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ORIGINAL ARTICLE

Diel, seasonal and vertical changes in the pelagic amphipod communities in the subarctic Pacific: insights from imaging analysis

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Vertically stratified day and night samples were collected from a depth of up to 1000 m during four seasons at a single station in the western subarctic Pacific. Subsequently, the abundance, biovolume, community structure and population structure of the pelagic amphipods were evaluated from the imaging analysis using ZooScan. The stable isotope ratio ($\delta^{15}\text{N}$) was also measured for each species. In total 10 amphipod species were identified in total, and their community structures were separated into four groups. The four communities were characterized by the upper-layer community dominated by *Themisto pacifica*, and the deep-water community that was characterized by the absence of *T. pacifica*. The other two groups were observed for the intermediate depths at night only, which was characterized by the low

abundance of *T. pacifica* performing diel vertical migration from those depths. The deep-dwelling species displayed higher $\delta^{15}\text{N}$ and positive relationships were detected with the habitat depth of each species. For the predominant *T. pacifica*, population structure (cohort) analysis was possible based on equivalent spherical diameter data from ZooScan. This study demonstrates that imaging analysis by ZooScan is applicable for analyses of minor taxonomic groups (amphipods) in zooplankton communities.

KEYWORDS: ZooScan; St. K2; amphipoda; imaging analysis

INTRODUCTION

In oceans worldwide, pelagic amphipods associate from the sea surface to the benthopelagic layer of the deep sea (Vinogradov, 1999). Pelagic amphipods are known to dominate zooplankton biomass, especially in higher-latitude oceans (Mayzaud and Boutoute, 2015). *Themisto japonica* is the dominant species in the waters surrounding Japan, specifically at Toyama Bay in the southern Japan Sea. The growth rate based on laboratory rearing (Ikeda, 1990; 1992), the budget of the assimilated carbon (Ikeda, 1991), diel vertical migration (DVM) and life cycle of the field population (Ikeda *et al.*, 1992), and production estimation (Ikeda and Shiga, 1999) of this species have been reported. For the second dominant species, *Primno abyssalis*, the vertical distribution, growth, maturation, brood size and life cycle of the field population have also been reported (Ikeda, 1995). Further, in the Oyashio region in the western subarctic Pacific, ecologies of the two sibling species, *Themisto pacifica* and *T. japonica* (Yamada *et al.*, 2004; Yamada and Ikeda, 2004), and *P. abyssalis* (Yamada and Ikeda, 2001; Yamada *et al.*, 2002) and *Cyphocaris challengerii* (Yamada and Ikeda, 2000; 2001) have also been previously studied. In addition, chemical composition, metabolic measurements and production estimations were made for the above-mentioned four dominant amphipod species (Yamada and Ikeda, 2003; 2006). Thus, the neighboring waters of Japan are characterized by intensively studied physiological and ecological characteristics of each dominant pelagic amphipod species. However, little information is available on the community structure of pelagic amphipods and the ecology of other minor pelagic amphipod species.

To evaluate the amphipod community and population structures of the dominant species, species identification and body size measurements are required. However, the body shapes of most amphipod specimens are rounded, making it difficult to measure their body size under ordinary microscopic observations. Thus, body-size measurements of amphipods are time-consuming, and they provide scarce information despite their importance (cf. Yamada *et al.*, 2004). Imaging analysis methods are available for accurate and rapid measurement of body sizes. Within the imaging methods, ZooScan can obtain taxonomic information (species identification) based on

images and size data from one scan. Thus, its usefulness has been applied to various regions (Gorsky *et al.*, 2010; Irisson *et al.*, 2022). Pelagic amphipods have large bodies; thus, species identification may be possible based on their images. Based on morphology, developmental stages (juveniles, immature, mature females and males) (Yamada *et al.*, 2004) may also be identified. However, no study has applied ZooScan to the community structure of pelagic amphipods or the population structure of the dominant species.

Various unique ecological characteristics of pelagic amphipods have been previously reported. Thus, some Hyperiid species are known to attach to gelatinous zooplankton. As host gelatinous zooplankton for Hyperiid species, taxa belonging to ctenophores, cnidarians, hydrozoans, salps and protozoan rhizarians have been reported (Harbison *et al.*, 1977; Madin and Harbison, 1977; de Lima and Valentin, 2001; Gasca *et al.*, 2007; Nakamura *et al.*, 2019). Species-specific associations are present between the host and amphipod species; thus, *Scina* spp. and *Vibilia* spp. are associated with hydrozoans and salps, respectively (Harbison *et al.*, 1977; Madin and Harbison, 1977; de Lima and Valentin, 2001; Gasca *et al.*, 2007). Sexual differences in associations with gelatinous zooplankton exist within each species; for example, *Hyperia galba* lives within the scyphozoans, feeding on the host tissue and their growth, reproduction, and early development are made in the host scyphozoans, although their adult males have no association with the scyphozoans (Fleming *et al.*, 2014).

The feeding modes of pelagic amphipods vary with species and developmental stages and include herbivores to carnivores (Sugisaki *et al.*, 1991; Espinosa-Leal *et al.*, 2020). As indices for evaluating the feeding modes of amphipods, stable isotope ratios, especially $\delta^{15}\text{N}$, are valuable (Sugisaki *et al.*, 1991; Søreide and Nygård, 2012; Fleming *et al.*, 2014). However, there is a lack of information on the stable isotope ratio of the whole pelagic amphipod species, and inter-species comparisons have been performed on this regard in the western subarctic Pacific.

A long-term time-series station in the western subarctic Pacific, station K2 (St. K2) (47°N, 160°E, depth 5230 m) was established by the Japan Agency for Marine-Earth

Science and Technology (JAMSTEC) (Honda *et al.*, 2017). Information on amphipods at St. K2 is limited due to various factors such as the DVM of the dominant species, night: day zooplankton abundance ratio showing 3.6-fold difference at 0–150 m depths, mesopelagic occurrence of *Phrosina* spp. and *Primno* spp. and *C. challengeri* exhibiting DVM of up to 200 m (Steinberg *et al.*, 2008). Other than the information on the abundance and community structure of amphipods, little information is available, especially regarding the seasonal changes and population structure of the dominant species.

In the present study, we used formalin-preserved samples collected from the day and night vertical stratification sampling at eight layers down to a depth of 1000 m at St. K2 in the western subarctic Pacific, covering four seasons in 1 year. For the sorted amphipod samples from the whole zooplankton samples, imaging data were captured by ZooScan and species identification and stage identification for *T. pacifica* were performed based on the images. From imaging data, abundance, biovolume, community structure and population structure of the dominant species (*T. pacifica*) were made. Based on the sorted specimens, dried with the species, the stable isotope ratio ($\delta^{15}\text{N}$) was measured and an inter-species comparison was made. For the gelatinous zooplankton taxa (Radiolaria/Phaeodaria, Cnidaria and Thaliacea) that have been reported as host associations for pelagic amphipods (Hyperiidia), their diel vertical distributions were conducted based on biomass data from the same samples (Kitamura *et al.*, 2016). Through this study, we will test whether we can obtain valuable information [abundance, biovolume, taxon (species and developmental stage) and body size] shortly and accurately by imaging analyses (ZooScan) on the sorted samples of the particular taxon (amphipods).

MATERIAL AND METHOD

Field sampling

Day and night vertically stratified oblique hauls of a 1.5 m² mouth opening Intelligent Operative Net Sampling System (IONESS, SEA Co. Ltd) equipped with 335 μm were made from eight depth strata (0–50, 50–100, 100–150, 150–200, 200–300, 300–500, 500–750 and 750–1000 m) at St. K2 (47°N, 160°E, depth 5230 m; Fig. 1) during four seasons over 1 year: 29 October 2010, 26 February, 22–23 April and 3–4 July 2011 (Electronic Supplement 1). Zooplankton samples were preserved in 4% formalin (v/v) immediately after collection. At each sampling date, environmental data [temperature, salinity and dissolved oxygen (DO)]

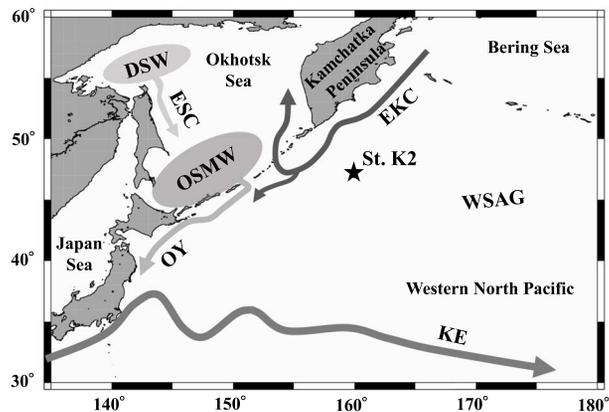


Fig. 1. Location of the sampling station: St. K2 in the western subarctic Pacific (solid star). Approximate positions and directions of the major current flows are shown with the arrows (cf. Yasuda, 2003). EKC: East Kamchatka Current, WSAG: Western Subarctic Gyre, DSW: Dense Shelf Water, ESC: East Sakhalin Current, OSMW: Okhotsk Sea Mode Water, OY: Oyashio, KE: Kuroshio Extension.

were measured using a CTD (SBE 911 plus; Sea-Bird Electronics Inc.).

ZooScan measurement

In the land laboratory, amphipod specimens were sorted from zooplankton subsamples at 1/2–1/64 based on the volume. For the sorted amphipod samples, we performed imaging analysis using ZooScan (ZooScan MIII, Hydroptic Inc.).

The protocols for sample measurement using ZooScan were based on Gorsky *et al.* (2010). Dehydrated water was used to examine the ZooScan cell, and a background scan was conducted. Subsequently, the sorted amphipod samples were filled for the scanning cell and images of the samples were captured. To obtain accurate images of the samples, floated samples and duplicated specimens were removed using tweezers to collect the individual specimen settled at the bottom of the scanning platform. The total numbers of captured images of amphipods were 6737. The scanned images were separated from the individual images using ZooProcess with ImageJ, Eco Taxa was updated (<http://ecotaxa.obs-vlfr.fr/>) via File Zila, and species identification and measurement of the equivalent spherical diameter (ESD, in mm) were performed. In addition to species identification, the developmental stages of the most abundant species (*T. pacifica*) were identified from the morphological characteristics mentioned below (Yamada and Ikeda, 2004):

Juvenile: body size <3.0 mm.

Immature males: antennules were segmented but not extended, and body size was 2.7–4.7 mm.

Immature females: antennules were not segmented; body size = 3.0–4.5 mm.

Mature males: antennules were segmented and extended; body size = 4.1–6.0 mm.

Mature females: antennules were not segmented, and spaces were present on the basis of the swimming legs; body size = 4.5–9.00 m.

These body sizes were used for the extended specimens (Yamada and Ikeda, 2004). While we did not measure the body sizes of the amphipods in the extended state, using the scales obtained for each image as a guide, approximate body sizes were estimated. It should also be noted that we applied ESD to the amphipod body size index. Images of the pelagic amphipods captured in this study are available as electronic supplements (Electronic Supplements 2–15).

Data analysis

Based on the split factors and filtered volumes of each sample, the abundance (ind. m⁻³) and biovolume based on ESD (mm³ m⁻³) were quantified for each species. Based on the abundance and biovolume data, Shannon-weaver species diversity (H') and Pielou's evenness index (J') were calculated using the following equations:

$$H' = - \sum_{i=1}^S p_i \times \ln(p_i)$$

$$J' = H' / \ln S$$

where S is the number of species and p_i is the relative composition of species i in each sample (Morishita, 1996).

For the community structure of the pelagic amphipods, Bray–Curtis (Bray and Curtis, 1957) cluster analysis connected with the complete linkage based on the abundance data (ind. 1000 m⁻³) standardized by the fourth root was performed. Similarities between the samples were evaluated using a two-dimensional plot of the nonmetric multidimensional scaling method (NMDS; Minchin, 1987), and interactions with environmental parameters (depth, temperature, salinity and DO) were analyzed by multiple regression analysis. For temperature, salinity and DO, integrated mean values at each sampling layer were calculated and applied for the multiple regression analysis. The effect of spatial and temporal environmental parameters (season, day/night and depth) and interactions within them (season × day/night, season × depth and day/night × depth) on the occurrence of each amphipod community was analyzed using PERMANOVA. These analyses were conducted using the PRIMERv7 software (PRIMER-E Ltd). Differences in amphipod abundance between the clusters were evaluated using one-way analysis of variance (ANOVA) and the subsequent post-hoc test (Tukey–Kramer test).

These analyses were performed using the OriginPro software (Light Stone Ltd).

The presence of DVM in pelagic amphipods was evaluated using the two-sample Kolmogorov–Smirnov test. To evaluate the vertical distribution depth of each species, the depths of 50% of the population ($D_{50\%}$) were calculated using the following equation (Pennak, 1943):

$$D_{50\%} = d_1 + d_2 \times \frac{50 - p_1}{p_2}$$

where d_1 is the upper depth (m) of the sampling layer where 50% of the population is distributed, d_2 represents the sampling interval (m) where 50% of the population is distributed, p_1 is the composition (%) of the cumulative abundance (ind. m⁻²) occurring in the upper layers of the sampling layer with 50% of the population distribution, and p_2 is the composition (%) of the cumulative abundance (ind. m⁻²) of 50% of the population distribution within the cumulative standing stock (ind. m⁻²) of the water column.

For the most numerous pelagic amphipod species at St. K2, *T. pacifica*, the cumulative numbers (n) of captured images in day or night samples at each sampling date were 93–1138 images, which allowed us to construct histograms of ESD and developmental stage composition in the population.

Stable isotope ratio ($\delta^{15}\text{N}$)

To quantify the stable isotope ratio ($\delta^{15}\text{N}$: ‰), specimens from the samples with the highest biovolumes (mm³ m⁻³), which varied with species, were chosen. The specimens were rinsed with distilled water and dried in an oven at 60°C for 5 h. After drying, the specimens were finely ground using a mortar and pestle. The nitrogen stable isotope (¹⁵N and ¹⁴N) was analyzed using an elemental analyzer (Flash EA1112-Delta V Plus He-Flow System, Thermo Fisher Scientific, Germany). The $\delta^{15}\text{N}$ value was calculated using the following equation.

$$\delta^{15}\text{N} = \left(\left[\frac{{}^{15}\text{N}_{\text{sample}} / {}^{14}\text{N}_{\text{sample}}}{{}^{15}\text{N}_{\text{standard}} / {}^{14}\text{N}_{\text{standard}}} \right] - 1 \right) \times 1000$$

where ¹⁵N_{sample} and ¹⁴N_{sample} are the values of the samples and ¹⁵N_{standard} and ¹⁴N_{standard} are the values of ¹⁵N and ¹⁴N of the standardized samples, respectively. Interspecies differences in $\delta^{15}\text{N}$ were evaluated using one-way ANOVA and the Tukey–Kramer test. To evaluate the effect of habitat depth on the $\delta^{15}\text{N}$ value, linear regression between $\delta^{15}\text{N}$ (‰) and nighttime $D_{50\%}$ (m) was

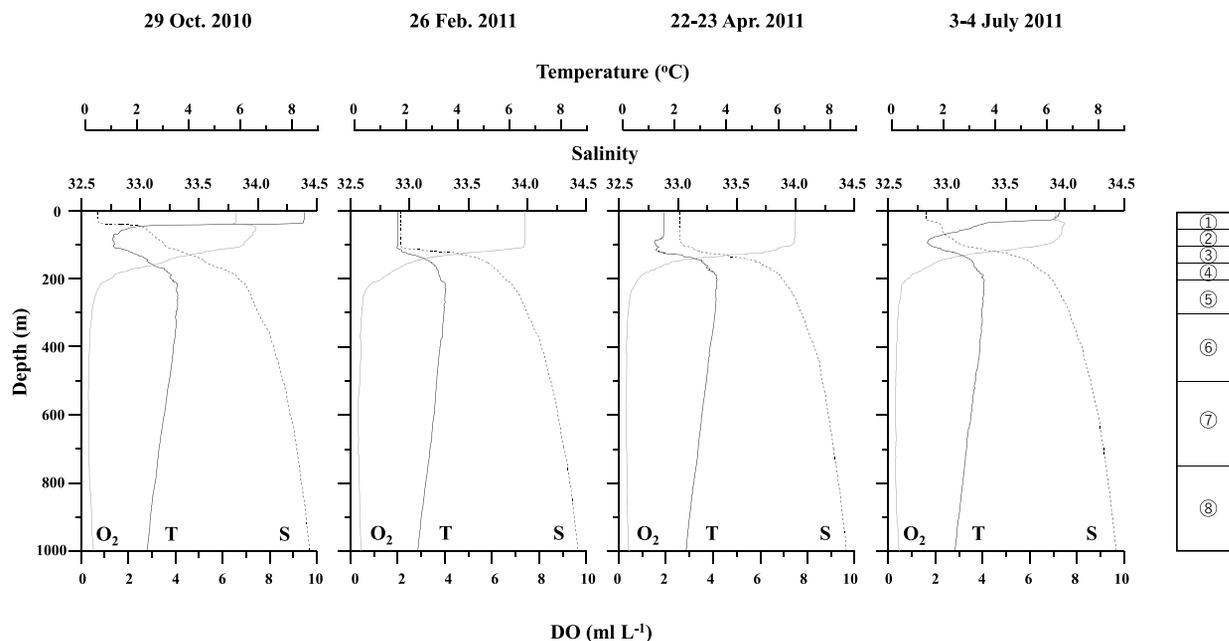


Fig. 2. Vertical distribution of temperature, salinity and DO (DO, O₂) at St. K2 in the western subarctic Pacific from October 2010 to June 2011. Circled numbers in the right column indicate sampling layers of oblique tows by IONESS.

performed. Since diel rhythm is available for the feeding activity of the amphipods (Froneman *et al.*, 2000), we applied the nighttime $D_{50\%}$ (m) as the depths where they perform the feeding.

RESULTS

Hydrography

Vertical changes in temperature, salinity and DO at St. K2 during the four sampling dates are shown in Fig. 2. Throughout the sampling layers and dates, temperature, salinity and DO ranged from 0.7–8.5°C, 32.5–34.5 and 0.3–7.5 mL L⁻¹, respectively. Seasonal thermoclines developed at depths of ~50 m in October and July, while temperatures were homogenous in the upper 100 m during February and April. For all sampling periods, the temperature showed a sub-minimum at 100 m and sub-maximum at 200 m, and then decreased with increasing depths below them. The salinity increased with increasing depth throughout the sampling period. Low salinity (<33) was observed in the upper 50 m in July and October. DO decreased rapidly from the sea surface to 200 m, was extremely low (2 mL L⁻¹) below 200 m depth and formed an oxygen minimum layer there.

Pelagic amphipod community

Throughout the year, 10 amphipod species belonging to 10 genera and 8 families occurred (Table I). The most abundant species was *T. pacifica* (annual mean: 363.9 ind.

1000 m⁻³). *T. pacifica* comprised 85.5% (annual mean) of the amphipod abundance. The next most abundant species were *P. abyssalis* (20.0 ind. 1000 m⁻³, 4.7%), *Scina borealis* (12.4 ind. 1000 m⁻³, 2.9%) and *C. challengeri* (10.0 ind. 1000 m⁻³, 2.4%).

The abundance density of pelagic amphipods at each sampling depth ranged between 4 and 17 057 ind. 1000 m⁻³ (Fig. 3). Based on abundance, species diversity (H') was 0–1.9, and species evenness (J') was 0–1.0. Both values were lowest near the surface layer where the single species (*T. pacifica*) predominated. During the day, the highest densities were seen at 0–500 m and varied seasonally. Thus, daytime abundance was high near the surface in July and October while was stable at 0–500 m in February and April. In contrast, the highest density during the night was observed at the shallowest depth (0–50 m) throughout the four seasons. The proportion of the dominant *T. pacifica* was high at 0–500 m during the day, while it was high at 0–100 m during night and was low below that layer at night. The shallowest depths (0–50 m) were dominated by *T. pacifica* during both day and night, which implied low species diversity (H') and evenness (J') in that layer. At depths below 300 m, *Koroga megalops*, *Rhachotropis natator* and *S. borealis* were distributed throughout the year, with high species diversity (H') and evenness (J').

In terms of biovolume, *T. pacifica* was also dominant (annual mean: 2931 mm³ 1000 m⁻³), but its annual mean composition (56.2%) was lower than that of the abundance (Table I). This was due to the dominance of small specimens of this species. For the species showing

Table I: List of pelagic amphipod species occurred for the 0–1000 m water column at St. K2 in the western subarctic Pacific from October 2010 to July 2011. Annual mean abundance and biovolume (mean ± 1 SE: ind. 1000 m⁻³ or mm³ m⁻³ at 0–1000 m water column) and the species composition (%) are also shown

Suborder	Family	Species	Abundance (ind. 1000 m ⁻³)	(%)	Biovolume (mm ³ 1000 m ⁻³)	(%)
Hyperiidea	Oxycephalidae	<i>C. scleroticus</i>	3.9 ± 3.0	0.9	120 ± 107	2.3
	Hyperiidae	<i>H. galba</i>	1.1 ± 0.6	0.3	141 ± 123	2.7
		<i>T. pacifica</i>	363.9 ± 289.1	85.5	2 931 ± 2 202	56.2
	Phrosinidae	<i>P. abyssalis</i>	20.0 ± 12.0	4.7	558 ± 235	10.7
	Scinidae	<i>S. borealis</i>	12.4 ± 7.1	2.9	87 ± 20	1.7
	Vibiliidae	<i>V. caeca</i>	1.2 ± 0.8	0.3	11 ± 9	0.2
Amphilochidea	Cyphocarididae	<i>C. challengerii</i>	10.0 ± 6.4	2.4	588 ± 370	11.3
	Eusiridae	<i>E. multicalceola</i>	1.2 ± 0.8	0.3	29 ± 27	0.6
		<i>R. natator</i>	3.7 ± 2.0	0.9	421 ± 459	8.1
	Uristidae	<i>K. megalops</i>	7.4 ± 1.9	1.7	313 ± 139	6.0
	Others		0.7	0.0	0.40	0.0

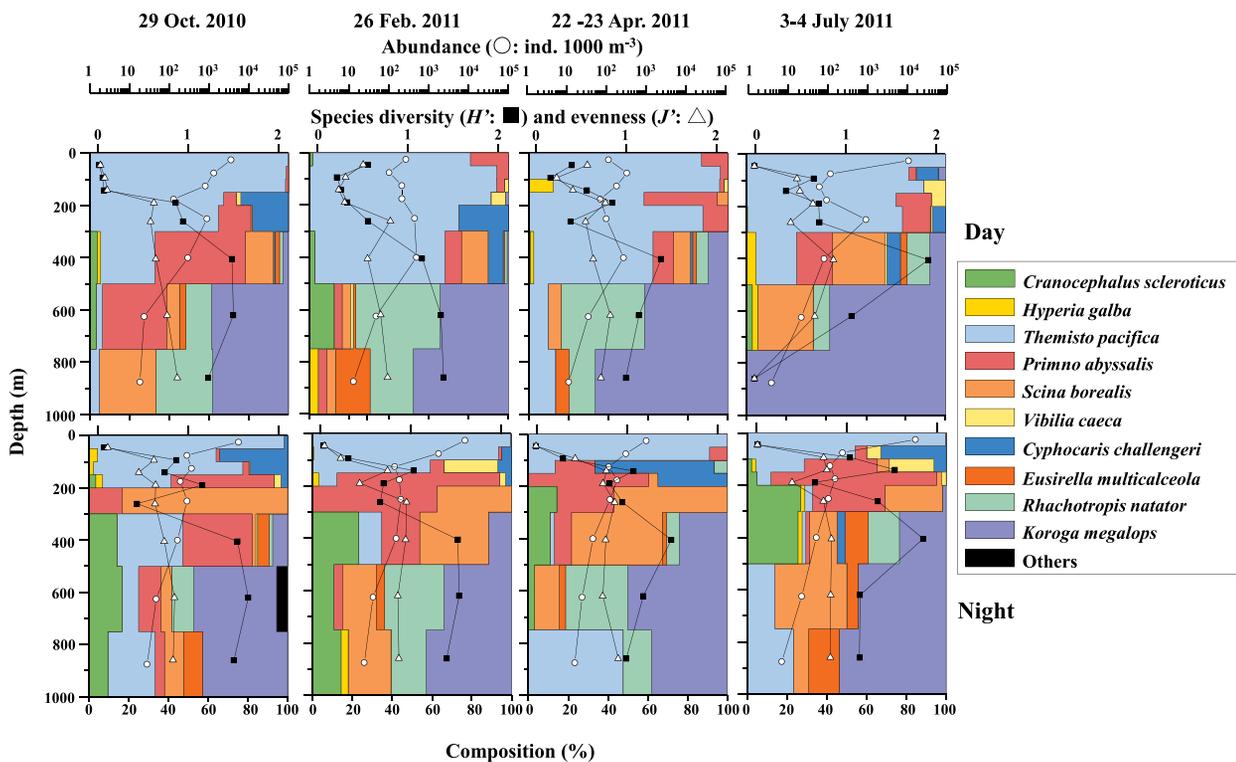


Fig. 3. Vertical changes in amphipod abundance, species diversity, evenness and species composition at day (upper) and night (lower) at 0–1000 m depths of St. K2 in the western subarctic Pacific during four occasions (October 2010, February, April and July 2011). Note that scales of abundance are in log scales.

*Table II: Comparison of amphipod abundances at four groups (A–D) identified by Bray–Curtis dissimilarity (cf. Fig. 4). Values are mean abundances at each group. Differences between groups were tested by one-way ANOVA and post-hoc Tukey–Kramer test. For the results of the Tukey–Kramer test, differences in superscript letters indicate significant differences ($P < 0.05$). Numbers in the parentheses indicate the number of samples included in each group. **: $P < 0.01$, ***: $P < 0.001$, NS: not significant*

Species	Abundance (ind. 1000 m ⁻³)				one-way ANOVA
	A (30)	B (8)	C (9)	D (17)	
<i>C. scleroticus</i>	0.1 ^a	10.7 ^b	4.1 ^{a,b}	1.2 ^a	***
<i>H. galba</i>	1.9	2.4	0	0.1	NS
<i>T. pacifica</i>	1785.9	61.9	0	1.8	NS
<i>P. abyssalis</i>	19.9 ^a	59.4 ^b	72.1 ^b	1.3 ^a	***
<i>S. borealis</i>	0.3 ^a	19.4 ^a	95.6 ^b	3.8 ^a	***
<i>V. caeca</i>	3.3	3.5	0	0	NS
<i>C. challengerii</i>	32.4 ^b	5.4 ^{a,b}	14.9 ^{a,b}	0 ^a	**
<i>E. multicalceola</i>	0 ^a	2.5 ^b	0 ^a	0.7 ^a	***
<i>R. natator</i>	0.2 ^a	3.7 ^b	0 ^a	4.6 ^b	***
<i>K. megalops</i>	0.3 ^a	6.5 ^b	0 ^a	10.1 ^b	***

high composition in biovolume, the large-sized two species: *C. challengerii* (588 mm³ 1000 mm⁻³, 11.3%) and *P. abyssalis* (558 mm³ 1000 mm⁻³, 10.7%) were marked. The biovolume densities of the pelagic amphipods in each sampling layer ranged between 0.1 and 130 mm³ m⁻³ (Electronic Supplement 16). During the day, the highest biovolume was observed at depths of 0–500 m and varied seasonally. However, the highest night-time biovolume was observed for the shallowest (0–50 m) throughout the four seasons.

From the cluster analysis based on abundance, the pelagic amphipod community was divided into four groups (A–D) with 68% dissimilarity (Fig. 4a). Each group contained 8–30 samples. In NMDS, each group was plotted in different areas, and all environmental parameters (temperature, salinity and DO) had a significant relationship with the NMDS ordination (Fig. 4b). For group A, the highest abundance and predominance of *T. pacifica* (>95%) was characteristic of the group. Abundances were low in groups B and C; group B was dominated by *T. pacifica* and *P. abyssalis*, and *P. abyssalis* and *S. borealis* were dominant in group C. The lowest abundance was observed in group D. *K. megalops*, *R. natator* and *S. borealis* were considered the abundant species of group D. Intergroup differences in abundance were observed for most groups of pelagic amphipods (Table II). Thus, *C. challengerii* was abundant in groups A–C, *K. megalops* was more abundant in groups B and D, *P. abyssalis* was high in groups B and C and *S. borealis* was abundant in group C.

Vertical distribution of each pelagic amphipod species

The seasonal, vertical and day–night occurrences of each pelagic amphipod group are shown in Fig. 5.

The pelagic amphipod communities varied vertically. Thus, throughout the season and day–night, only group A was seen in the upper 0–100 m. For the deepest layer (500–1000 m), group D was seen throughout the season and day–night. The occurrence of groups B and C was observed at intermediate depths of 100–500 m. In terms of the season, group B was absent in July. On the other three sampling occasions, all four groups occurred. A diel change was observed in group C. Thus, group C was specifically observed at 150–300 m at night. The PERMANOVA indicated that the three environmental parameters (day/night, depth and interaction of day/night × depth) had a significant effect on the separation of each group ($P < 0.001$) (Electronic Supplement 17). Weak effects from the season and the interaction of season and depth were also observed ($P < 0.05$).

For the 10 amphipod species that occurred at St. K2, the day–night vertical distributions during the four seasons of the five major species and the five minor species are shown in Figs 6 and 7, respectively. The vertical distribution center ($D_{50\%}$) and magnitude of DVM ($\Delta D_{50\%}$ [D–N]) are presented in Table III. Three species (*T. pacifica*, *P. abyssalis* and *C. challengerii*) were distributed at depths of 0–500 m (Fig. 6a–c). For *T. pacifica*, DVM characterized by nocturnal ascent was observed for the three seasons ($P < 0.05$), and the mean DVM magnitude was computed as 55 m (Table III). For *P. abyssalis*, nocturnal ascent of the DVM was observed in July, but no DVM behavior was observed in the remaining three seasons. For *C. challengerii*, the nocturnal ascent of DVM was observed during the three seasons, and their mean DVM magnitude was 203 m that was the largest among the three species.

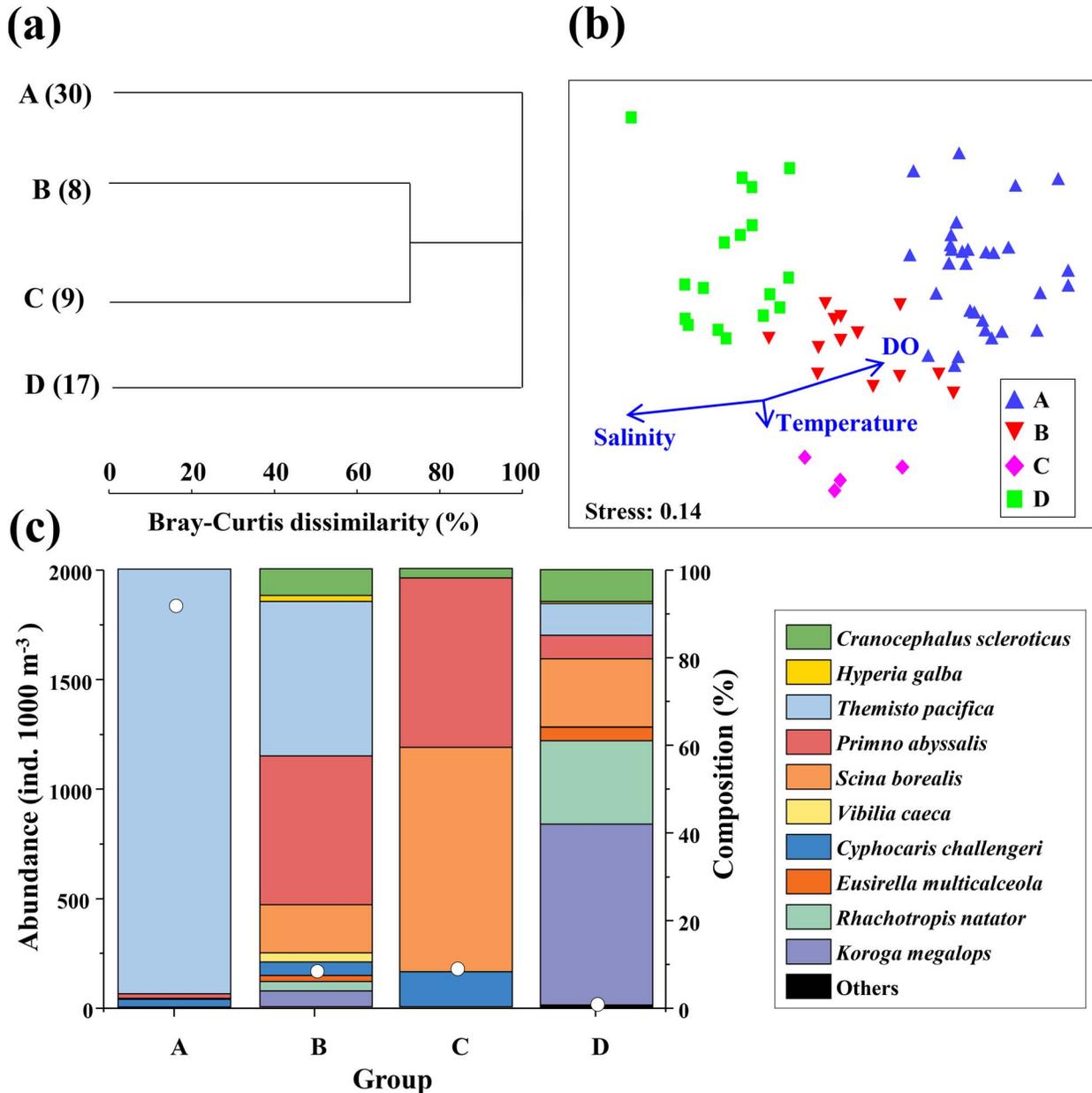


Fig. 4. (a) Results of cluster analysis based on amphipod abundance at St. K2 in the western subarctic Pacific. Four groups (A–D) were identified at 68% Bray–Curtis dissimilarity connected with the complete linkage method. Numbers in the parentheses indicate the number of samples each group contained. (b) NMDS plots of each group. Arrows indicate directions of significant environmental parameters. (c) Mean abundance (white circle) and species composition of each group.

Except for the above-mentioned three species, the remaining seven species had no significant DVM behavior in either the whole season or day–night (Figs 6 and 7). The vertical distribution of the remaining seven species was classified into two patterns. Thus, the four species (*K. megalops*, *Cranocephalus scleroticus*, *R. natator* and *Eusirella multicalceola*) that occurred at deeper depths (750–1000 m) had broader vertical distribution depths and $D_{50\%}$ at depths of ~400–500 m (Table III).

However, the remaining three species (*S. borealis*, *Vibilia caeca* and *H. galba*) did not occur at the deepest depths of this study (750–1000 m) and had a $D_{50\%}$ at the intermediate 150–300 m depths (Figs 6d, 7b and d). Among the three species, *S. borealis* and *H. galba* displayed shallower vertical distribution depths at night because of the smaller abundance in the samples; it was not significant in most of the cases, and only one season of *S. borealis* was evaluated as the nocturnal ascent DVM (Figs 6d and 7d). For *K.*

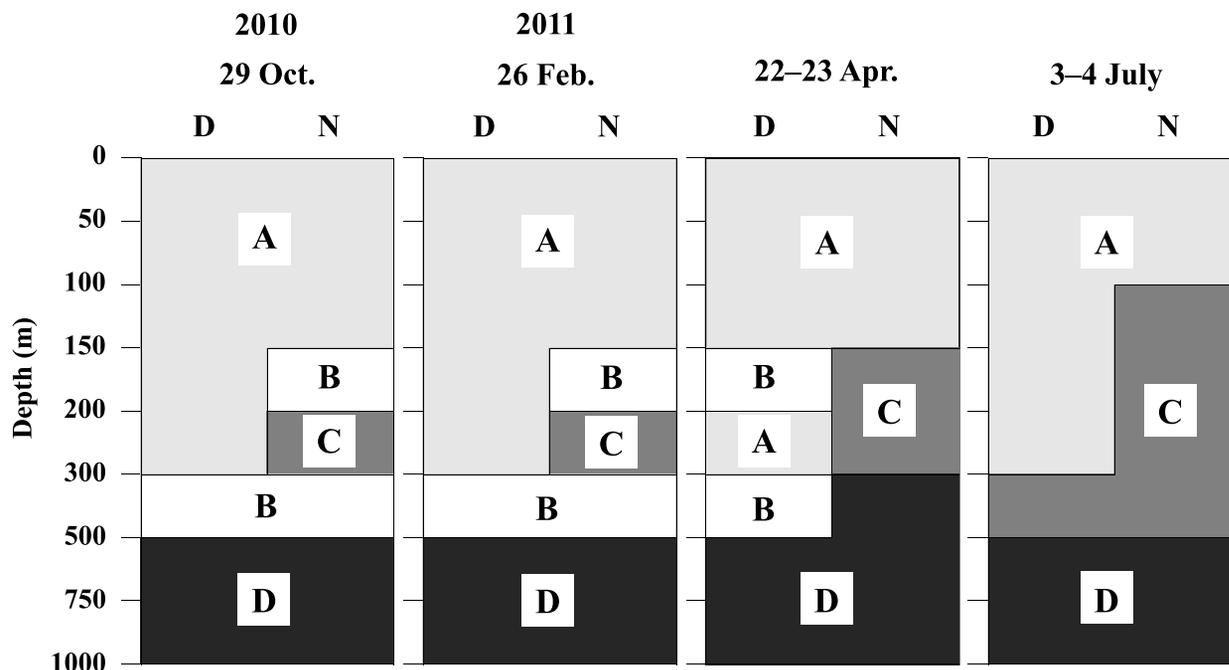


Fig. 5. Seasonal, vertical and diel changes in the occurrence of the four amphipod community groups (A–D) identified by Bray–Curtis dissimilarity based on their abundance (cf. Fig. 4a) at St. K2 in the western subarctic Pacific during October 2010 to July 2021. D: day, N: night.

*Table III: Summary of vertical distribution cores ($D_{50\%}$) of the 10 amphipod species at St. K2 in the western subarctic Pacific during four occasions between October 2010 and July 2011. Day and night differences were tested by Kolmogorov–Smirnov test. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$. Grand mean was calculated based on the data of day and night. Values are means \pm SE. Species order was arranged with the order of the grand mean of daytime $D_{50\%}$. For calculation of the grand mean, the datum shown with the underline was omitted. - : no occurrence*

Species	29 October 2010		26 February 2011		22–23 April 2011		3–4 July 2011		Grand mean (m)		$\Delta D_{50\%}$ (D–N)
	Day	Night	Day	Night	Day	Night	Day	Night	Day	Night	
<i>T. pacifica</i>	45	28*	162	30*	101	33**	27	25	84 \pm 26	29 \pm 1	55
<i>V. caeca</i>	184	164	165	129	125	-	162	107	159 \pm 11	133 \pm 12	26
<i>P. abyssalis</i>	262	192	53	186	166	166	241	161***	181 \pm 41	176 \pm 7	5
<i>H. galba</i>	314	103	<u>875</u>	92	131	-	321	208	255 \pm 44	134 \pm 26	121
<i>C. challengerii</i>	235	60***	267	62***	400	149	236	58**	285 \pm 34	82 \pm 19	203
<i>S. borealis</i>	390	252**	404	293	381	254	401	243	394 \pm 5	261 \pm 10	133
<i>E. multicalceola</i>	412	429	492	625	415	477	400	438	430 \pm 18	492 \pm 39	-62
<i>C. scleroticus</i>	407	391	388	422	400	266	625	242	455 \pm 49	330 \pm 39	125
<i>R. natator</i>	548	533	579	670	500	350	420	400	512 \pm 30	488 \pm 62	24
<i>K. megalops</i>	557	576	590	526	486	540	531	467	541 \pm 19	527 \pm 20	14

caeca, their $D_{50\%}$ was at restricted depths of 107–184 m (Table III) throughout the season and day–night (Fig. 7b).

Nitrogen stable isotope ($\delta^{15}\text{N}$) of each pelagic amphipod species

The $\delta^{15}\text{N}$ values of each pelagic amphipod species at St. K2 ranged between 6.8 and 14.0‰, and the lowest

and highest values were observed for *C. challengerii* and *R. natator*, respectively (Fig. 8). Common for pelagic amphipods, $\delta^{15}\text{N}$ values were lower for the shallower dwelling species and higher for the deeper dwelling species. These depth-related trends in $\delta^{15}\text{N}$ values implied a significant relationship between $\delta^{15}\text{N}$ and nighttime habitat depth ($D_{50\%}$): $\delta^{15}\text{N} = 0.00904 \times \text{nighttime } D_{50\%} + 8.028$ ($r^2 = 0.542$, $P < 0.0001$, Fig. 8). While such

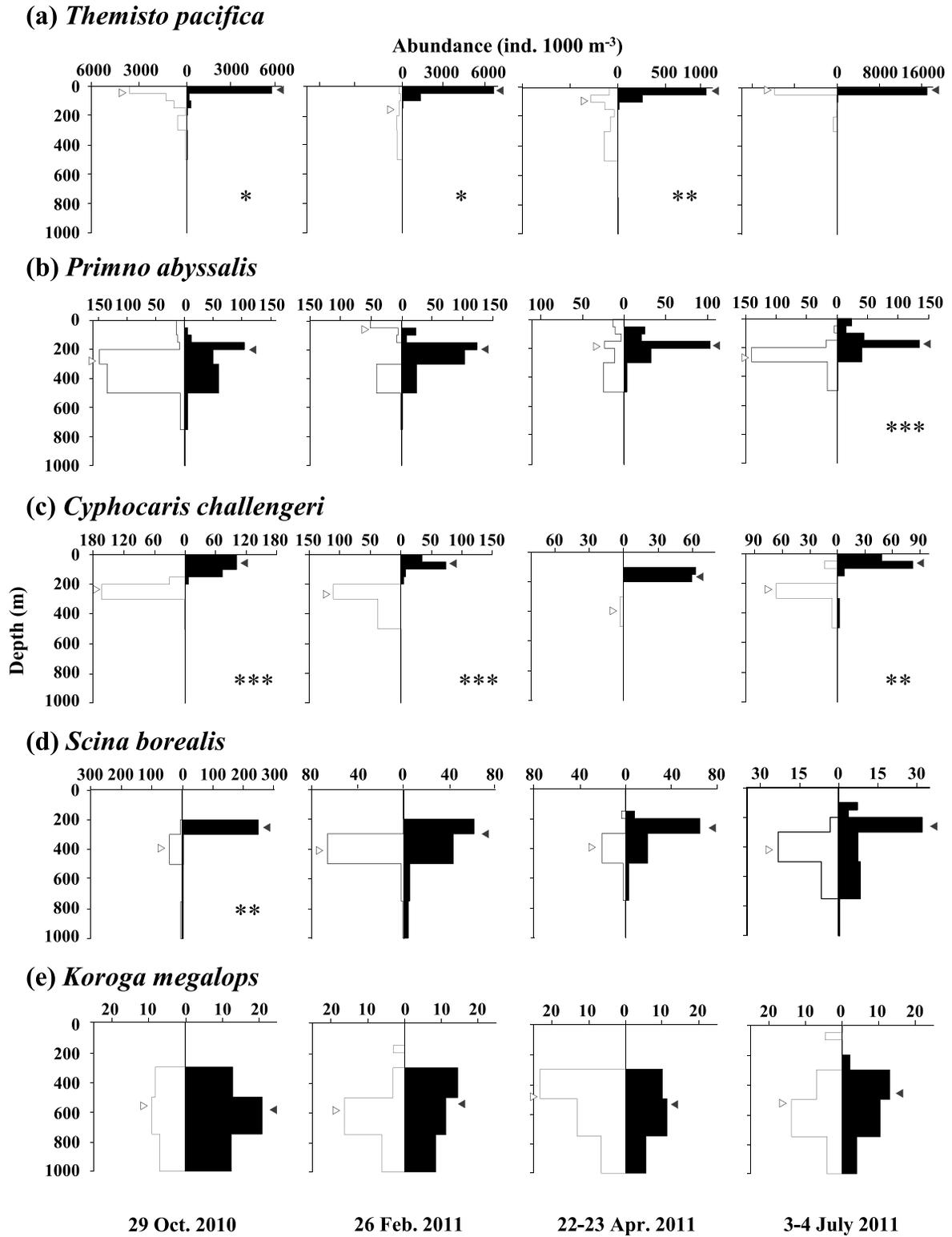
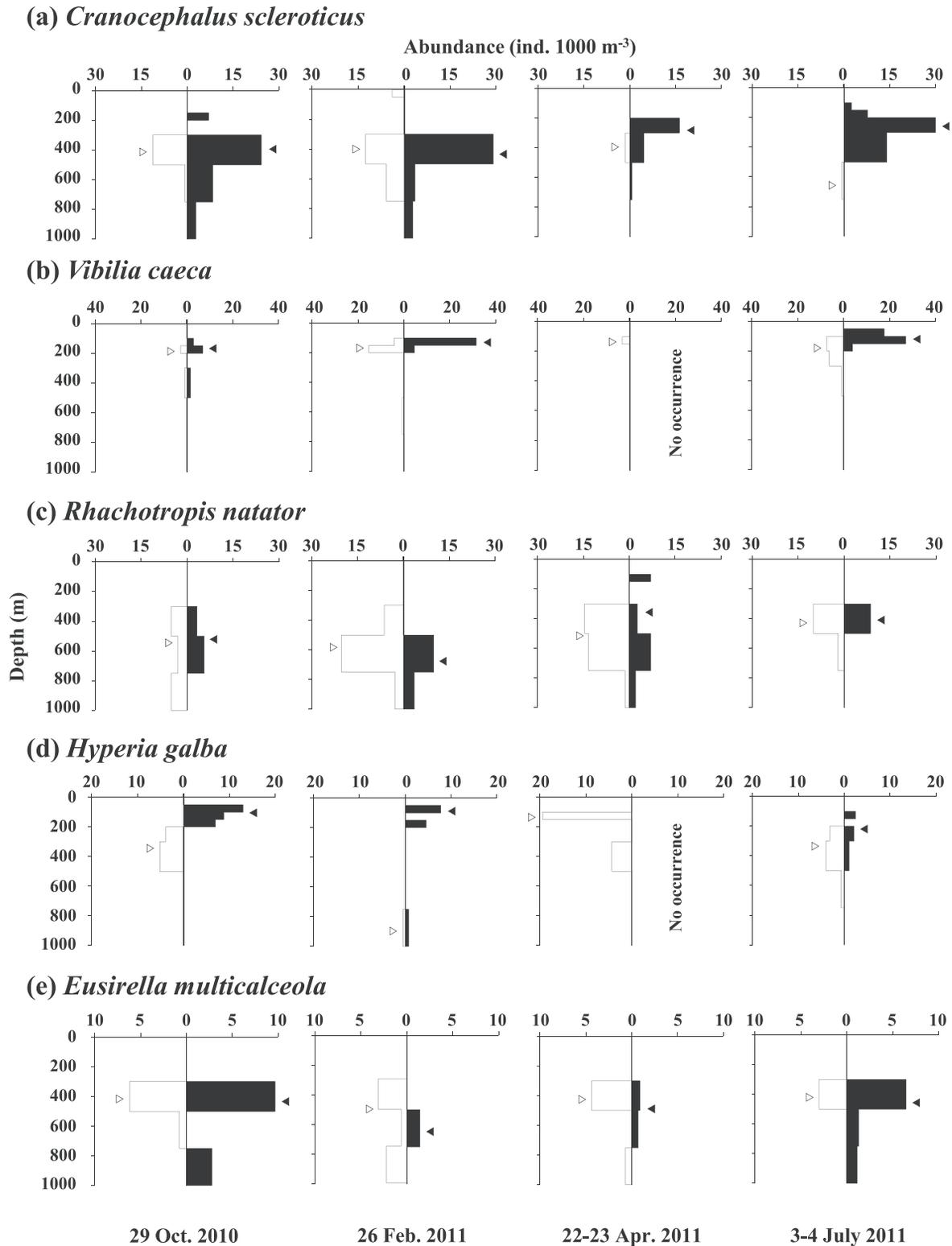


Fig. 6. Day (open, left) and night (solid, right) vertical distribution of the five amphipod species (arranged with the order of abundance, cf. Table II): *T. pacifica* (a), *P. abyssalis* (b), *C. challengerii* (c), *S. borealis* (d) and *K. megalops* (e) at St. K2 in the western subarctic Pacific during four sampling occasions between October 2010 and July 2011. Triangles represent vertical distribution cores ($D_{50\%}$). Diel changes were tested by Kolmogorov-Smirnov test. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.



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Fig. 7. Day (open, left) and night (solid, right) vertical distribution of the five amphipod species (arranged with the order of abundance, cf. Table II): *C. scleroticus* (a), *V. caeca* (b), *R. natator* (c), *H. galba* (d) and *E. multicalceola* (e) at St. K2 in the western subarctic Pacific during four sampling occasions between October 2010 and July 2011. Triangles represent vertical distribution cores ($D_{50\%}$). Diel changes were tested by Kolmogorov–Smirnov test. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

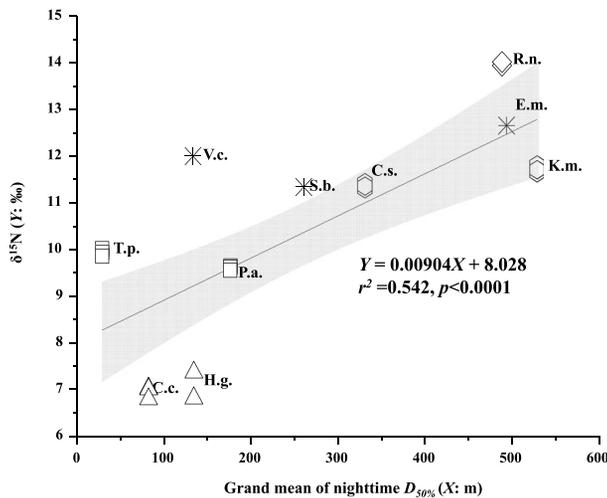


Fig. 8. Scatter plot between $\delta^{15}\text{N}$ and grand mean of nighttime $D_{50\%}$ of the 10 amphipod species at St. K2 in the western subarctic Pacific. Differences in symbols denote significant differences in $\delta^{15}\text{N}$ values (one-way ANOVA and Tukey–Kramer test). The line was regression based on the whole species data. The shaded area represents a 95% confidence interval. T.p.: *T. pacifica*, C.c.: *C. challengeri*, H.g.: *H. galba*, V.c.: *V. caeca*, P.a.: *P. abyssalis*, S.b.: *S. borealis*, C.s.: *C. scleroticus*, R.n.: *R. natator*, E.m.: *E. multicalceola*, K.m.: *K. megalops*.

depth-related patterns were present, the $\delta^{15}\text{N}$ values of some species were outside the 95% confidence limit. Thus, low $\delta^{15}\text{N}$ values were observed in *C. challengeri* and *H. galba*. For the high $\delta^{15}\text{N}$ values, three species, *T. pacifica*, *V. caeca* and *R. natator*, exhibited similar patterns.

Population structure of the dominant *T. pacifica*

ESD of *T. pacifica* was at 0.2–9.0 mm (Fig. 9a). For ESD in each sampling period, two–three cohorts were identified. The recruitment of the small-sized ESD was observed for two periods (October and April). The subsequent growth of each cohort was traceable for 9 months (October–July) or 1 year (April–April). The population structures in February and April, with the small abundances of the small-sized ESD specimens, were characterized by the dominance of mature females and males (Fig. 9b). On the other hand, juveniles composed more than half an abundance in July and October.

DISCUSSION

Community structure

There were three notable characteristics of the pelagic amphipod community in this study. First, both abundance and biovolume showed the highest values at the shallowest

depths of 0–50 m at night, which is comparable with the other region (Espinosa-Leal *et al.*, 2020). Secondly, the community structure showed diel changes, particularly at depths of 150–300 m. At that depth, the community was dominated by *T. pacifica* during the day, whereas the composition of *T. pacifica* substantially decreased during nighttime, and the composition of the other species that remained stable occurred at the depth without increased DVM. Such DVM behavior of *T. pacifica* in this region has been reported (Yamada *et al.*, 2004). Third, species diversity had high values at 300–500 m during both day and night. These depths are comparable with the other regions (Roe *et al.*, 1984; Espinosa-Leal *et al.*, 2020). Among the three characteristics, the first two are caused by the DVM behavior of *T. pacifica*, the dominant species in both abundance and biovolume. The DVM behavior of *T. pacifica* and *T. japonica* is well documented in the western subarctic Pacific (Yamada *et al.*, 2004; Hanamiya *et al.*, 2020) and Japan Sea (Ikeda *et al.*, 1992). Furthermore, the nocturnal ascent DVM of *T. pacifica* induces a decrease in its composition at depths of 150–300 m during the night, which causes diel changes in the community structure at that layer. Finally, because both deep-water species and upper-layer species occurred at 300–500 m, the species diversity may have peaked at that layer.

Evaluation of the separation of the pelagic amphipod community into four groups (A–D) is also a notable finding of this study (Fig. 4). The numbers of the pelagic amphipod community classification have well corresponded with those in the worldwide oceans (Lavaniegos and Hereu, 2009; BurrIDGE *et al.*, 2017; Espinosa-Leal *et al.*, 2020; 2021). In this study, group A, dominated by *T. pacifica*, was observed down to 300 m during the day, while their occurrence depth was restricted to <150 m during the night. *T. pacifica* dominated the pelagic amphipod community, comprising 86 and 56% of the total abundance and biovolume, respectively. Thus, their dominance and DVM behavior (Yamada *et al.*, 2004) alter the amphipod community. At 150–300 m depth at night, group C, dominated by *S. borealis* and *P. abyssalis* instead of *T. pacifica*, was observed. This is due to the upward migration of *T. pacifica* at night, then the community at 150–300 m is composed of the remaining species at the depth during the night. Deeper distribution of *P. abyssalis* than those of *T. pacifica* has been reported (Ikeda, 1995; Yamada *et al.*, 2002). The depths with high species diversity (300–500 m) corresponded well with the depths of various zooplankton communities (B, C and D). For groups B and D, the vertical distribution depths of the dominant species, *E. multicalceola*, *R. natator* and *K. megalops*, corresponded well with those of a previous study (Barnard, 1964).

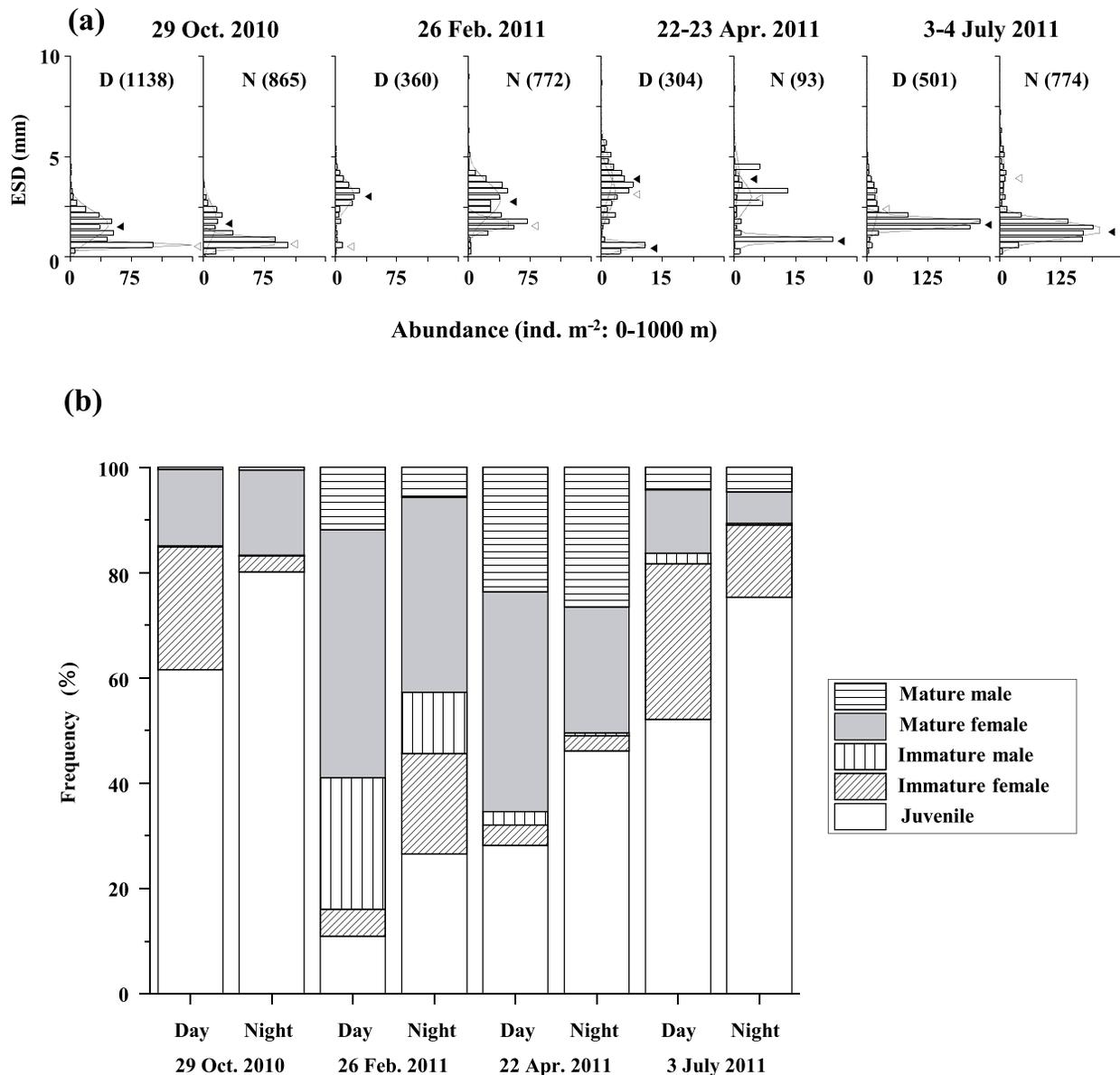


Fig. 9. Seasonal changes in the histogram on ESD **(a)** and developmental stage composition **(b)** of *T. pacifica* at St. K2 in the western subarctic Pacific. The triangles in **(a)** show the means of each cohort in ESD, and solid and open triangles represent possible differences in generations. Numbers in the parentheses in **(a)** indicate the number (*n*) of captured images.

Vertical distribution of each species

As new findings of this study, evaluation of the diel vertical distribution and magnitude of DVM for all 10 amphipod species is also important (Figs 6 and 7). For dominant amphipod species, DVM behavior characterized by nocturnal ascent has been reported for *T. pacifica/japonica* (Ikeda *et al.*, 1992; Yamada *et al.*, 2004), *P. abyssalis* (Ikeda *et al.*, 1995; Yamada *et al.*, 2002) and *C. challengeri* (Yamada and Ikeda, 2000). Among the three species, the magnitude of the DVM was the largest (203 m) for *C. challengeri* (Table III). The DVM behaviors

of *P. abyssalis* and *C. challengeri* at St. K2 have also been documented in a previous study (Steinberg *et al.*, 2008).

Within the 10 pelagic amphipods presented in this study, associations with gelatinous zooplankton have been reported for all three species. Thus, *Scina* spp. have been reported to attach to jellyfishes (Madin and Harbison, 1977; Gasca *et al.*, 2007), *Vibilia* spp. have been reported to attach to salps (Harbison *et al.*, 1977; Gasca *et al.*, 2007; Quigley *et al.*, 2015), and *H. galba* has also been reported to attach to jellyfishes (Madin and Harbison, 1977; Gasca *et al.*, 2007; Quigley *et al.*, 2015). The $D_{50\%}$ of these three

species occurred mainly at 150–300 m (Table III). These three species did not occur at the sea surface or the deepest depths in this study, and their vertical distribution patterns were classified for the same, occurring at intermediate depths (Figs 6d, 7b and d).

The zooplankton biomass of each taxon used in the samples in this study has been previously reported (Kitamura *et al.*, 2016). The day/night vertical distributions of radiolarians/phaeodarians, cnidarians and thaliaceans reported by Kitamura *et al.* (2016) are shown in Electronic Supplement 18. The $D_{50\%}$ of the above-mentioned three species was 133–159 m (*V. caeca*), 134–255 m (*H. galba*), 261–394 m (*S. borealis*) and shallower at night (Table III). The $D_{50\%}$ of cnidarians was 102–154 m and that of thaliaceans was 200–250 m. Thus, the vertical distributions of the host and three amphipod species nearly correspond. However, clear associations between them (i.e. occurrence of amphipod species in gelatinous zooplankton) were not observed in this study. It is possible to drop off the amphipods from the gelatinous zooplankton during oblique net towing of IONESS. To evaluate the intensity of pelagic amphipod attachment on gelatinous zooplankton, an *in-situ* observation system made by nondestructive visual devices such as a video plankton recorder may be a suitable instrument for this purpose (Takahashi *et al.*, 2013; 2015a; b).

Nitrogen stable isotopes ($\delta^{15}\text{N}$)

The nitrogen stable isotopes ($\delta^{15}\text{N}$) of the pelagic amphipods have a linear depth-related relationship: they increase with increasing depth (Fig. 8). $\delta^{15}\text{N}$ is an index of the trophic level, and their differences of 3–4% suggest differences in trophic levels (Aita *et al.*, 2011). The depth-related increase in $\delta^{15}\text{N}$ of mesozooplankton has been reported for the Mediterranean and the Arabian Sea. Moreover, $\delta^{15}\text{N}$ values near the surface layer (2%) are reported to increase ~12% at 4000 m, and it is interpreted that the particulate organic materials reach deeper depths through several-time prey-predation interactions (Koppelman and Weikert, 2000; 2003; Koppelman *et al.*, 2009).

Bearing this in mind, the depth-increasing patterns observed for $\delta^{15}\text{N}$ in the pelagic amphipods in this study may be related to the accumulation of prey-predation interactions that may increase with increasing depth. Interestingly, several amphipod species had extremely low (*C. challengerii* and *H. galba*) or high (*T. pacifica*, *V. caeca* and *R. natator*) $\delta^{15}\text{N}$ values than the regression line (Fig. 8). Since the $\delta^{15}\text{N}$ values are indices of trophic levels, the former species may have a herbivorous tendency, whereas the latter species would have a carnivorous tendency.

As feeding modes of these species, small-sized juveniles of *Themisto* spp. are reported to become carnivores as they grow (Sugisaki *et al.*, 1991; Pakhomov and Perissinotto, 1996; Froneman *et al.*, 2000; Haro-Garay, 2003). Thus, based on gut pigment and $\delta^{15}\text{N}$ values, Sugisaki *et al.* (1991) reported that the feeding modes of *T. japonica* change from herbivores to carnivores as they grow. For *C. challengerii*, carnivorous (Haro-Garay, 2003) or scavenging (Yamada and Ikeda, 2003) feeding modes have been proposed. *H. galba* has been reported to be associated with jellyfish (Madin and Harbison, 1977; Gasca *et al.*, 2007; Fleming *et al.*, 2014; Espinosa-Leal *et al.*, 2021). Associations with salps have been reported for *V. caeca* (Harbison *et al.*, 1977; Roe *et al.*, 1984; Gasca *et al.*, 2007; Quigley *et al.*, 2015). *R. natator* is reported to be distributed at depths of 930–5500 m in the Gulf of Alaska (Barnard, 1964).

Thus, while the $\delta^{15}\text{N}$ values of the pelagic amphipods showed a clear depth-related relationship, the feeding modes of the species plotted out of the regression did not correspond with the reported feeding modes of each species. As an alternative explanation, the $\delta^{15}\text{N}$ values of the species with exoskeletons are reported to have lower values of the exoskeleton, whereas internal tissues may have relatively high $\delta^{15}\text{N}$ values, and the internal tissue values may express the feeding modes of each species. Therefore, the low $\delta^{15}\text{N}$ values observed for *C. challengerii* and *H. galba* might reflect the development of the exoskeleton of these species, while the high $\delta^{15}\text{N}$ values of *T. pacifica*, *V. caeca* and *R. natator* might reflect the thinner exoskeleton of these species. Because of the smaller body sizes of the target species, we did not separate the exoskeleton and internal tissues in this study for measurement of $\delta^{15}\text{N}$ values that is termed the “bulk method” for the pelagic amphipods (Søreide and Nygård, 2012). Thus, for evaluation of the exact trophic levels, measurements that separate the exoskeleton and internal tissue, and measurement of $\delta^{15}\text{N}$ only on the internal tissue might be required for pelagic amphipods in future studies.

Population structure of the dominant species

In the present study, using the images captured by ZooScan, developmental stages of the dominant amphipod species, *T. pacifica*, were identified, and histogram drawing applying the ESD as an index of the body size of the individuals, and cohort analyses on the ESD were possible (Fig. 9). Because of their rounded body shapes, body size measurements of pelagic amphipods are time-consuming. However, with the initially sorted taxa samples, captured zooplankton images and species

or stage identification performed on the images, they are relatively easy and rapidly analyzed (Irisson *et al.*, 2022). Thus, this study showed the usefulness of ZooScan analyses for community and population structure analyses of dominant zooplankton taxa. When sorted samples on each taxon are available, ZooScan analysis allows us to obtain each species' abundance and biomass (biovolume) data by capturing images. The biovolume is easily converted to ESD, which is used as an index of body size. It should be noted that the numbers in body size measurements in Fig 9a were maximum of 1138 individuals per one occasion. Measuring their body length with a microscope is very time-consuming, and accuracy and precision may be similar to those of the ZooScan data (Gorsky *et al.*, 2010). Because of these reasons, the usefulness of ZooScan using sorted samples on specific taxon is easily understood.

For the population structure data of *T. pacifica*, the main recruitment of juveniles was observed in July–October which corresponded to the period in the Oyashio region (Yamada *et al.*, 2004). While each cohort of ESD seems to be traceable from small juveniles to adults, it should be noted that the population structure analyses based on the more frequent sampling interval (16 times per 2 years: 8 times per year) showed that the recruitment of young *T. pacifica* in the Oyashio region and their generation length varies with temperature, and 32 days at 12°C and 224 days at 2°C have been reported (Yamada *et al.*, 2004). Considering these time scales, the rough sampling interval of this study (four times per year) may make it difficult to accurately trace the cohort of *T. pacifica* in this region. Thus, while the cohort trace of this study in Fig. 9 is doubtful, the sampling interval of the time-series samples applied for this study is not adequate; if the samplings were conducted at a sufficient time interval, population structure analyses using ZooScan may provide solid insight into their life cycles. Thus, this study indicates the usefulness of ZooScan for community and population structure analyses of certain zooplankton taxa.

CONCLUSION

This study revealed the ecology of pelagic amphipods in the western subarctic Pacific by applying image-scanned data of the sorted pelagic amphipod samples from the day/night samples collected between 0 and 1000 m depths on four occasions covering 1 year. Through the analytical methods used in this study, the abundance, biovolume and community structure of pelagic amphipods were evaluated. In addition, the day/night vertical distribution patterns of all 10 amphipod species were evaluated and classified into

three patterns. For the most dominant pelagic amphipod species (*T. pacifica*), estimation of life cycle patterns is possible by using ESD data as an index of body size. Vertical changes in $\delta^{15}\text{N}$ of all species are novel attempts and findings in this field. For example, the alternative nitrogen stable isotope ($\delta^{15}\text{N}$) analyses showed increases in $\delta^{15}\text{N}$ values with increasing habitat depths at night. In this study, imaging analyses of the sorted samples with taxa were helpful for species identification, size quantification and estimation of the accurate biovolume of each individual.

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SUPPLEMENTARY DATA

Supplementary data can be found at *Journal of Plankton Research* online.

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DATA AVAILABILITY

Data will be provided upon request from the corresponding author.

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