16.2 | 0.7 | 12.0 | 0.6 | 16 | −15.8 | 1.0 | 12.0 | 0.5 | 12 | −16.0 | 0.6 | 12.2 | 0.5 |
| November | | | | | | | | | | | | | | | |
| Suspension feeder | 23 | −18.7 | 0.5 | 5.4 | 0.6 | 19 | −18.6 | 0.5 | 5.6 | 0.5 | 16 | −19.0 | 0.4 | 5.6 | 0.5 |
| Deposit feeder | 15 | −16.4 | 0.6 | 7.4 | 1.2 | 12 | −16.8 | 0.8 | 7.3 | 1.0 | 12 | −16.7 | 0.8 | 7.5 | 0.9 |
| Grazer | 26 | −15.2 | 1.4 | 7.7 | 0.9 | 20 | −15.5 | 1.3 | 8.1 | 0.7 | 23 | −14.8 | 1.0 | 8.1 | 0.8 |
| Omnivore | 12 | −15.8 | 1.8 | 9.8 | 0.4 | 12 | −15.6 | 1.0 | 9.9 | 0.4 | 12 | −15.7 | 1.0 | 9.9 | 0.4 |
| Carnivore | 12 | −16.8 | 0.6 | 11.0 | 0.3 | 16 | −16.5 | 0.8 | 11.0 | 0.3 | 12 | −16.6 | 0.6 | 11.1 | 0.4 |

| PERMANOVA | Season | | Site | | Interaction | |
|---|---|---|---|---|---|---|
| | pseudo-*F* | *p*-value | pseudo-*F* | *p*-value | pseudo-*F* | *p*-value |
| Suspension feeder | 104.90 | 0.001 | 0.55 | 0.663 | 1.01 | 0.391 |
| Deposit feeder | 35.58 | 0.001 | 1.07 | 0.355 | 0.36 | 0.735 |
| Grazer | 36.33 | 0.001 | 1.47 | 0.212 | 0.36 | 0.815 |
| Omnivore | 33.20 | 0.001 | 0.58 | 0.626 | 0.27 | 0.816 |
| Carnivore | 50.05 | 0.001 | 0.79 | 0.533 | 0.11 | 0.901 |

**Table 4.** Isotopic niche areas of each feeding group (suspension feeder, deposit feeder, grazer, omnivore, carnivore) estimated as the total area (TA) and standard ellipse area (SEAc) and isotopic niche overlaps (percentage) between the heavily oil-impacted (Gurepo, G and Padori, P) and non-impacted reference (Yeonpo, Y) sites using the Stable Isotope Bayesian Ellipse in R (SIBER) procedure.

| | Isotopic Niche Metrics | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Feeding Group** | **Gurepo** | | **Padori** | | **Yeonpo** | | **Percentage Overlap (%)** | |
| | **TA** | **SEAc** | **TA** | **SEAc** | **TA** | **SEAc** | **G and Y** | **P and Y** |
| July | | | | | | | | |
| Suspension feeder | 2.32 | 0.80 | 2.77 | 0.99 | 2.01 | 0.92 | 80 | 84 |
| Deposit feeder | 3.05 | 1.51 | 2.92 | 1.64 | 2.67 | 1.23 | 60 | 82 |
| Grazer | 8.18 | 2.98 | 8.31 | 2.40 | 9.45 | 3.23 | 76 | 62 |
| Omnivore | 6.38 | 3.45 | 4.00 | 2.20 | 3.27 | 1.89 | 71 | 60 |
| Carnivore | 2.73 | 1.48 | 3.30 | 1.70 | 2.13 | 0.99 | 66 | 55 |
| November | | | | | | | | |
| Suspension feeder | 2.04 | 0.80 | 1.97 | 0.70 | 1.31 | 0.66 | 41 | 40 |
| Deposit feeder | 3.89 | 1.83 | 3.62 | 1.95 | 3.21 | 1.70 | 54 | 83 |
| Grazer | 7.29 | 3.17 | 6.02 | 2.86 | 6.08 | 2.71 | 48 | 62 |
| Omnivore | 5.22 | 2.53 | 2.34 | 1.24 | 2.55 | 1.38 | 57 | 92 |
| Carnivore | 1.37 | 0.77 | 2.09 | 0.91 | 1.57 | 0.80 | 61 | 61 |

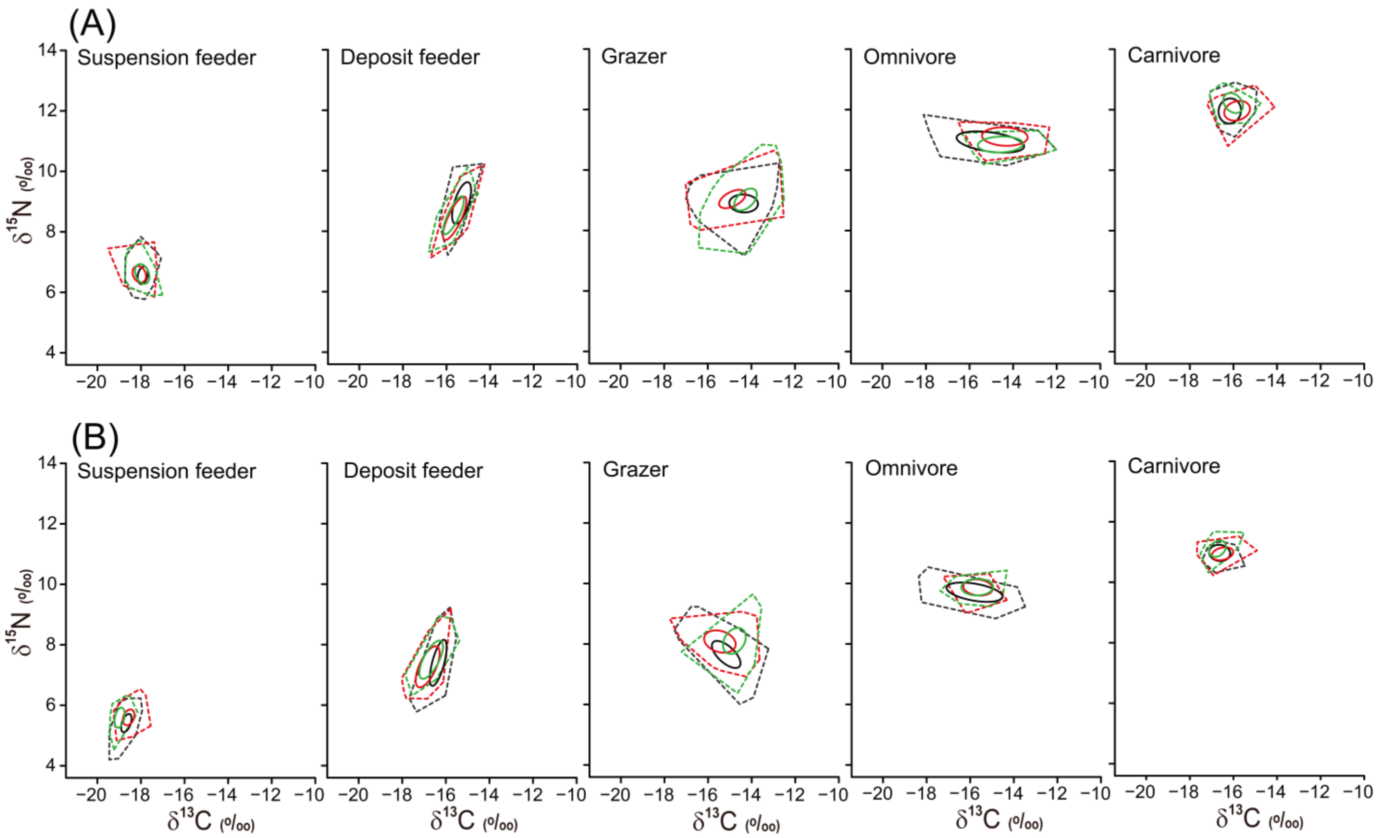


**Figure 3.** Isotopic niche comparisons of each feeding group (suspension feeder, deposit feeder, grazer, omnivore, and carnivore) estimated as the total area (TA, dotted lines) and standard ellipse area (SEAc, solid lines) among the two oil-impacted sites (Gurepo, red lines and Padori, green lines) and one non-impacted reference site (Yeonpo, black lines) in July (**A**) and November (**B**) using the Stable Isotope Bayesian Ellipse in R (SIBER) procedure.

## 4. Discussion

Our isotopic data showed that there were no significant differences in the carbon and nitrogen isotope signatures of consumer feeding groups and their isotopic niches between the test and control sites. Oil contamination has been considered to influence multiple trophic levels, and their relationships arise from top–down or bottom–up effects [33]. Therefore, similar isotopic signatures and niches of macrobenthic consumers between polluted and control sites provide evidence for the trophic recovery of rocky shore food webs related to the HSOS [7,17]. A comprehensive assessment of recovery time following the HSOS impacts revealed that damaged intertidal and subtidal benthic communities could recover their ecological status completely to the normal level after approximately 6 years [11]. Although the benthic communities on rocky shores were not consistent between the oiled and control sites at the sampling times used, the high similarity in isotope characteristics of the same macrobenthic feeding groups between the sites suggests almost complete recovery of the functions of the food web in terms of carbon and energy flows.

### *4.1. Comparison of Isotopic Signatures of Organic Matter Sources and Microbenthic Consumers between Oil-Impacted and Control Sites*

Coastal ecosystems impacted by direct or indirect anthropogenic activities can exhibit changes in dietary sources for macrobenthic consumers, thereby causing modification in the trophic structure [1,34]. However, in the present study, regardless of season, the $\delta^{13}C$ and $\delta^{15}N$ values of consumer species and their potential food sources at the oil-impacted sites were very similar to those at the control site. These isotopic similarities suggest that impacts on the food web through the feeding relationships between basal resources and their consumers after HSOS did not differ between the two sites [17,35]. In addition, the isotopic values of organic matter sources and animals at the two sites were within the ranges previously observed in the natural coastal waters off the Korean Peninsula [36–38]. When organic matter sources are still contaminated by crude oil at polluted sites, their $\delta^{13}C$ values may be decreased by 1‰ to 4‰ relative to those at the control site [39]. Such altered $\delta^{13}C$ values of organic matter sources by petrogenic hydrocarbons might be reflected in their incorporation into the macrobenthic food webs through the feeding of consumer species [17,40]. Here, the spatial isotopic consistency between the two sites suggests that there was no contribution from petrogenic hydrocarbon to the macrobenthic food webs. The concentrations of residual oils in seawater, sediments, and bivalves were estimated quantitatively through long-term assessment, and the results showed that they dropped to background levels [11]. The residual oils in seawater and sediments are decomposed mostly by microbial degradation; therefore, petrogenic hydrocarbon may not be incorporated into the food webs based on the carbon uptake by primary producers [41]. Accordingly, trophic consistencies between the two sites suggest that the rocky shore food web impacted by the HSOS could have been rehabilitated by natural conditions to that of the levels of unaffected environments in response to the modification of energy flow through food web structures.

Seasonal variations in community (both autotrophs and heterotrophs) and food web structures are observed commonly in coastal ecosystems in response to seasonal changes in environmental factors [42,43]. Normal coastal ecosystems constitute very complex environments and a variety of primary producers, resulting in seasonal variations in the trophic structure caused by the changes in the importance of dietary sources of consumer species. Similarly, our study also demonstrated seasonal variations in the isotopic ratios of macrobenthic consumers and their potential food sources regardless of the sites impacted by the HSOS. The seasonal differences in the isotopic ratios of consumer species might be linked to food availability, which can vary with seasons because of the change in the relative contribution of pelagic and benthic sources to the macrobenthic food webs. In addition, isotopic ratios of consumers were well classified by feeding strategies at both sites in both seasons, which was aligned with the general trend in natural ecosystems. The seasonal isotopic shifts of organic matter sources and feeding groups are consistent with the characteristics of the trophic base and feeding strategy of macrobenthic consumers reported for Korean coastal ecosystems [35–37,44]. As a result, seasonal variations in the isotopic ratios of all items might be exhibiting the same trend between the two sites, which suggests consistent normal trophic transfers from basal resources to higher trophic levels within the food webs in the oil-impacted sites.

### *4.2. Isotopic Niche Comparison of Microbenthic Consumers between Oil-Impacted and Control Sites*

Trophic functional modifications to macrobenthic communities following an environmental disturbance are likely to occur along with changes in the densities of dominant species [1]. Oil contamination can lead to a trophic niche shift with consequent changes in trophic levels and food chain lengths of consumers [17,45]. The trophic niche of consumers can be estimated by their isotope values, which provide information on the changes in the trophic structure caused by a variety of natural and anthropogenic disturbances [25,46,47]. Our results showed similar isotopic niche parameters in all the feeding groups between the oil-impacted and natural sites, suggesting that consumer species composed of the same feeding groups utilize similar nutritional resources under common trophic pathways regardless of the presence of oil spillage [48]. In addition, here we found high degrees of isotopic niche overlaps (58% to 84% in July and 40% to 92% in November) of each feeding group between the two study sites. The proportion of isotopic niche overlap represents a quantitative measure of trophic redundancy and segregation between consumer species or groups of consumers [49]. These results suggest that the same feeding groups might exploit similar basal resources and show high trophic redundancy between the oil-impacted and control sites [50]. In this respect, the overlap proportions suggest that the macrobenthic food web on the oil-impacted rocky intertidal habitats might be functionally similar to those in the natural habitats approximately 4 years after the HSOS. Accordingly, a comparison of the isotopic niche assessment of feeding groups of the oil-affected and natural sites suggests that the HSOS did not affect the trophic structure of the rocky shore community in the long term because of the trophic recovery of the ecological components.

### *4.3. Ecological Implication of Trophic Recovery after the HSOS*

The density and biomass of macrobenthic invertebrates decrease suddenly after oil spills, which tend to gradually recover with natural attenuation of residual oils with varying recovery periods in different cases [12]. Macrobenthic communities on the oil-impacted rocky shores in our study areas were fully recovered compared with the reference habitats by 67 months after the HSOS [11]. By contrast, Jung et al. [27] reported that, despite the evidence of macrobenthic recovery on the impacted rocky shores, there were noticeable differences in the abundance of dominant species between the oil-impacted and natural sites during the first 5 years after the HSOS because of the proliferation of the Pacific oyster *Crassostrea gigas* and the high abundance of *Lottia* spp. and *Odostomia aomori* at the oil-impacted site. These studies mostly focused on the environmental and structural recovery of the oil-impacted ecosystem by the approach based on residual oils and community analyses. However, assessments of the recovery of community- and ecosystem-level responses following oil spills should consider the structure of the food web [5,7]. Our results show functional recovery of the macrobenthic food web structure on the rocky shores based on similar trophic niches and higher overlap proportions of feeding groups between the oil-affected and natural sites. Following the impact of diverse pollution incidents, the recovery process in marine ecosystems can occur through the recolonization of organisms and increasing robustness of the trophic structure [17]. Overall, despite the differences in the compositions of the macrobenthic community in the oil-affected and natural sites, our results reveal the trophic recovery of rocky shore habitats from the impacts of the HSOS, as evidenced by the high equivalency of the trophic structure between the sites.

## 5. Conclusions

Our study provides reasonable evidence for the trophic recovery of an ecosystem approximately 4 years after the HSOS as observed by similar trophic characteristics of the oil-impacted rocky habitat to that of the natural habitat, with similar resource use by consumers and their trophic relationships. Furthermore, the similarity in isotopic niche indices of consumer feeding groups between the oil-affected and natural rocky shores suggests that the oil-impacted habitat might achieve the ecological function of energy transfers from basal resources to higher trophic levels in the natural ecosystem. Despite the controversy in the recovery of the macrobenthic community, these results indicate the natural conditions of the rocky shore ecosystem and its services due to the recovery of the food web structure. The recovery assessment based on the comparison of feeding groups between the oil-impacted and natural habitats using stable isotope data can provide valuable information on trophic recovery and ecological function of the ecosystem. Finally, this study enhances our understanding of the ecological processes of trophic recovery in coastal ecosystems that are impacted by oil spills and provides insights into the responses of such ecosystems to large-scale oil contamination using proper and effective assessment procedures.

**Author Contributions:** Conceptualization, H.J.P. and C.-K.K.; methodology, H.J.P., C.K. and C.-K.K.; formal analysis, H.J.P. and C.K.; investigation, H.J.P.; data curation, H.J.P. and C.K.; writing—original draft preparation, review and editing, H.J.P. and C.-K.K.; visualization, H.J.P. and C.K.; supervision, C.-K.K.; project administration, C.-K.K. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was supported by “Predictable Effects of Climate Change on Marine Ecosystem in The Tsushima Warm Current System of Korean Waters” program of Korea institute of Marine Science & Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (KIMST-20220558).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

**Acknowledgments:** Special thanks should be given to Dong Hoon Shin for his effort for field sampling.

**Conflicts of Interest:** The authors declare no conflict of interest.

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