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Species Composition and Distribution of Hull-Fouling Macroinvertebrates Differ According to the Areas of Research Vessel Operation

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Abstract: Global ecological concern regarding the transfer of fouling organisms to ship hulls is increasing. This study investigated the species composition, dominant species, distribution patterns, community structure, and life-cycle differences of hull-fouling macroinvertebrates on five research vessels (R/Vs: Isabu, Onnuri, Eardo, Jangmok 1, and Jangmok 2) operated by the Korea Institute of Ocean Science and Technology (KIOST). Hull-fouling macroinvertebrates were collected three to five times on quadrats from the upper and middle sectors of the hull sides, bottom, and niche areas (the propellers, shafts, and thrusters). A total of 47 macroinvertebrate species were identified, represented by 8519 individuals (ind.)/m^2 and a biomass of 1967 gWWt/m^2 on the five vessels. The number of species, density, and biomass were greater on the coastal vessels Eardo, Jangmok 1, and Jangmok 2 than on the ocean-going vessels the Isabu and Onnuri. Among the coastal vessels, barnacles were the most abundant and had the greatest density, while mollusks had the highest biomass. Differences between hull sectors showed that the highest species abundance and density appeared on all hulls in ports and bays where the Jangmok 1 operated, while the highest species abundance, density, and biomass were identified in the niche areas of the Eardo, which operated farther from the coast. The hull-fouling macroinvertebrates that exceeded 1% of all organisms were the barnacles *Amphibalanus amphitrite*, *Balanus trigonus*, and *Amphibalanus improvisus*; the polychaete *Hydroides ezoensis*; the bivalves *Magallana gigas* and *Mytilus galloprovincialis*; and the amphipod *Jassa slatteryi*. The dominant species were cosmopolitan and globally distributed, and many of them were cryptogenic. Six native species were identified: *M. gigas*, *H. ezoensis*, the amphipod *Melita koreana*, the isopod *Cirolana koreana*, and the barnacles *B. trigonus* and *F. kondakovi*. Eight non-indigenous species (NIS) were detected: the barnacles *A. amphitrite* and *A. improvisus*, the bivalve *M. galloprovincialis*, the polychaete *Perinereis nuntia*, the amphipods *J. slatteryi* and *Caprella californica*, and the bryozoans *Bugulina californica* and *Bugula neritina.* Of the fouling macroinvertebrates found on the vessel hulls, 13% were native, and 17% were NIS. More diverse communities developed on the hulls of vessels that operated locally rather than globally or in deep oceans. The species diversity index correlated positively with the total number of anchoring days and coastal operation days and negatively with the total number of operation days and ocean operation days. The macroinvertebrates differed by the area of operation, the port of anchorage, the number of days in operation and at anchor, and the hull sectors. There is no previous research data on hull-fouling macroinvertebrates in the Republic of Korea, and this study provides a basis for future studies to identify introduced species and their differences based on operation area.

Keywords: hull-fouling; macroinvertebrates; species composition; non-indigenous species (NIS); KIOST research vessels; Korea

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1. Introduction

As human activities and international trade expand [1], biodiversity and economic losses caused by introduced marine species are increasing, with non-indigenous species (NIS) being one of the major components of polluting communities [2]. NIS can cause biodiversity loss and change community structure through the local elimination of native species [2–4]. NIS can be introduced by ships and negatively impact marine ecosystems by destroying the habitats of native species, changing species compositions and reducing marine resources [5,6]. Ports are the main focus of NIS, and artificial structures are becoming spots of introduction [2,7]. Marine biofouling of ships docked in ports not only directly damages hulls via corrosion and deformation; it also greatly compromises sailing power, increasing fuel consumption, reducing speed, and lowering propeller efficiency by 15–20% [5,8].

Ship ballast water and hull-fouling organisms may be invasive and harmful, and hull-fouling is of particular concern, as ships are primary vectors for introducing NIS into new localities [9,10]. It has also been argued that floating structures such as hulls serve as major introduction and dispersion vectors with specific niches, anchors, propellers, and sea chests [11–14]. The colonization success of non-indigenous species is not seen to be driven primarily by habitat and local propagules rather than international traffic effects [11]. Quantitative techniques for assessing the transport of species through hull-fouling are not very widespread, although some data have been used to predict the abundance and diversity of organisms transported by ocean-going yachts in New Zealand and Scotland [15,16]. The expiration of paint efficiency and long periods of inactivity/reduced sailing increase the risk of hull macrofouling development [15–18].

Several risk assessment studies have been carried out abroad based on predictive tools for the prevention of the accidental introduction of marine NIS [15,16] and hull-fouling organisms as introduction vectors [10]. In Germany, species introductions by international ships were assessed from 1992 to 1996. NIS were found in 38% of all ballast water samples, 57% of all sediment samples, and 96% of all hull samples, indicating that hull-fouling is an important source of introduction; 57% of the species were not native to the North Sea [6]. Of percentages, all species found in New Zealand, 69% of NIS were found on hulls compared with 3% in ballast water [19]. In Japan, 26 species have been unintentionally introduced, and 42.3% of these are presumed to have been introduced by hull-fouling [20]. In Vladivostok, Russia, it was reported that spatially disconnected macrofouling communities of tropical and boreal origin may simultaneously coexist on the same ocean-going ships in the Far East Sea basin [21,22].

The International Maritime Organization has issued recommendations for the control and management of ship biofouling to minimize the transfer of introduced aquatic species [23]. The Korean Ministry of Oceans and Fisheries (MOF) reported 84,015 ship movements in 2017, of which 21,597 were domestic, and 62,418 were of foreign origin [24]. Korea has established a Second Basic Plan (2019–2028) for marine ecosystem conservation and management, as dictated by the Marine Ecosystem Act of 2009 [25]. Marine ecosystem disturbances were monitored, and harmful marine organisms were detected in 10 domestic ports in 2008–2012; research on how to manage the issues commenced in 2014 [26]. Therefore, although port-monitoring systems have been developed and regulations have been established for the systematic management of non-indigenous marine species, hull-fouling has not been addressed.

Habitat and native organism disturbance caused by NIS is decreasing in Korea [26–28], and interest in research on marine-introduced organisms has recently increased. In a survey of structures in 19 ports along the Korean coast between 2008 and 2013, a total of 26 species of invasive marine benthic organisms were recorded, of which 20 species of macroinvertebrates were found to be marine NIS [27]. However, in Korea, hull-fouling as a vector has received little attention, and no research has been conducted on hull-fouling macroinvertebrates due to research difficulties. Surveys of hull-fouling communities and settlement plates in ports provide suitable data for studying interactions between NIS due to spatial limitations and the abundance of species [29]. In the future, it is very likely that these will be considered environmental risks, as are organisms in ballast water. Therefore, it is important to proactively consider the regulatory framework of the IMO to limit the introduction of harmful organisms from abroad [23,30,31]. The International Maritime Organization (IMO) is a specialized agency of the United Nations that is responsible for measures to improve the safety and security of international shipping and to prevent pollution from ships.

To date, there has been no previous research on hull-fouling macroinvertebrates in Korea, and studies have only been conducted on artificial structures, floating objects, the rocky bottom, and intertidal zones in various ports. Therefore, the purpose of this study is (1) to reveal differences in the species composition and distribution of attached organisms depending on the operating area; (2) to identify domestically introduced species using ship hulls as a vector and to distinguish between native and non-native species; (3) to determine the correlation between the operating–anchoring conditions of ships and the distribution of attached organisms; (4) to determine and present ships' hull pollution status, prepare a preemptive response strategy to IMO regulations, and provide basic data for policy development.

2. Materials and Methods

2.1. Hull Sampling

In 2016 and 2017, we studied five research vessels currently in use by the Korea Institute of Ocean Science and Technology (KIOST) in Busan, Korea. All vessels were cleaned annually in a dry dock, and the sample survey was carried out immediately after the removal of all seawater from the shipyard dock just prior to cleaning. More samples and species were collected in dry docks than in water, for which the method of Meloni et al. [32] was followed. The hull-macrofouling samples were collected from four hull sectors in the dry dock (Supplementary Tables S1 and S2): the upper, middle, and bottom sectors (defined by reference to the waterline) and from the thrusters or propeller shafts (niche areas). Samples were collected in three to five replicates using a scraper from all samples within quadrats (15 × 15 cm) for four sectors of the five ship hulls (69 samples in total). Sampling was performed using an electric table lift on the upper, middle, and propeller-shaft sectors of large ship hulls. The samples were fixed in plastic bags containing a diluted 10% formalin solution and transported to the laboratory (Figures 1 and 2). All collected organisms were counted and identified to the species level by experts using a microscope and weighed to an accuracy of up to 0.001 g in the laboratory. The identification of hull-fouling macroinvertebrates followed the literature guidelines: Mollusks in Korea [33], Marine Mollusks in Japan [34], Annelida Polychaetes I, II, III [35–37], Marine Crustaceans in Korean Coasts [38,39], and Echinoderms of Korea [40]. Biomass was measured after the complete removal of moisture from the surface of the organism with strong absorbent paper. Individual numbers and biomass were converted to per unit area for comparison by each ship hull and sector. When collecting hull-fouling macroinvertebrates, photographs were taken to confirm the collection area before and after sample collection. Bottom and side gap samples were collected on both sections, and the propeller shaft was collected by rotating the quadrat, taking into account the curvature. Finally, a species list was generated using Microsoft Office Professional Plus 2019 Excel v1808 software (Supplementary Appendices S1–S5).

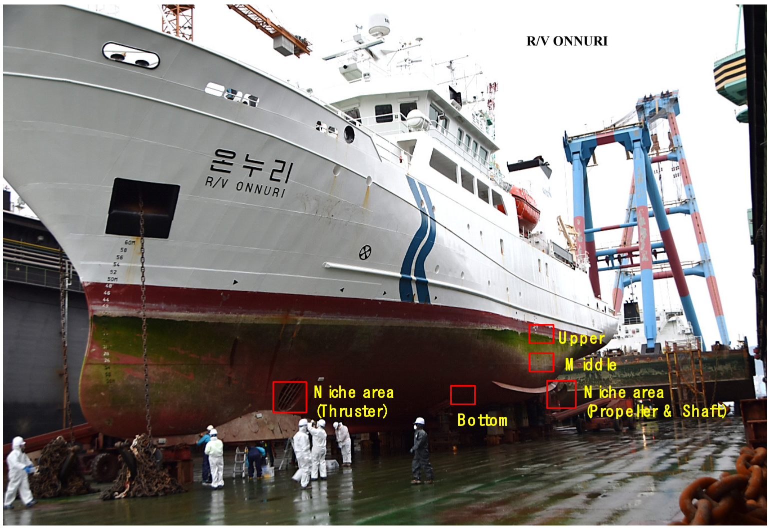

Figure 1. Sampling locations for attached hull-fouling macroinvertebrates. Samples were collected 3–5 times repeatedly using quadrats (15 × 15 cm) in each sector: upper, middle, bottom, and niche areas (propeller shaft or thruster).

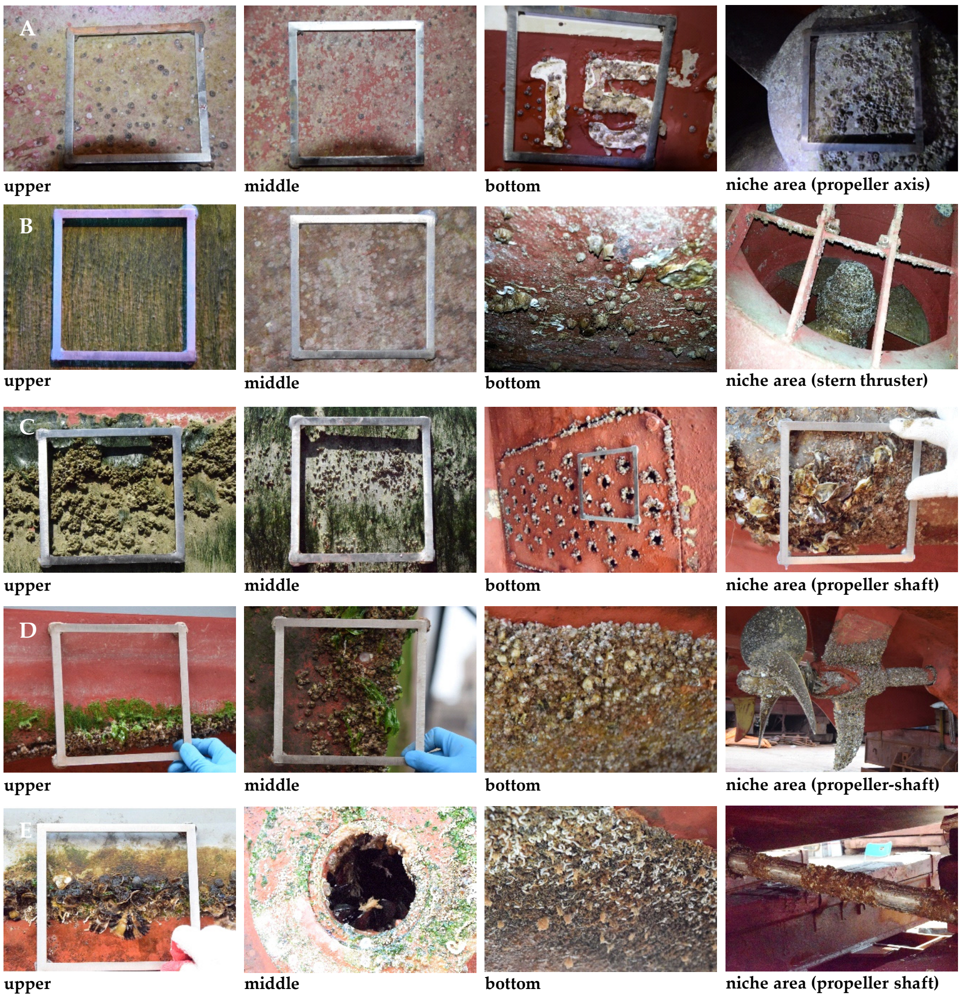

Figure 2. Attached hull-fouling macroinvertebrates on the five research vessels ((**A**): Isabu, (**B**): Onnuri, (**C**): Eardo, (**D**): Jangmok 1, (**E**): Jangmok 2; **upper**: waterline on the hull side, **middle**: between the waterline and bottom line, **bottom**: including the seawater inlet, **niche areas**: propeller, shaft, and thruster; quadrats: 15 × 15 cm).

2.2. Navigation Areas

The five vessels were divided into three categories according to navigation area and tonnage (Supplementary Table S2). The ocean-going Isabu and Onnuri, which principally survey the ocean worldwide, anchored in Guam in the Northwest Pacific Ocean, Colombo (Sri Lanka) in the North Indian Ocean, and Mauritius in the Southwest Indian Ocean. The coastal class Eardo mainly investigates the South and East Seas of South Korea. The local class Jangmok 1 mainly operates inshore, such as in ports and bays in the South Sea. The coastal-local class Jangmok 2 investigates the coastal, port, and bay areas of the East Sea, West Sea, and South Sea. In particular, the Jangmok 2 frequently operates in the Ulleung island area, 120 km away in the East Sea. After the research surveys, the Jangmok 2 anchored at an East Sea port and the other research vessels at the Jangmok port (adjacent to the South Sea) when they were not operating. The operational records of these research vessels for one year from 2016 to 2017 are presented (Supplementary Table S3, Figure 3).

Figure 3. Map showing the navigation areas of the Korea Institute of Ocean Science and Technology (KIOST) research vessels during 2016–2017 (green line: ocean worldwide class the Isabu, yellow line: ocean class the Onnuri, blue line: coastal class the Eardo, magenta line: local class the Jangmok 1, ocher line: coastal-local class the Jangmok 2; http://oceandata.kiost.ac.kr/realtime (accessed on 11 February 2023)).

2.3. Data Analysis

The faunal matrix resulting from abundances per species by sample was employed for subsequent analyses. The data were subjected to fourth-root transformation to focus on rare species and decrease the significance of common species during analysis. The number of individuals was converted into a per-square-meter measure [41]. Univariate analysis was conducted on community structure data, such as number of species, number of individuals, and biomass. In addition, the Shannon–Wiener diversity index ($\log_e H'$) and the Pielou evenness index (J') were calculated using the Primer-e v7 software [42,43]. Major species were selected as the species having an abundance of more than 1% of the total number of individuals. Before multivariate analysis, the null hypothesis that the sample follows a normal distribution was rejected, as the *p*-value was lower than the significance level alph = 0.05 ($p < 0.0001$), so non-parametric statistics were performed. This normality test was carried out using the Shapiro–Wilk, Anderson–Darling, and Lilliefors methods with the XLSTAT Basic 19.02 Addinsoft.

We examined community similarity using a nonmetric multidimensional scaling (nMDS) plot of the fouling communities based on Bray–Curtis similarities, which were generated per hull sector of each vessel. The similarity measure was calculated based on fourth-root transformed data of the number of individuals. Similarity profile tests (SIMPROF) from nMDS analysis were used for sample clusters. Similarity percentage (SIMPER) analysis was performed to identify the contribution of each species to the similarity and dissimilarity between the communities [44]. Community composition differences among vessels were tested using one-way ANOSIM (analysis of similarity) on the fourth-root transformed similarity matrix of each hull [45]. Furthermore, the heterogeneous dispersion among vessel groups was tested with permutation multivariate ANOVA (PERMANOVA) based on resemblance measures using permutations. All of the multivariate analyses were performed using PRIMER v.7 [46]. The Spearman rank correlations between the number of species, number of individuals, biomass, diversity index, and evenness index of macroinvertebrates on the hull and the total operation days (TODs), total anchoring days (TADs), coastal operation days (CODs), and ocean operation days (OODs) were analyzed using the XLSTAT Basic 19.02 Addinsoft. The total operation days and total anchoring days represent the number of days the five survey vessels operated and anchored, respectively, in one year. Coastal operation days and ocean operation days indicate the number of days each of the five survey vessels operated on the coast and ocean in one year. Ship operating records for this period (2016–2017) were downloaded from http://oceandata.kiost.ac.kr/realtime (accessed on 11 February 2023) (Supplementary Table S3).

3. Results

3.1. ISABU

The Isabu hull-fouling macroinvertebrates included seven species, with a mean density of 2516 ind./m^2 and a biomass of 220 gWWt/m^2. Only arthropods were detected: four species of amphipods and three cirripedes were found on the hull (Figures 4–6). The mean density was significantly lower compared with that of the other vessels. The barnacle *Amphibalanus amphitrite* Darwin, 1854, was distributed across the hull and somewhat higher on the side of the hull. The barnacle *Fistulobalanus kondakovi* Tarasov and Zevina, 1957, was found in propeller shaft crevices (six individuals), and the lepad *Conchoderma auritum* Linnaeus, 1767, was discovered on the bottom of the hull (15 individuals). Amphipods *Caprella equilibra* Say, 1818, and *Crassicorophium crassicorne* Bruzelius, 1859, were also found in that sector (one of each), and one individual each of *Monocorophium acherusicum* A. Costa, 1853, and *Pareurystheus anamae* Gurjanova, 1952, were discovered on the side of the hull. The biomass was highest on the bottom of the hull where *Conchoderma auritum* Linnaeus, 1767, was distributed (Figures 7–9). The major species (>density 1%) on Isabu were the barnacle *A. amphitrite* (96.9%), followed by the lepad *C. auritum* (1.9%) and the barnacle *F. kondakovi* (1%) (Figures 10 and 11). The species diversity indices of hull-attached macroinvertebrates were lower than those of other vessels (average 0.14). The bottom of the hull exhibited a low value of 0.33, followed by the propeller and shaft niches (0.16) and the sides (0.03). The evenness indices were very low compared with those of the other vessels (average 0.14) but were relatively high (0.24) on the bottom of the hull and the propeller shaft (Figure 12).

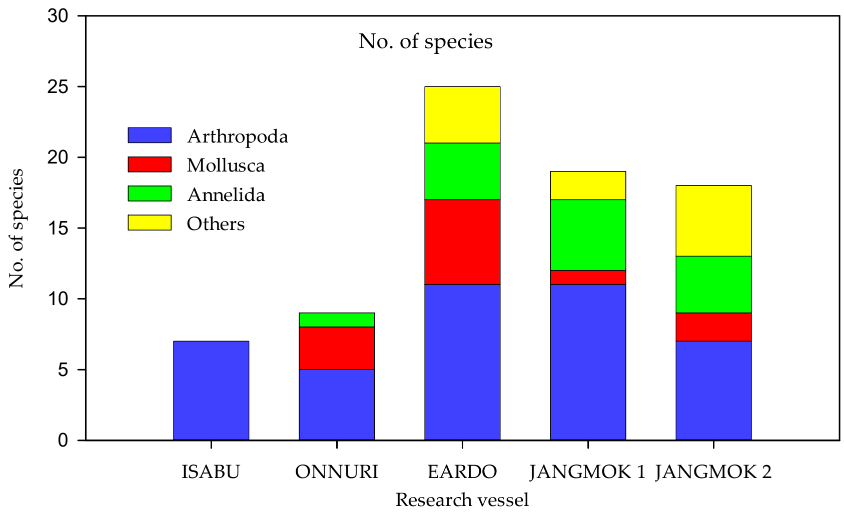

Figure 4. The number of species by taxa of attached macroinvertebrates on the vessels.

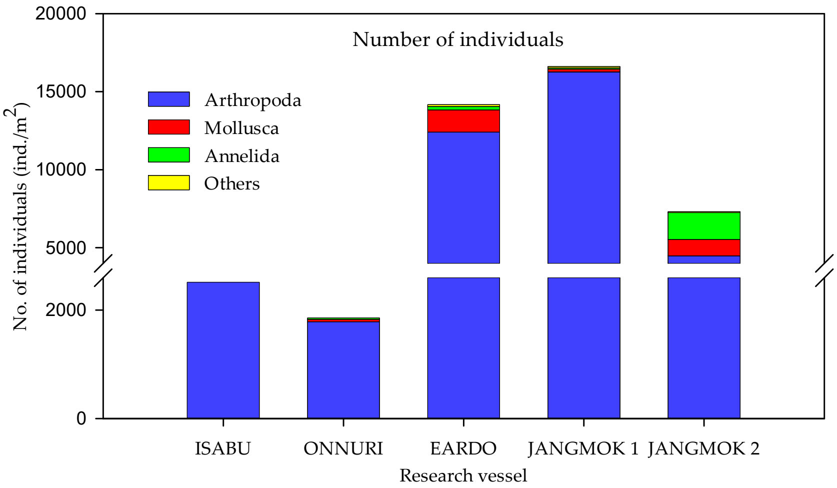

Figure 5. The number of individuals by taxa of attached macroinvertebrates on the vessels.

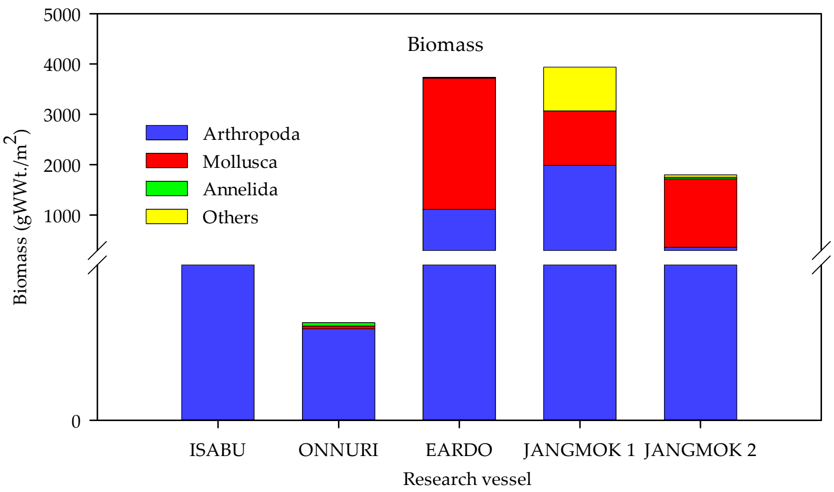

Figure 6. The biomass by taxa of attached macroinvertebrates on the vessels.

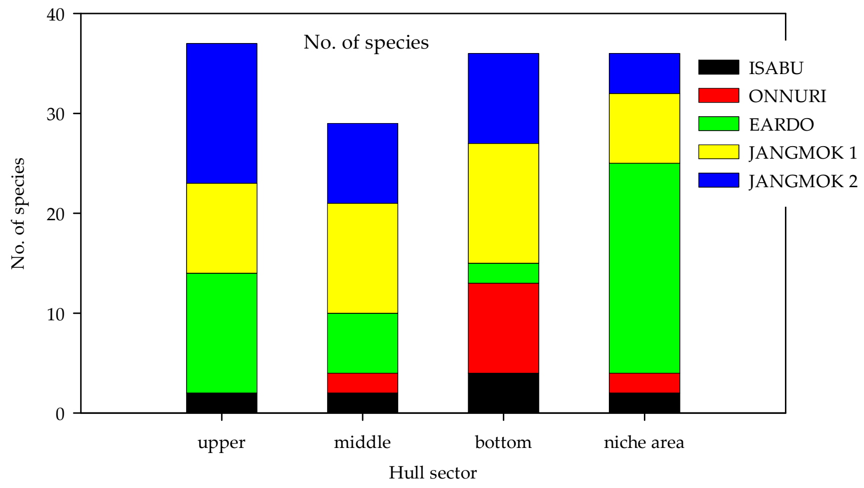

Figure 7. The number of species by hull sector of attached macroinvertebrates on the vessels.

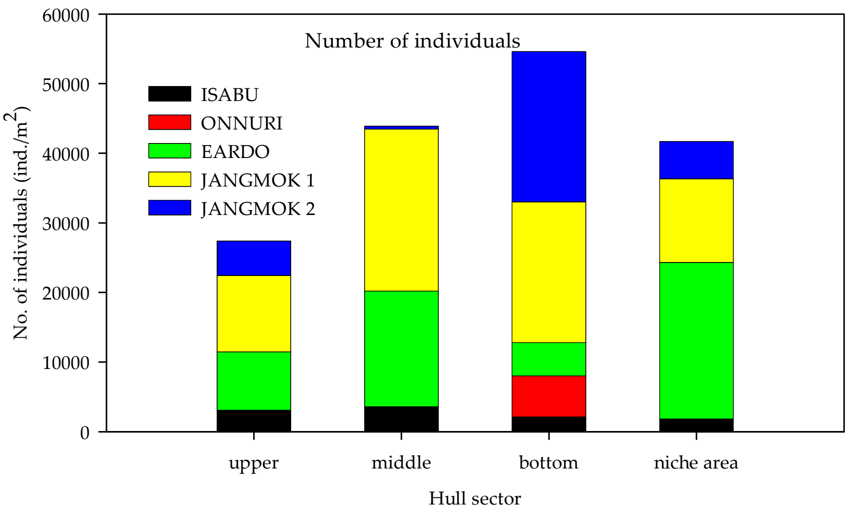

Figure 8. The number of individuals by hull sector of attached macroinvertebrates on the vessels.

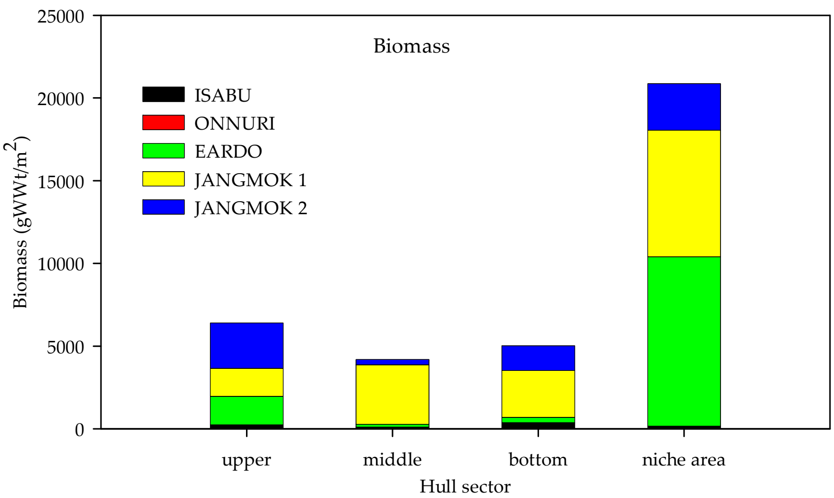

Figure 9. The biomass by hull sector of attached macroinvertebrates on the vessels.

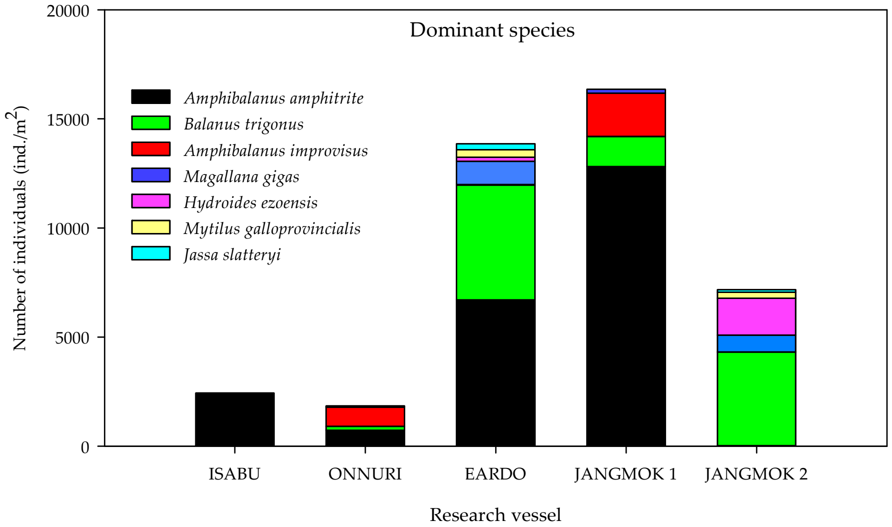

Figure 10. Distribution of dominant species of hull-fouling macroinvertebrates by vessel.

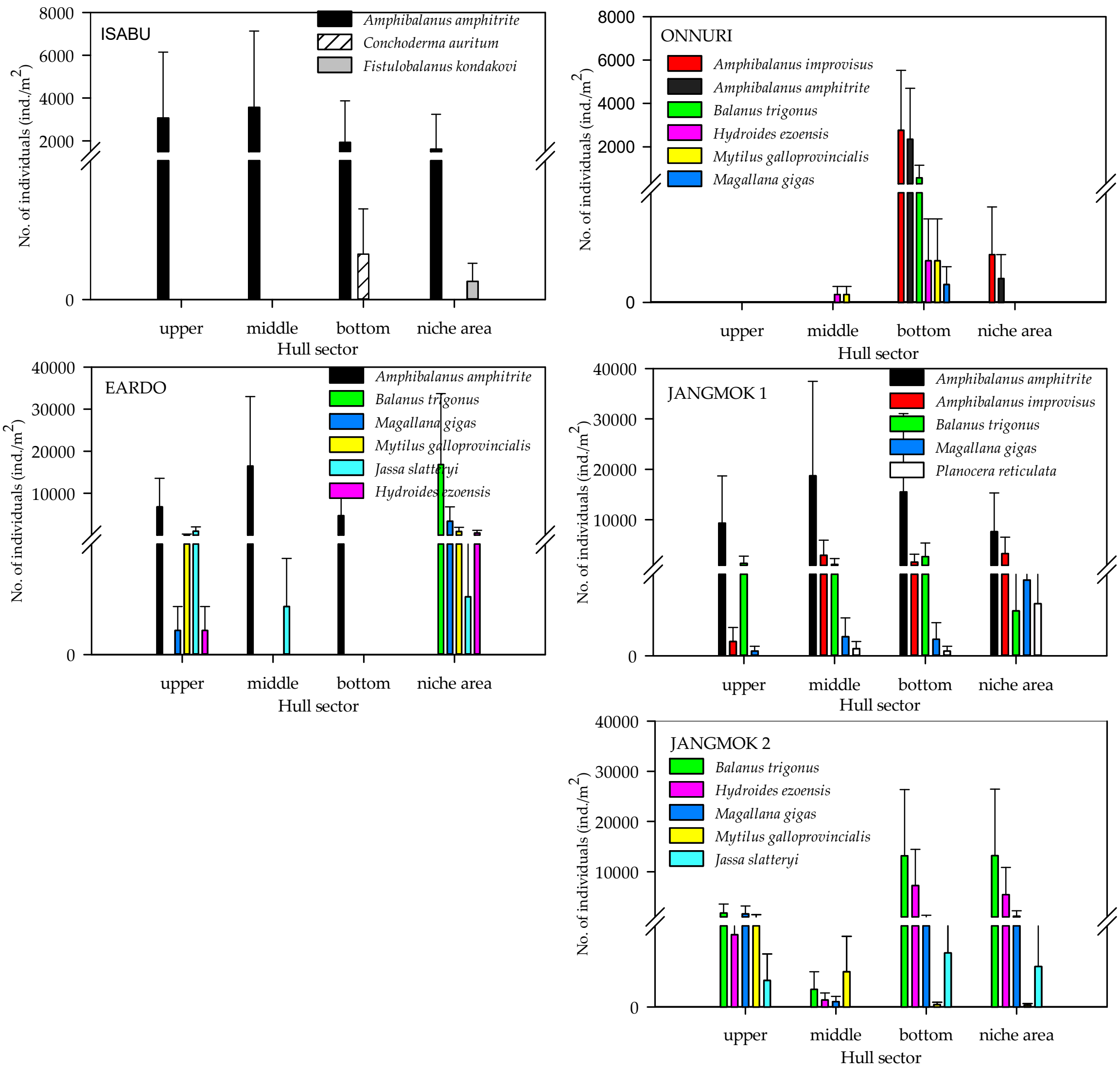

Figure 11. Distribution of dominant species of hull-fouling macroinvertebrates in each ship hull sector. The Isabu and Onnuri have a different scale than the other three vessels. Vertical lines are standard errors.

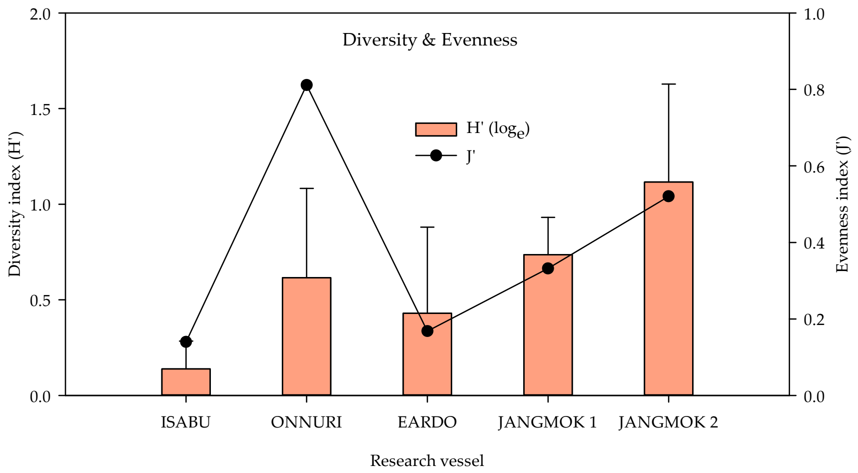

Figure 12. Diversity and evenness indices of hull-fouling macroinvertebrate communities on the vessels. Vertical lines are standard errors.

3.2. ONNURI

The Onnuri hull-fouling macroinvertebrates included nine species, with a mean density of 1856 ind./m^2 and a biomass of 31 gWWt/m^2. The taxonomic groups included five species of arthropod; the three barnacles *A. amphitrite*, *Amphibalanus improvisus*, and *Balanus trigonus* Darwin, 1854; the amphipods *Ericthonius pugnax* Dana, 1852, and *M. acherusicum*; the mollusks *Magallana gigas* Thunberg, 1793, *Mytilus galloprovincialis* Lamarck, 1819, and *Hiatella arctica* Linnaeus, 1767; and the polychaete *Hydroides ezoensis* Okuda, 1934. The mean densities were as follows: arthropods 1786 ind./m^2 (96.2%), mollusks 43 ind./m^2 (2.3%), and polychaetes 27 ind./m^2 (1.5%). Arthropods made up most of the biomass at 29 gWWt/m^2 (93.7%), followed by polychaetes at 1 gWWt/m^2 (3.4%) and mollusks at 0.9 gWWt/m^2 (2.9%) (Figures 4–6). By hull sector, nine species were on the bottom of the hull, and two were present on each of the middle of the hull sides and the niche areas (propeller and shaft). The mean densities on each hull sector were in the order of 5913 ind./m^2 for the bottom, 130 ind./m^2 for the niche areas, and 29 ind./m^2 for the middle sector. The biomass was the highest on the bottom (27 gWWt/m^2) and was low at 0.5 gWWt/m^2 in the niche areas and 0.1 gWWt/m^2 in the middle sector (Figures 7–9). The major species (>density 1%) were the barnacles *A. improvisus* 870 ind./m^2 (46.9%), *A. amphitrite* 732 ind./m^2 (39.5%), and *B. trigonus* 177 ind./m^2 (9.6%), followed by the polychaete *H. ezoensis* at 27 ind./m^2 (1.4%), the bivalves *M. galloprovincialis* at 27 ind./m^2 and *M. gigas* at 10 ind./m^2 (1.0%). The dominant species, *A. improvisus* and *A. amphitrite*, exhibited the highest densities on the bottom of the hull (Figures 10 and 11). The Onnuri species diversity indices averaged 0.62, with a maximum of 1.13 on the bottom of the hull, 0.69 in the middle sector on one side, and 0.64 in the niche areas (propeller and shaft). The evenness indices averaged 0.61, being relatively high in the middle sector of the hull sides (1.00), 0.92 for the niche areas, and low on the bottom (0.52) (Figure 12).

3.3. EARDO

The Eardo hull-fouling macroinvertebrates included 25 species, with a mean density of 14,171 ind./m^2 and a biomass of 3736 gWWt/m^2. The taxonomic groups included arthropods (11 species), mollusks (6), polychaetes (4), and other taxa (4). The mean densities were as follows: arthropods 12,408 ind./m^2 (87.6%) (mostly barnacles), mollusks 1424 ind./m^2 (10.0%), polychaetes 220 ind./m^2 (1.6%), and other taxa 120 ind./m^2 (0.8%). The biomass was highest for mollusks (2603 gWWt/m^2) (69.7%), particularly bivalves; this was followed by the arthropods at 1116 gWWt m^2 (29.9%), other taxa at 12 gWWt/m^2 (0.3%), and polychaetes at 6 gWWt/m^2 (0.1%) (Figures 4–6). By hull sector, there were 21 species in the niche areas (the aft propeller shaft), 12 species in the upper sector of the hull around the waterline, six species in the middle sector, and two species on the bottom.

The mean densities by hull sector were 22,496 ind./m^2 in the niche areas, given the high density of *B. trigonus*, followed by 16,598 ind./m^2 in the middle sector (principally *A. amphitrite*), 8402 ind./m^2 on the upper, and 4754 ind./m^2 on the bottom. The biomass by hull sector was highest at 10,231 gWWt/m^2 in the niche areas (principally *M. gigas* and *B. trigonus*), followed by 1732 gWWt/m^2 in the upper sector (mostly *M. galloprovincialis*), 319 gWWt/m^2 in the bottom sector, and 185 gWWt/m^2 in the middle sector (Figures 7–9). The six dominant species (>1%) were the barnacles *A. amphitrite* at 6704 ind./m^2 (47.3%) and *B. trigonus* at 5.266 ind./m^2 (37.2%), the bivalves *M. gigas* at 1065 ind./m^2 (7.5%) and *M. galloprovincialis* at 342 ind./m^2 (2.4%), the amphipod *Jassa slatteryi* Conlan, 1990 at 269 ind./m^2 (1.9%), and the polychaete *H. ezoensis* at 190 ind./m^2 (1.3%). By hull sector, the barnacle *A. amphitrite* dominated in the middle sector of the hull on both sides, and *B. trigonus* and *M. gigas* dominated in the niche areas on the propeller shaft (Figures 10 and 11). The species diversity indices were low (average 0.43); the highest values were 0.90 in the niche areas and 0.73 in the upper sector around the waterline. The figures for the middle and bottom sectors were very low at 0.05 and 0.04. The evenness indices were also very low (average 0.17); the values for the upper and niche areas were 0.29 and 0.30, and those for the middle and bottom sectors were 0.03 and 0.05 (Figure 12).

3.4. JANGMOK 1

The Jangmok 1 hull-fouling macroinvertebrates included 19 species, with a mean density of 16,616 ind./m^2 and a biomass of 3937 gWWt/m^2. The groups included arthropods (11 species), polychaetes (5), mollusks (2), and other taxa (2). The mean densities were as follows: arthropods 16,257 ind./m^2 (97.8%) with many barnacles, mollusks at 178 ind./m^2 (1.1%), polychaetes at 80 ind./m^2 (0.5%), and other taxa at 101 ind./m^2 (0.6%). The arthropod biomass was highest at 1985 gWWt/m^2 (50.4%), principally for the barnacles *A. amphitrite* and *A. improvisus*, followed by the mollusks at 1080 gWWt/m^2 (27.4%) (mostly *M. gigas*), other taxa at 869 gWWt/m^2 (22.1%), and polychaetes at 2.1 gWWt/m^2 (0.1%) (Figures 4–6). By hull sector, 12 species were on the bottom, followed by 11 in the middle sector of the hull side, nine on the upper around the waterline, and seven in the niche areas. The mean densities by hull sector were 23,246 ind./m^2 in the middle and 20,246 ind./m^2 on the bottom, attributable to the barnacles *A. amphitrite* and *A. improvisus*, followed by 12,029 ind./m^2 in the bottom and 10,942 ind./m^2 in the upper sectors. The biomass by hull sector was highest at 7647 gWWt/m^2 in the niche areas (mostly the bivalve *M. gigas*), followed by 3589 gWWt/m^2 in the middle sector and 2822 gWWt/m^2 in the bottom sector (many barnacles) (Figures 7–9). The dominant species (density > 1%) were barnacles (three species), a bivalve (one species), and turbellarians (one species); the barnacles were *A. amphitrite* at 12,812 ind./m^2 (77.1%), *A. improvisus* at 1986 ind./m^2 (11.9%), and *B. trigonus* at 1380 ind./m^2 (8.3%). The value for the bivalve *M. gigas* was 178 ind./m^2 (1.1%), and that for *Planocera reticulata* was 98 ind./m^2 (0.6%). All dominant species were on the hull. In particular, the most dominant, *A. Amphitrite*, was distributed at high densities on the sides and bottom; the values were 18,725 ind./m^2 for the middle and 15,522 ind./m^2 for the bottom; the densities in the upper and niche areas were lower at 9348 and 7652 ind./m^2, respectively. The number of individuals of the second most dominant species, *A. improvisus*, was 3290 ind./m^2 on the bottom (the propeller and shaft), 2971 ind./m^2 on the middle of the hull side, and 1594 ind./m^2 on the bottom of the hull. The number of individuals of the third most dominant species, *B. trigonus*, was 2696 ind./m^2 on the bottom, 1391 ind./m^2 in the upper area, and 1159 ind./m^2 in the middle. The number of individuals of the fourth most dominant species (the bivalve *M. gigas*) was 464 ind./m^2 in the niche areas on the propeller shaft (Figures 10 and 11). The species diversity indices averaged 0.74, being highest at 0.97 for the niche areas, followed by 0.79 for the bottom, 0.68 for the middle, and 0.50 for the upper sector. The evenness indices were relatively high (average 0.33). The values were 0.50 for the niche areas, 0.32 for the bottom, 0.28 for the middle, and 0.23 for the upper sector (Figure 12).

3.5. JANGMOK 2

The Jangmok 2 hull-fouling macroinvertebrates included 18 species, with a mean density of 7307 ind./m^2 and a biomass of 1801 gWWt/m^2. The taxa were arthropods (seven species), other taxa (five), polychaetes (four), and mollusks (two). The mean densities were as follows: arthropods at 4475 ind./m^2 (61.2%) (many *B. trigonus* barnacles), followed by polychaetes at 1720 ind./m^2 (23.5%) (many of the serpulid polychaete *H. ezoensis*), mollusks at 1053 ind./ m^2 (14.4%) (many bivalves, i.e., *M. gigas* and *M. galloprovincialis*), and other taxa at 59 ind./m^2 (0.8%). The biomass was the highest for mollusks (1358 gWWt/ m^2; 75.4%), principally the bivalves *M. gigas* and *M. galloprovincialis*. The values were 361 gWWt/m^2 (20.1%) for the arthropods, 46 gWWt/m^2 (2.5%) for other taxa, and 36 gWWt/m^2 (2.0%) for the polychaetes (Figures 4–6). By hull sector, there were 14 species in the upper sector around the waterline, nine on the bottom, eight in the middle, and four in the niche areas (propeller and shaft). The mean densities by hull sector were 21,580 ind./m^2 on the bottom (many *B. trigonus* and *H. ezoensis*); meanwhile, the upper and bottom sectors exhibited relatively low densities of 4449 and 5333 ind./m^2, respectively, and the middle sector had a very low density of 446 ind./m^2. By hull sector, the biomass was the highest (2814 gWWt/m^2) in the niche areas (propeller and shaft) (principally *M. gigas* and *B. trigonus*). It was slightly lower for the upper sector (2737 gWWt/m^2, principally *M. gigas* and *M. galloprovincialis*) and at the bottom (1508 gWWt/m^2, mostly *M. gigas*, *H. ezoensis*, and *B. trigonus*). The value in the middle sector was very low (324 gWWt/m^2) (Figures 7–9). The dominant species (density > 1%) were the barnacle *B. trigonus* at 4314 ind./m^2 (59.0%), the polychaete *H. ezoensis* at 1689 ind./m^2 (23.1%), the two bivalves *M. gigas* at 780 ind./m^2 (10.7%) and *M. galloprovincialis* at 273 ind./m^2 (3.7%), and the amphipod *J. slatteryi* at 118 ind./m^2 (1.6%). The density of *B. trigonus* was highest (13,217 ind./m^2) in the niche areas and at the bottom (13,174 ind./m^2) of the hull. The second dominant species, *H. ezoensis*, exhibited the highest density of 7232 ind./m^2 on the bottom and a density of 5424 ind./m^2 in the niche areas. The number of individuals of the third most dominant species, *M. gigas*, was 1587 ind./m^2 in the upper section of the hull waterline and 1109 ind./m^2 in the niche areas. See Figures 10–12 for other data.

3.6. Non-Indigenous Species

According to a report by MOF [27] based on a survey of several domestic ports, it was revealed that 26 marine benthic NIS, including animals and plants, were introduced into Korea. Among these, five species appeared repeatedly in this survey: the bivalve *M. galloprovincialis*, the barnacles *A. amphitrite* and *A. improvisus*, and the bryozoans *B. neritina* and *Celleporaria brunnea* Hincks, 1884. However, among the 47 hull-fouling macroinvertebrates that appeared in this survey, six native species and eight NIS appeared (Supplementary Table S4). The native species were the bivalve *M. gigas*, the polychaete *H. ezoensis*, the amphipod *Melita koreana*, the isopod *Cirolana koreana*, and the barnacles *B. trigonus*, and *F. kondakovi*. The NIS (Figure 13) were the bryozoans *Bugulina californica* and *B. neritina*, the bivalve *M. galloprovincialis*, the polychaete *Perinereis nuntia*, the amphipods *Caprella californica* and *J. slatteryi*, and the barnacles *A. amphitrite* and *A. improvisus*. Non-indigenous barnacle species, i.e., *B. trigonus* and *F. kondakovi*, appeared in abundance on the hulls of the ocean-going vessels the Isabu and Onnuri and the coastal vessels Eardo and local Jangmok 1. In addition, the bivalve *M. galloprovincialis* and amphipod *J. slatteryi* appeared in abundance on the hull of the coastal–local Jangmok 2. Among the 47 species of fouling macroinvertebrates that were found on the hulls of five vessels, native species accounted for 13%, NIS accounted for 17%, and species of unknown origin accounted for 70%.

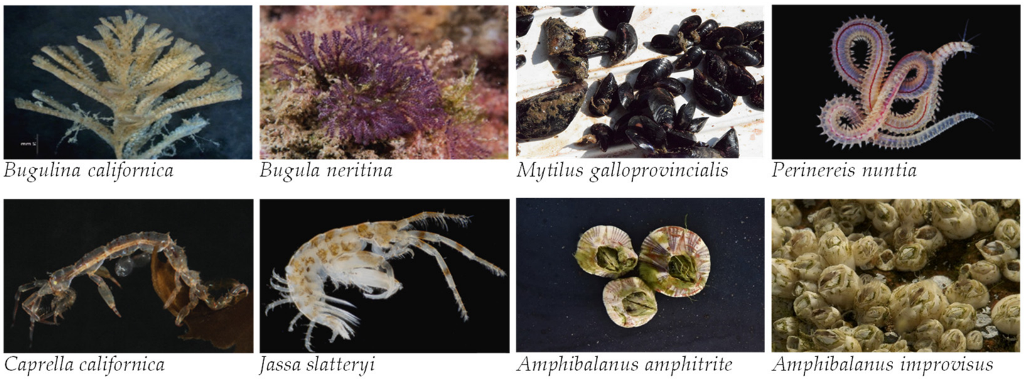

Figure 13. Eight NIS of hull-fouling macroinvertebrates introduced by five research vessels among the species introduced into Korea: bryozoans *B. californica and B. neritina*; bivalve *M. galloprovincialis*; polychaete *P. nuntia*; amphipods *C. californica and J. slatteryi*; barnacles *A. amphitrite* and *A. improvisus*.

3.7. Community Analysis

Sixty-nine samples of macroinvertebrates attached to the hulls of five research vessels were analyzed. The nMDS ordination analysis of the five vessels revealed four clusters of fouling invertebrate communities (ANOSIM global R-value: 0.90, $p < 0.001$): two ocean clusters (the Isabu and Onnuri), one coastal cluster (the Eardo and Jangmok 2), and one local cluster (the Jangmok 1). Vector and species overlays were produced using Pearson correlations between the species' variables and the ordination MDS 1 and MDS 2 axes. This is offered purely as an exploratory tool to visualize potential linear or monotonic relationships between a given set of variables and ordination axes (Figure 14).

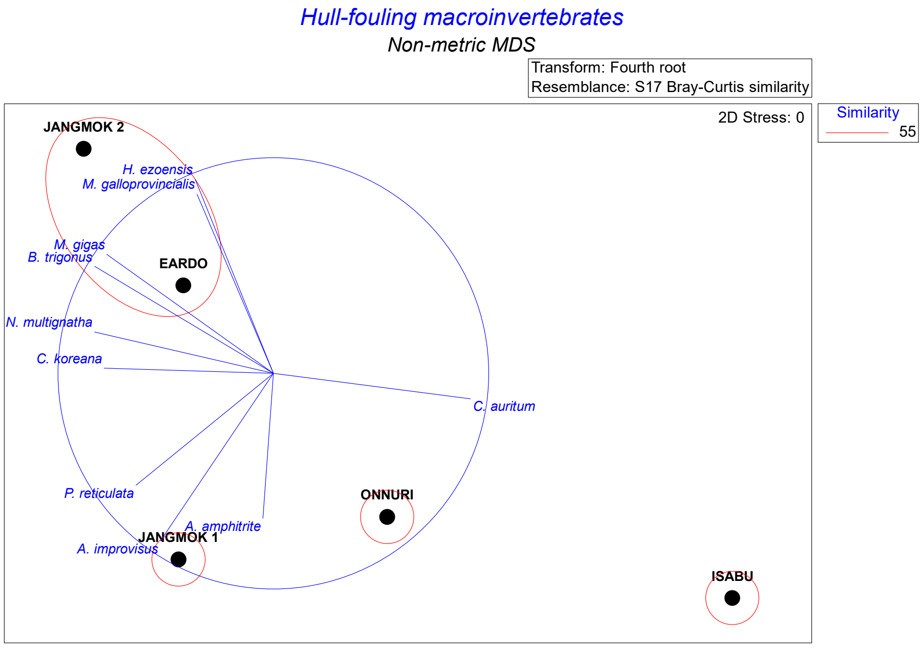

Figure 14. Nonmetric multidimensional scaling (nMDS) ordination of Bray–Curtis similarities of fourth-root transformed species abundance data from the five KIOST research vessels (SIMPROF test: the groups are separated by red lines; ANOSIM results: Global test R = 0.90, $p < 0.001$). The stress value is a representation of the spatial dispersion based on resemblances among 69 samples. Vector and species overlays were produced using Pearson correlations between the species variables and the ordination MDS 1 and MDS 2 axes (blue lines). This is offered purely as an exploratory tool to visualize potential linear or monotonic relationships between a given set of variables and ordination axes.

SIMPER analysis was performed to identify the contribution of each species to the dissimilarity between the vessel communities. As a result, the following species that contributed most to community composition were identified. The Isabu and Onnuri (group av. dissimilarity: 69.0%): *A. improvisus* (18.6%), *B. trigonus* (12.5%), *Conchoderma auritum* (9.1%); the Isabu and Jangmok 1 (group av. dissimilarity: 75.5%): *A. improvisus* (12.5%), *B. trigonus* (11.4%), *M. gigas* (6.8%), *A. amphitrite* (6.2%); the Isabu, Eardo, and Jangmok 2 (group av. dissimilarity: 90.6%): *B. trigonus* (12.3%), *M. gigas* (8.1%), *H. ezoensis* (7.7%), *A. amphitrite* (7.3%), *M. galloprovincialis* (6.2%); the Onnuri and Jangmok 1 (group av. dissimilarity: 51.3%): *A. amphitrite* (13.2%), *Planocera reticulata* (7.8%), *Nereis multignatha* (6.7%), *Cirolana koreana* (6.4%), *B. trigonus* (5.9%); the Onnuri, Eardo, and Jangmok 2 (group av. dissimilarity: 51.3%): *B. trigonus* (8.9%), *A. amphitrite* (8.8%), *A. improvisus* (8.1%); and the Jangmok 1, Eardo, and Jangmok 2 (group av. dissimilarity: 61.6%): *A. amphitrite* (8.9%), *A. improvisus* (8.0%), *H. ezoensis* (7.7%), and *M. galloprovincialis* (6.2%) (Supplementary Table S5).

One-way ANOSIM was performed to test the null hypothesis that there were no significant differences between the macroinvertebrate communities among the vessels without considering clusters divided by nMDS analysis (global R-value: 0.532, $p < 0.001$). In the pairwise test, the Isabu cluster showed differences from the Onnuri cluster (R = 0.34, $p < 0.05$), the Jangmok 1 cluster (R = 1.0, $p < 0.05$), and the Jangmok 2 cluster (R = 1.0, $p < 0.05$). The Onnuri cluster showed differences from the Jangmok 1 cluster (R = 0.32, $p < 0.05$) and the Jangmok 2 cluster (R = 0.39, $p < 0.05$). The Eardo cluster showed differences from the Jangmok 1 cluster (R = 0.46, $p < 0.05$) and the Jangmok 2 cluster (R = 0.64, $p < 0.05$). Additionally, the Jangmok 1 and Jangmok 2 clusters were different (R = 1.0, $p < 0.05$). However, pairwise tests showed no significant differences between the Isabu and Eardo clusters (R = 0.35, $p > 0.05$) and the Onnuri and Eardo clusters (R = 0.08, $p > 0.05$) (Supplementary Table S6); R-values in the range of $0.25 < R < 0.5$ means differences with some overlap, and $0.5 < R$ means differences between clusters. Permutation multivariate ANOVA (PERMANOVA) tests showed significant differences between vessel groups (df = 4, F = 4.47, $p < 0.001$), and pairwise tests also showed similar results to ANOSIM, with no significant differences between the Onnuri and Eardo clusters (t = 1.08, $p > 0.05$).

Spearman rank correlations between the biological indices and vessel operating conditions were analyzed (Supplementary Table S7). Operating conditions were analyzed using one year's operating records for the five vessels, which included the total operation days (TODs): the Isabu, 219; the Onnuri, 165; the Eardo, 180; the Jangmok 1, 141; the Jangmok 2, 138. Total anchoring days (TADs) were the following: the Isabu, 146; the Onnuri, 200; the Eardo, 185; the Jangmok 1, 224; the Jangmok 2, 227. The coastal operation days (CODs) were the following: the Isabu, 157; the Onnuri, 255; the Eardo, 365; the Jangmok 1, 365; the Jangmok 2, 365. Moreover, ocean operation days (OODs) were the following: the Isabu, 208; the Onnuri, 110; the Eardo, 0; the Jangmok 1, 0; the Jangmok 2, 0 (Supplementary Table S3). Biological indices such as the number of species, number of individuals, and biomass correlated highly and positively with the number of days of coastal operation and negatively with the number of days of ocean operation. The species diversity index (H′) correlated positively with the total numbers of anchoring and coastal operation days and negatively with the total numbers of operation days and ocean operation days.

4. Discussion

4.1. Hull-Fouling Macroinvertebrates

Meloni et al. [32] reported that more samples and species were collected in dry docks than in water. In this study, 47 species of hull-fouling macroinvertebrates were identified, represented by 8519 ind./m^2 and a biomass of 1967 gWWt/m^2. The 47 species of macroinvertebrates (Supplementary Table S4) that appeared in this study were slightly higher than the 6–42 species found on ship hulls operated in the Far East basin, as reported by Moshchenko and Zvyagintsev [21,22]. In terms of density, the barnacles were the most numerically abundant on all surveyed vessels and were dominated by the suspension- or filter-feeding barnacles *A. amphitrite*, *B. trigonus*, and *A. improvisus*. In particular, barnacles are greatly resistant to dislodgement by currents and waves, as their adhesion strength is very high (0.02 MPa in juveniles to over 2.00 MPa in adults), although variable depending on the substratum, species, and age/size of the individuals [17,18,47]. These results are believed to be one of the reasons why barnacles, along with attached organisms, take over ship hulls and spread worldwide.

Relatively more species, individuals, and biomass were found on coastal-operated hulls than on ocean-going vessels. Conversely, the ocean-going Isabu and Onnuri, with fewer anchorage days, exhibited low values. In particular, the coastal-operated Eardo and the local-operated Jangmok 1 hulls, which had 151 and 186 long anchorage days in the South Sea, with a high average annual water temperature of 15.7–18.8 °C [48], had high biomass, driven by the bivalve mollusk *M. gigas* and the barnacle *A. amphitrite*. Conversely, the hull of the coastal- and local-operated Jangmok 2, which had a long stationary period of 115 days in the East Sea with a low average annual water temperature of 8.6–15.9 °C, had a low number of individuals and biomass [48–50]. These results suggest that prolonged port stays of ships increase the risk of fouling attachment to the hulls [29,51–53] and that seawater temperature is a major geographical determinant of the composition of marine communities, including biofouling composition, influencing the spawning period, settlement, growth, and reproduction of organisms [54]. In addition, it was confirmed through settlement plate experiments that salinity and port significantly affect the composition of the fouling assemblages [55]. However, prolonged ocean operations may reduce biofouling by creating low-food, high-flow conditions that stress hull-adhering organisms [52,56].

The attachment level of hull-fouling macroinvertebrates shows different distribution patterns depending on the sectors of the hull. Submerged sections and niche areas (i.e., in the cavities and metallic parts) of the hull were the best predictors for NIS abundance on ships [12,13]. Studies on these hull sections and niche areas may show differences in attached organisms depending on the various operating conditions of the ship [12,13,52,53,57]. This study also showed similar results. The Eardo, operating in coastal areas, exhibited very high biomass on the propeller shaft, with organisms more commonly attached to the niche areas than to the hull. However, the Jangmok 1 (141 days of operation, 224 days of anchorage), which operates in the warm southern seas, had a high number of species and individuals on the side and bottom of the hull. The Jangmok 2 (138 days of operation, 227 days of anchorage), which operates in the East Sea in low water temperatures, had a high number of species on the sides of the hull and individuals on the bottom of the hull (Supplementary Table S3, Figures 7–9). Therefore, it is highly likely that the attachment pattern of hull-fouling macroinvertebrates will vary depending on the vessel's operating area, anchorage port, number of anchorage days, and hull sector.

4.2. Dominant and Non-Indigenous Species

Eight NIS were found on the hulls of the five vessels during the survey periods (Supplementary Table S4). The 26 marine benthic NIS reported in Korea [27] include all animals and plants attached to floating structures, rocks, nets, etc., in 19 domestic ports with frequent ship traffic, while the eight species in this study can be understood through differences in research results for only hull-attaching organisms. Among these non-indigenous, the barnacle *A. amphitrite* was dominant on the ocean-going Isabu hull, and two species, along with *A. improvisus*, were dominant on the Onnuri hull (Figures 10 and 11). In addition, *A. amphitrite* is widely distributed in Korea, with many individuals found on the hulls of the coastal-operated Eardo and Jangmok 1, which operate in ports and bays. Non-native species are believed to have already been introduced, settled, and widely distributed in the country. In this study, 47 species, of which 13% were native and 17% were non-indigenous, were found, which is lower than the 60 species, of which 40% were native and 25% were non-indigenous in New Zealand [14], that are located at the same mid-latitude. This aspect can be attributed to the geographical differences between the southern and northern hemispheres and differences in the number and duration of survey vessels.

Most of the dominant species and NIS that are attached to the hull and spread worldwide are known to be highly adaptable to environmental changes such as temperature, salinity, flow rate, space, competition, substrate, etc. [29]. Based on the track records of research vessels in tropical to temperate regions (Figure 3), the main hull-fouling macroinvertebrates listed in Supplementary Table S4 are presumed to be highly adaptable to changes in water temperature and salinity [58–60]. Spawning may be induced by changes in temperature or salinity upon entry into a port, according to seasons [50,54,55,61]. If the spawning season of the attached organisms does coincide with the time the ship anchors and stays in port, they will be able to settle. Therefore, organisms can be spread by ships that frequently enter and leave domestic and foreign ports [62]. In addition, species that are resistant to various environmental conditions, such as water temperature and salinity in the anchorage areas, and have a strong survival capacity can successfully colonize, so these species will attach to the hull of the ship and play the role of invaders [63,64]. Sessile taxa can withstand high water velocities and protect adjacent mobile organisms from water forces, increasing introduced species diversity [52,65]. These results demonstrate how dominant species and NIS can spread globally through the hulls of ships. It is true that most dominant species have various geographical distribution forms and are spread widely around the world, so there are limits to determining whether they are native and NIS.

The most dominant species, barnacle *Amphibalanus amphitrite* Darwin, 1854 [66], was once found in the Cenozoic stratum of Japan [67] but then disappeared and reappeared in the Far East [68]. However, the organism is now found in the intertidal zones of southeastern Korea. It has been introduced to tropical/warm-temperate waters worldwide, primarily via hull-fouling and possibly also with oyster *M. gigas* shipments or in ballast water [69]. As early as 1854, Darwin noted that this species and a few other barnacles "which seem to range over nearly the whole world (excepting the colder seas)" could have been transported to some regions via ship fouling [70]. This species was absent from Korea before 1970 but is now the most widespread species along the entire coast, including brackish waters. It was introduced in ballast water or on hulls in the early 1970s when international trade began to increase; it was the first introduced species found in Korea [70]. *A. amphitrite* competes with the native barnacle *Fistulobalanus albicostatus*; in some parts of the southern coast, *F. albicostatus* is disappearing [19]. A previous study [71] reported that *A. amphitrite* was the dominant sessile species on artificial submerged polyvinylchloride (PVC) substrates from June to September in Jangmok Bay, Geoje Island, southern Korea. In our study, *A. amphitrite* dominated on four of the research vessels but not on Jangmok 2, which operates mainly in the Korean East, West, and South seas; the longest stay at an East Sea port was 115 days. In particular, *A. amphitrite* was present at high densities on the sides of the hull of the Eardo and the bottom of the Jangmok 1 but at low densities on the hulls of the Isabu and Onnuri. This species is thought to have spread along the entire Korean coast [71].

The barnacle *Balanus trigonus* Darwin, 1854 [66] is a cosmopolitan species; large numbers have been observed on the spiny lobster *Panulirus gracilis* in western Mexico [72]. In the eastern Pacific, it spreads from Monterey, California, to Peru, including the entire Gulf of California [73]. It does not appear to have been in the Atlantic Ocean basin prior to the 1850s. These common fouling barnacles may have been introduced to the South Atlantic by ships from the Pacific and Indian oceans sailing via Cape Horn and the Cape of Good Hope [74]. This species is endemic to the Indo-Pacific region and is known to be an opportunistic fouling species. Therefore, it is likely to be a native species. On the New Zealand coast, *B. trigonus* is an opportunistic species that attains sexual maturity within 14 days; the high growth rate, early maturity, and high predation level are associated with a short generation time and high population turnover [75]. In the laboratory, the time from the newly hatched nauplius stage to the cyprid stage is 9–13 days (average 11 days) [76]. *B. trigonus* juveniles settle on hulls when ships visit tropical ports [77]. The densities were high in the niche areas and bottoms of the Eardo, Jangmok 1, and Jangmok 2, but the species was not found on the hull of the Isabu. In general, *B. trigonus* attaches at high densities to objects in lower intertidal zones, underwater structures, fishing nets, and the shells of mussels in shallow water, which is clear and warm along all Korean coasts.

The barnacle *Amphibalanus improvisus* Darwin, 1854 [66], is found in temperate and tropical oceans from the Atlantic coasts of America to Europe. It is not known where the species originated, perhaps in North America or Europe [78]. The organism has colonized many oceans, including Indo-Pacific and Australasian waters, via ship biofouling [79]. The organism has been reported from underwater structures in brackish lakes (salinity 8 psu) of the eastern coast of the South Sea and in an East Sea port; in the mid-1980s, it was found at high levels in the brackish Lake Jumunjin of the Korean East Coast. The organism attaches to oyster/mussel culture frames and powerplant drains. In the future, it is likely to spread to western coastal areas, posing a risk to native species; continuous monitoring and management are necessary [70,80]. We found that this species was most common on the bottom of the Onnuri but the entire hull (principally the propeller and shaft) of the Jangmok 1; it was absent from the Isabu and Jangmok 2. The reason why it was present in high amounts on the bottom of the Onnuri is probably because the amount of adhesion increased as seawater was sucked in from the bottom intake part. The average annual water temperatures in South Korea are 8.6–15.9 °C on the east, 10.9–14.9 °C on the west, and 15.7–18.8 °C on the south coasts [48]. The Jangmok 2 anchored for 34 days in the West Sea, where the current is strong at 1 m/sec, and the average tidal difference is 7 m, and for 115 days in the East Sea, where the water temperature is low. Therefore, hull larval settlement was unlikely.

The polychaete *Hydroides ezoensis* Okuda, 1934 [81], is a tube-forming annelid that is native to the temperate Northern Pacific, Northwest Pacific, and the central Indo-Pacific; it is widely distributed in the intertidal zone and on submerged rocks, shells, and hulls in Korea, Russia, China, and Japan [82]. This species was first described in 1934 in waters around Hokkaido, Japan, but later became established in seas around Great Britain [83] and Australia. Therefore, this species is likely to be native to Korea. It commonly adheres to hulls, docks, industrial water intakes, aquaculture facilities, and sometimes muddy gravel substrates. It was first recorded in Geomun-do and Baek-do in the South Korean Sea in 1978 [84] and spawns from April to December. The trochophore larvae hatch 20 h after fertilization and settle 6 days later [85]. The species forms calcareous tubes on the shells of oysters in hanging cultures (damaging the oysters) [86] and is the dominant sessile species on artificial PVC substrates from May to October in Jangmok Bay, Geoje Island, southern Korea [71]. We found that this species dominated the bottom seawater intake plate and propeller shaft of the Jangmok 2 and was also found (in lower numbers) on the hulls of the Onnuri and Eardo. The larvae of this species settle in regions with good water flow and water temperatures above 10–11 °C. Off Pohang, in the southern part of the Korean East Sea, this temperature is maintained from mid-April to mid-December [85]. Therefore, if a vessel is anchored in port at this time, the likelihood of attachment increases.

The bivalve *Magallana gigas* Thunberg, 1793 [87], grows rapidly and tolerates a wide range of environmental conditions (temperature 1.8–35 °C; salinity 20–38 psu). It originated in the Northwest Pacific in Korea, China, and Japan but spread to the west coast of the United States in the 1920s and Western Europe in the 1960s, attached to hulls and in ballast water [88]. In Korea, the hanging culture of *M. gigas* commenced in the 1930s and increased in 1969. Spawning off the coast of Korea is optimal at a water temperature of 23–25 °C and thus occurs from the end of May to the end of October. Settling occurs within 2 weeks of hemiplankton hatching; copper (Cu) ions (at 0.05–0.06 mg/L in seawater) facilitate settlement [89]. *M. gigas* was extensively attached to the propeller shaft of the Eardo and to the entire hull of the Jangmok 2. We presume that when the vessels were anchored, the water temperature and salinity allowed the organism to attach to propeller shafts (which lack protective paint) and settle and grow around the hole on the side of the hull from which organic matter is discharged.

NIS, such as the bivalve *Mytilus galloprovincialis* Lamarck, 1819 [90], are widely distributed from the temperate to the subarctic coasts of both the Northern and Southern

Hemispheres and often dominate the hard substrates of intertidal and nearshore habitats. This species was introduced to the coasts of Korea, Japan, Vladivostok in Russia, and the northern Pacific from Europe via human activity in the early 20th century [27]. This mussel is confined to submerged artificial substrates from March to April and becomes dominant in July [71]. It excludes other macroalgae recruiters by overgrowing them to occupy the entire substrate surface and maintains a high population density by preventing the settlement of other species until late autumn [71]. It has replaced the native Korean bivalve *M. unguiculatus* (Valenciennes, 1858) and prevents the attachment of the ascidiacean *Halocynthia roretzi* [91]. On the Eardo and Jangmok 2 operating along domestic coasts, the organism was densely distributed in the drainage holes on the hull sides, and many individuals were noted in the propeller shaft cover of the Eardo. The organism may optimally settle and grow when seawater over a substrate is stirred.

The amphipod *Jassa slatteryi* Conlan, 1990 [92], was first recorded on the propeller of an international trader of the Hanjin Shipping Company; it differs from the native *Jassa falcata* [93] of the southern Korean coast [94]. This hull fouler is also found in ballast water. The life history pattern is annual and iteroparous; there are two principal juvenile recruitment periods during the year, namely, spring (March to May) and fall (October to December) [95]. The organism was principally found on the hull side of the Eardo (with a higher number of individuals on the waterline) and on the hull sides, bottom, and propeller shaft of the Jangmok 2. The former ship had sailed the East and South Seas and stayed in South Sea ports for 151 days; the latter had sailed the entire coast of South Korea and stayed in the East Sea ports for 115 days. However, the organism was absent from the Jangmok 1, which operated for 102 days and anchored for 186 days off the South Coast in 2016–2017.

4.3. Differences among Vessels

Between 2016 and 2017, hull-fouling macroinvertebrates were studied on five research vessels that operated in the ocean, coastal, and local areas for one year after cleaning (Supplementary Tables S2 and S3). Two ocean-going vessels mainly operated in low-latitude tropical climate regions, while three coastal vessels operated in the temperate regions of mid-latitudes. There were differences in the attached organisms depending on the navigation area; i.e., the abundance of attached organisms was relatively lower on vessels operating in low latitudes than on vessels sailing in mid-latitudes. Such species composition, community structure, and distribution patterns are thought to reflect the unique ecological characteristics of the shipping area [21,22]. However, conflicting research results are presented. Ships operating at low latitudes may be exposed to greater numbers of fouling organisms because the productivity of biological communities is generally higher in warmer climates [96]. Moreover, in tropical to sub-tropical locations of low latitudes, biofouling is generally more intense since the warmer temperatures (>20 °C) allow for continuous reproduction throughout the year and an increased growth rate of the biofouling organisms [59,60]. On the other hand, in temperate areas of mid-latitudes with mild temperatures (5–20 °C), biofouling occurs throughout the year and shows strong seasonality, with most spawning and growth occurring from spring (beginning of April) to early autumn (end of October) [59–61]. In this study, the species composition of the ocean-going vessel community was poor, while the coastal vessel community composition was richer. The reason is that the ocean navigation type of two research vessels had very short anchorage days at ocean ports (22 days/year for the Isabu and 9 days/year for the Onnuri) and long operating days (186 days/year for the Isabu and 101 days/year for the Onnuri) (Supplementary Table S3) [16,96,97]. Therefore, it is assumed that the short anchorage days are because there are fewer opportunities for fouling organisms to attach to the operational hull.

In nMDS ordination based on similarity, the reason why Jangmok 2 and Eardo were classified as similar clusters (Figure 14) was interpreted as the result of periodic survey activities in the offshore areas of the East and South Seas. SIMPER analysis (Supplementary Table S5) showed high dissimilarity values between groups, and the reason why the dissimilarity between the ocean-going Onnuri and local Jangmok 1 groups was relatively low is presumed to be due to the very long anchoring days of the two vessels in the South Sea port [51]. Significant differences between the vessel groups were verified based on ANOSIM and PERMANOVA pairwise tests (Supplementary Table S6). However, the Isabu and Eardo's cluster and the Onnuri and Eardo's cluster did not show significant differences. The Isabu and Onnuri operated on the ocean from July to September and anchored at a South Sea port in winter, while the Eardo operated in the South Sea and East Sea and anchored at a South Sea port (Supplementary Table S3). In this way, the groups of ocean-going vessels and coastal-going vessels showed various aspects with no clear statistical differences. Presumably, it is related to the similarity in species composition due to Eardo's survey activities at long distances from the coast or to the number of days of operation, port of anchorage, and period of stay.

In general, seawater temperature affects the settlement and spread of fouling organisms and is related to latitude and seasonality [29,54], and long stationary periods and reduced navigation activity increase the risk of macrofouling species attaching to hulls [16]. This was confirmed by the positive correlation between the number of operating days and the number, density, and biomass of hull-attached organisms for vessels that frequently operate at mid-latitudes with clear seasonal changes and by a negative correlation with the number of operating days for vessels with few anchorage days in low latitudes with low water temperature changes (Supplementary Tables S3 and S7). Moreover, even within the mid-latitude region, port location and period of stay can be identified through differences in the adherent organisms of ships operating in low-water temperatures in the north and high-water temperatures in the south (Supplementary Table S7). The species diversity index (H') positively correlated with the total numbers of anchoring and coastal operation days and negatively with the total numbers of operation and ocean operation days (Supplementary Table S7). Thus, a spatially disconnected macrofouling community of tropical and temperate region origin may simultaneously coexist on the same ship [21,22]. This possibility may also be related to the current sub-tropicalization of the Korean Peninsula.

5. Conclusions

Ship biofouling is one of the main vectors for the introduction and spread of harmful marine species worldwide. However, its importance in Korean coastal ecosystems has not been fully studied. This study is expected to be very valuable as a preliminary study in the absence of previous research due to the difficult conditions of domestic ship surveys, such as owner permits, port access restrictions, and on-site risk. Previous studies were conducted on ports, intertidal zones, artificial structures, and floating objects, but this is the first study to investigate organisms attached to hulls. In connection with the research project, we investigated hull-fouling macroinvertebrates on KIOST research vessels and identified the species composition, dominant species, life history, native species, non-native species, and community structure of hull-fouling macroinvertebrates among research vessels. These hull-fouling macroinvertebrates showed differences in species composition depending on the vessel's operating area, number of days of operation, anchorage area, and number of days at anchor, and differences in the distribution of attached organisms were also confirmed along the coasts of mid-latitude regions due to differences in water temperature depending on latitude. There were six native species and eight exotic species that were identified during the survey period, and the major dominant species were confirmed to be species that have already been introduced into Korea and are widely distributed around the world. This will be an important basis for research on ship fouling in response to future IMO restrictions [23]. In conclusion, the introduction of NIS through hull-fouling is expected to increase, and future long-term studies will be needed. In addition, to minimize NIS transfers, it will be necessary to introduce standards and regulations such as the level of fouling organisms on the hull, hull cleaning practices and collection methods, docking time and location records, and antifouling paint conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse12040613/s1, Table S1: Sampling sectors of hull-fouling macroinvertebrates on the research vessels.; Table S2: Information on the research vessels such as the navigation area, tonnage, dimension, and sampling date based on KIOST operating guidelines.; Table S3: Operational records of the research vessels for one year from 2016 to 2017 (Abbreviations: Sail, sailing days; Anchor, anchoring days).; Table S4: List of native and NIS found among 47 species of hull-fouling macroinvertebrates in this study. The numbers are the sum of the number of individuals of macroinvertebrate species attached to the hull of each vessel. Asterisks indicate six native and eight NIS. Others are species of unknown origin.; Table S5: Similarity percentage (SIMPER) results showing the average abundance, the average dissimilarity, the percentage contribution of each species to the dissimilarity among the vessel community groups, the percentage cumulative dissimilarity, and the average dissimilarity of the groups (Group A: Isabu, Group B: Onnuri, Group C: Jangmok 1, Group D: Eardo and Jangmok 2).; Table S6: One-way ANOSIM pairwise tests for differences between vessel groups for hull-fouling macroinvertebrates (Global R = 0.532, $p < 0.001$).; Table S7: Spearman rank correlation (ρ) of the variables of the biological indices and operating conditions of vessels (Abbreviations: TODs, total operation days; TADs, total anchoring days; CODs, coastal operation days; OODs, ocean operation days).; Appendix S1: Hull-fouling macroinvertebrate specimens identified to species on the Isabu research vessel.; Appendix S2: Hull-fouling macroinvertebrate specimens identified to species on the Onnuri research vessel.; Appendix S3: Hull-fouling macroinvertebrate specimens identified to species on the Eardo research vessel.; Appendix S4: Hull-fouling macroinvertebrate specimens identified to species on the Jangmok 1 research vessel.; Appendix S5: Hull-fouling macroinvertebrate specimens identified to species on the Jangmok 2 research vessel.

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