

Seasonal changes in composition of zooplankton communities along the coast of Sado Island in the Sea of Japan

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Abstract

Information on zooplankton abundance is essential to evaluate the health of marine ecosystems, but zooplankton remain poorly studied in many regions. This study describes seasonal changes and community composition of zooplankton on the coast of Sado Island, in the Sea of Japan, which has unique geographic features. Zooplankton diversity and environmental parameters were surveyed monthly from April 2022 to March 2023 on the coast of Sado Island. The zooplankton community comprised 113 morphologically distinguished operational taxonomic units (OTUs). Zooplankton diversity was maximal in late winter whereas abundance peaked in early spring. Small harpacticoid copepods, copepod nauplii, and meroplankton (bivalves and fish larvae) were dominant throughout the year. Cyclopoid nauplii and bivalve larvae (veliger spp.) were more abundant in spring and summer, whereas small harpacticoid copepods were abundant in autumn and winter. Radiolarians and foraminifera were also found in summer and autumn along the coast. Moreover, several polychaetes were abundant in summer and winter. Distinct seasonal changes in zooplankton community composition and abundance were observed at Sado Island, which provide general information of zooplankton in the central Sea of Japan.

Key words: zooplankton; community; diversity; seasonality; coastline; Sado Island

Introduction

Sado Island, located in the east central part of the Sea of Japan, located west of Niigata Prefecture, is the second-largest offshore island of Japan. Sado Island has various coastal landforms, including sandy beaches and rocky reefs, with a coastline of 280 km. The island also offers a rich variety of seasonally different aquatic organisms and presents unique biodiversity. Sado island is surrounded by the Tsushima warm current (TWC) (Fig. 1), which brings rich fishery resources such as yellow tail (*Seriola quinqueradiata*) and bluefin tuna (*Thunnus orientalis*) from the East China Sea into the Sea of Japan. The TWC also affects the zooplankton community around Sado Island, but this has not been well studied.

Senkaku Bay, is situated on the northwestern coast of

Sado Island, near Tassha village, with its beach and harbor area. This bay is noted for its many fish species and other aquatic organisms, including plankton, such as radiolarians and mollusc and gastropod larvae, which occur there in abundance due to the TWC and the regular inflow of freshwater from a small river (Tassha River) (Fig. 1) (Abe et al. 1984; Matsuoka et al. 2001, 2002; Kurihara and Matsuoka 2011). The semi-enclosed bay includes various shore-protection structures that provide stable nursery sites and a wide range of food for fishes and other aquatic organisms. It also provides an ideal setting for investigating seasonal diversity of zooplankton and community composition in a relatively small geographic area.

The seasonal abundance and composition of zooplankton taxa at Sado Island have remained un-

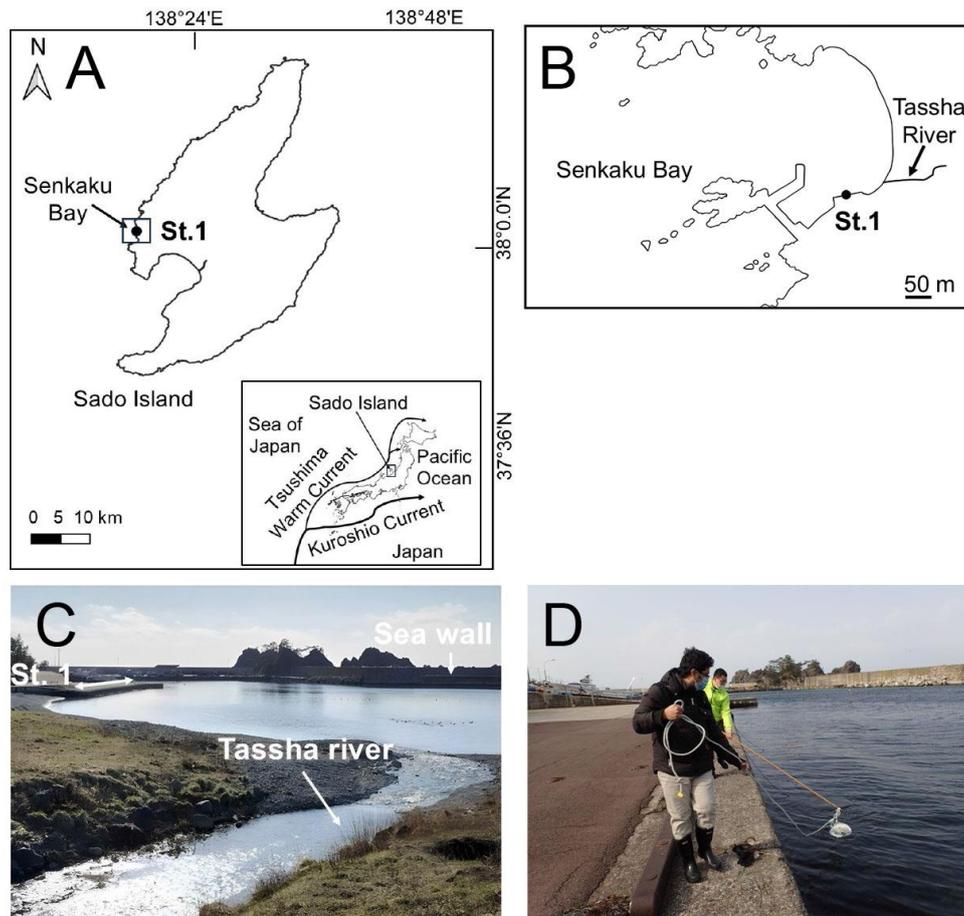


Fig. 1. The location of the sampling station at Sado Island, and the location of Sado Island along the coast of Japan (A, B). Photos of the landscape of the sampling station with the Tassha River (C) and hand-towed sampling using a plankton net (D).

explored. Only a few studies have focused on zooplankton (Abe et al. 1984) and other single-cell marine communities such as radiolarians (Matsuoka et al. 2001, 2002; Kurihara and Matsuoka 2009, 2010, 2011). Abe et al. (1984) listed 150 species of zooplankton at Tassha, which were dominated by copepods (Families Calanidae, Paracalanidae, Corycaeidae, and Acartiidae) from spring to autumn. In addition, radiolarians, such as the Orders Spumellaria and Nassellaria were dominant at Tassha, Senkaku Bay (Matsuoka et al. 2001, 2002; Kurihara et al. 2009, 2010, 2011). However, seasonal abundance of zooplankton is totally unknown. Therefore, the objectives of this study were to investigate seasonal changes of zooplankton in relation to environmental characteristics along the coast (Tassha, Senkaku Bay) of Sado Island.

Materials and Methods

Sampling sites

Sado Island (38.04°N, 138.33° E) is located offshore of Niigata Prefecture in the Sea of Japan, about 70–96 km from mainland Japan. The island has clear waters and rocky shores, and Senkaku Bay, including Tassha village on the inside of the bay, is on the west coast of the island. To understand seasonal changes of zooplankton communities, one sampling station (St. 1) was set on the coast (38°05.535' N, 138°14.806' E) of Tassha in Senkaku Bay (Fig. 1). St. 1 was along a concrete shoreline inside an area protected by a seawall (approximately 20 m height) next to the outlet of the Tassha River (approximately 3 m width and shallower than 1 m at the river mouth) (Fig. 1). St. 1 was located adjacent to a mountainous area. The water is shallow (1–2 m) and the site is influenced by strong

wind, especially in winter, but relatively low waves in comparison with offshore due to the sea wall (Fig. 1). There are stones and pebbles on the bottom, with various green and brown algae. Transparency is high and larvae and juveniles of various aquatic organisms such as fish, crabs, copepods, snails and annelids are abundant, either floating, swimming or attached to the concrete shoreline.

Environmental data

A CTD, (conductivity, temperature and depth profiler, RINKO-Profilor, ASTD102, JFE Advantech, Japan) was used to measure conductivity, temperature, and depth at the time of zooplankton sampling (details below). Environmental parameters included water temperature (°C), dissolved oxygen (DO, mg/L), pH, chlorophyll-*a* (Chl-*a*, µg/L), and salinity, which were measured every 1 m in depth. The CTD was lowered into the sea 1–2 m at St. 1.

Field sampling and preservation of zooplankton

Monthly zooplankton sampling was carried out from April 2022 to March 2023 at the sampling station using a plankton net. Sampling was done about 1 m below the surface along the coast at approximately 9:00 to 10:00 am. At St. 1, a plankton net (a Kitahara surface plankton net, XX13, mesh size 100 µm and mouth diameter 0.3 m, Rigo-sha Ltd.) was towed by hand by two people using a long pole. The towing distance was 90 m along the artificial concrete shoreline. The volume of water filtered (*V*) was calculated using the formula below.

$V = \pi r^2 h$, where *r* is the radius of the net ring, and the *h* is the distance towed (Karmakar et al. 2022). As a result, 84.8 m³ water were filtered each time.

Sampling was conducted once each month throughout the year and one pass with the net was made at each sampling. Collected samples were brought to the laboratory (Marine Biological Station, Niigata University: SMBS) and were examined under a stereomicroscope (Olympus, SZX16) to observe

movements and characteristics of zooplankton. Afterward, samples were preserved in 3 % formaldehyde for identification and quantitative observations.

Taxonomic identification of zooplankton

Taxonomic identification of zooplankton was done in the SMBS laboratory using a stereomicroscope (Olympus, SZX16) and camera system (Olympus, DP23). Zooplankton were identified to the lowest possible taxonomic level using publications (Yamaji 1984; Chihara and Murano 1997; Larink and Westheide 2011; Iwakuni City Micro Life Museum 2013) and treated as operational taxonomic units (OTUs). Zooplankton are diverse, and there are no diagnostic features that can be used for all zooplankton taxa, including their developmental stages. Moreover, counting and observation of body parts are needed for species or generic-level identification for some taxonomic groups. However, small body parts, for example, grasping spines on the head region and fins of Sagittidae and long antennae and appendages of calanoid copepods, were sometimes broken during sampling. Therefore, we used OTUs instead of species, as done in other plankton studies (Costa et al. 2011; Gallego et al. 2014; Pineda-Metz and Montiel 2021; Costello et al. 2023; Killeen et al. 2023; Yamamae et al. 2023). For the sake of accuracy, we identified some small zooplankton to the order level. Taxonomic status of identified OTUs was confirmed using the World Register of Marine Species database (WoRMS Editorial Board 2023). Though many zooplankton were identified to family or order, not species, sp. 1 or spp. for OTU was used for convenience. Single-cell organisms (such as radiolarians and foraminiferans) were also included in this study as they are vital members of plankton communities in this region.

After observation and taxonomic identification, 5 mL subsamples were transferred to petri dishes 5 times using pipettes. In total, 25 mL of concentrated, preserved sub-samples were used for counting the

abundance of each zooplankton OTU. They were calculated as individuals/10 m³ using the counted results of the 25 mL preserved samples, as some zooplankton OTUs were very rare. We developed a large list of identified heterotrophic zooplankton including offshore samples, but we only included zooplankton OTUs found during this field survey. Note that in this study, “zooplankton community” refers to zooplankton collected in each month or season.

Statistical analysis

We defined seasons as follows: April to June, spring; July to September, summer; October to December, autumn; and January to March, winter, according to general seawater temperature changes in temperate regions.

The Shannon diversity index (Shannon 1951), which is based on information theory (Morris et al. 2014), was used to summarize plankton diversity. It reflects the rarity or commonness of species in given communities (Sarker et al. 2021), while including uncertainty about individual identities. This index was calculated according to the following equation:

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

where, H' = observed diversity index, P_i = proportion of individuals of i th OTU in a whole community. A cluster dendrogram was used to find distinct groups or clusters in monthly zooplankton datasets. All statistical analyses were done with the vegan package in R ver. 4.2.2 (R core team, 2022).

Results

Environmental parameters

Environmental parameters such as water temperature, DO, chlorophyll-*a*, and salinity were relatively similar among months in the same season, but showed seasonal changes (Table 1). Water temperature was the highest in August (27.3 °C in summer) and the lowest in February (10.2 °C in winter). Salinity ranged from 29.5 in April (spring) to 34.0 in August (summer). The concentration of chlorophyll-*a* was noticeably higher in April (3.9 µg/L in spring) and July (3.5 µg/L in summer). Comparatively higher DO was measured in April (10.6 mg/L in spring) and February (10.9 mg/L in winter) (Table 1).

Table 1. Monthly and seasonal sea-surface environmental variables in the top one meter from April 2022 to March 2023 at Sado Island.

Season	Month	Temperature (°C)		Salinity		Chlorophyll- <i>a</i> (µg/L)		DO (mg/L)	
		Monthly	Average	Monthly	Average	Monthly	Average	Monthly	Average
Spring	April	11.2		29.5		3.9		10.6	
	May	16.0	15.6	33.2	31.8	0.3	1.5	8.9	8.8
	June	19.6		32.9		0.3		6.9	
Summer	July	25.5		33.2		3.5		6.9	
	August	27.3	25.8	34.0	32.6	0.5	1.5	6.5	6.7
	September	24.6		30.4		0.4		6.6	
Autumn	October	20.1		32.5		0.3		7.3	
	November	18.8	16.9	33.4	32.5	0.2	0.2	7.4	7.7
	December	11.8		31.7		0.2		8.3	
Winter	January	10.8		32.7		0.2		9.2	
	February	10.2	11.1	33.2	31.9	0.1	0.2	10.9	9.9
	March	12.4		29.9		0.2		9.6	

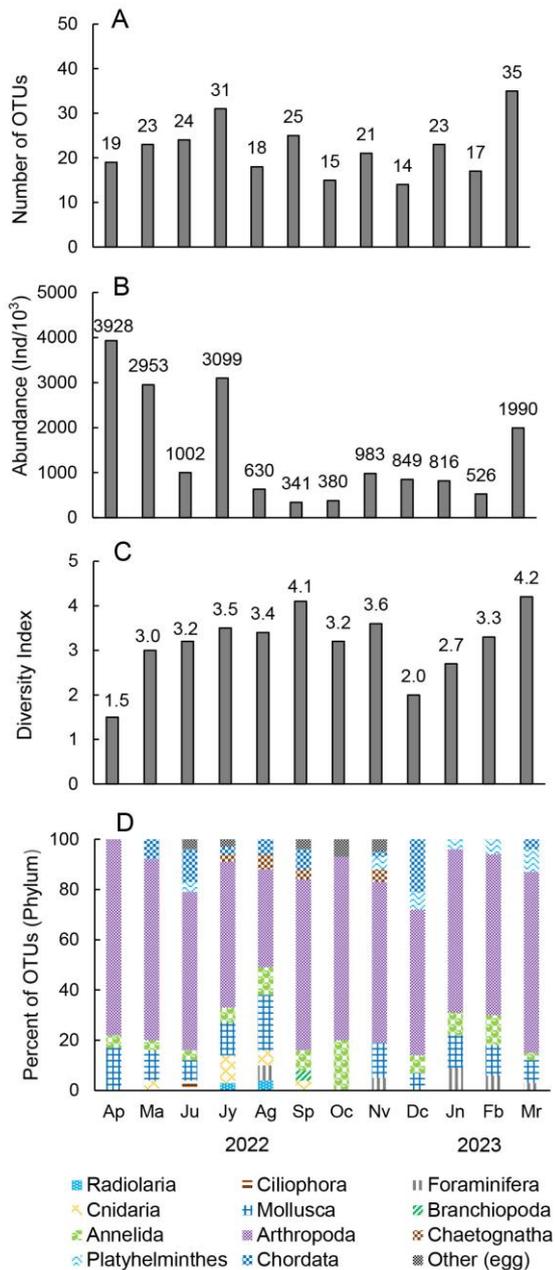


Fig. 2. Monthly variations in number OTUs (A), abundance (B), diversity index (C) and percentage of zooplankton OTUs by phylum (D) from April 2022 (spring) to March 2023 (winter) along the coast of Sado Island.

Overview of taxonomic composition

A total of 113 zooplankton OTUs were identified from zooplankton samples collected during 12 sampling months at Tassha, Senkaku Bay (Supplementary Table S1). OTU composition showed clear seasonal variation. The largest number of OTUs was found in March (35 in winter), but the smallest

occurred in December (14 in autumn) (Fig. 2A). Zooplankton comprised mostly copepods, nauplii, gastropods, veligers (early developmental stage of bivalves), and polychaetes (Table 2). At the phylum level, the largest numbers of OTUs were arthropods, followed by molluscs, annelids, and chordates (Fig. 2D). The contribution of each phylum differed by month and season. Arthropods were most numerous throughout the year, especially in autumn (highest in October), molluscs in summer (August), chordates in autumn (December) and annelids in autumn (October) (Fig. 2D). Arthropods were abundant throughout the year and dominated by harpacticoid copepods and cyclopoid nauplii (Supplementary Table S2). Among molluscs, littorinimorpha (sp. 1) and veliger (sp. 1) were abundant (Supplementary Table S2). Some phyla were collected only or mainly in certain months/seasons, such as Ciliophora in late spring (June), and Radiolaria in summer (July–August) (Fig. 2D).

Variation in abundance and diversity index

Zooplankton abundance varied seasonally at the study site (Supplementary Table S2). Abundance peaked in early spring (3928 Ind./10 m³ in April), while it was lowest in late summer (341 Ind./10 m³ in September) (Fig. 2B). The Shannon diversity index was high in late winter (4.2 in March) and late summer (4.1 in September) whereas it was lowest in early spring (1.5 in April) (Fig. 2C). In terms of seasonality, zooplankton communities were generally more diverse in summer, including copepods (*Oncaea* sp. 1, *Corycaeus* sp. 1 and *Microsetella* sp. 1), radiolarians (*Acanthometron pellucidum*), foraminifera (*Rotaliida* sp. 2), cladocerans (*Evadne spinifera*), and tunicates (*Oikopleura* sp. 1).

Zooplankton community

Abundant zooplankton groups were relatively similar among months in a given season, but clearly fluctuated between seasons. For example, cyclopoid nauplii (sp.

Table 2. The five most abundant zooplankton OTUs and their total abundance (Ind./10 m³) from April 2022 to March 2023 at Sado Island.

Spring		2022 Summer		Autumn		2023 Winter	
OTU	Abundance	OTU	Abundance	OTU	Abundance	OTU	Abundance
Cyclopoid nauplii sp. 1	3781	Veliger sp. 1	933	Harpacticoida sp. 9	466	Harpacticidae sp. 1	515
Harpacticoida sp. 4	669	Littorinimorpha sp. 1	512	Cyclopoid nauplii sp. 1	310	Polychaeta sp. 3	338
Veliger sp. 1	557	Cirripedia nauplii sp. 1	491	Harpacticoida sp. 3	221	Harpacticoida sp. 3	230
Harpacticoida sp. 1	418	Polychaeta sp. 3	280	Littorinimorpha sp. 1	164	Harpacticoida sp. 4	211
Littorinimorpha sp. 1	335	Calanoida sp. 16	221	Cyclopoida sp. 1	154	Calanoid nauplii sp. 3	207

1) was abundant in April (early spring), veligers were abundant in July (early summer), and harpacticoid copepods were numerous in late autumn (December) and early winter (January) (Supplementary Table S2). For the five dominant zooplankton OTUs and their total seasonal abundance (Ind./10 m³) (Table 2), cyclopoid nauplii sp. 1 and harpacticoid sp. 1 were highly abundant in April, May and June, but their abundance was low in other seasons (Supplementary Table S2). In spring, some calanoid copepods (spp. 1–5, 13 and 15) were also found (Supplementary Table S2). In summer, the largest numbers of bivalve veliger sp. 1 and cirriped nauplii sp. 1 were observed (Table 2) and some radiolarians (*Acanthometron pellucidum*) and foraminifera (*Rotaliida* sp. 2) were abundant (Supplementary Table S2). In autumn, harpacticoida sp. 9 and some cyclopoid copepods were abundant (Supplementary Table S2). In winter, Harpacticidae sp. 1 and polychaete sp. 3 were dominant (Table 2). There were some unique OTUs

observed in winter such as *Polychaete* sp. 12 and *Pseudoblennius* sp. 1 (Supplementary Table S2), which were not observed in other seasons. A dendrogram from the cluster analysis shows that zooplankton communities in spring (April and May) and early autumn (October) were different from those of other seasons (Fig. 3).

Discussion

Community composition, diversity, and seasonal abundance of zooplankton

This is the first study of year-round, seasonal changes of zooplankton communities along the coast of Sado Island. During the survey, 113 zooplankton OTUs were identified. Abe et al. (1984) identified 150 species of zooplankton from 2 sampling stations located offshore near Senkaku Bay, Sado Island. The number of zooplankton OTUs identified was probably lower in the present study because Abe et al. (1984) worked 2 offshore stations vertically, whereas we

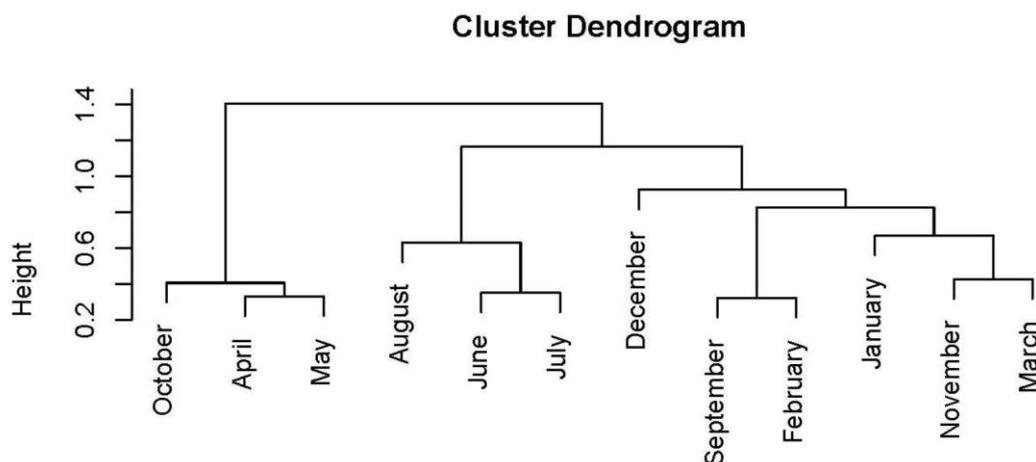


Fig. 3. Dendrogram of cluster analysis of monthly changes in the zooplankton community from April 2022 (spring) to March 2023 (winter).

surveyed a coastal station. Our results were similar to those of Abe et al. (1984) in terms of major copepods of harpacticoid and cyclopoid.

Zooplankton communities showed monthly and seasonal variation. The zooplankton communities in spring (April and May) and early autumn (October) were distinctly different from those of other seasons (Fig. 3). That was probably due to the high abundance of cyclopoid nauplii (sp. 1) and bivalve veliger (sp. 1) in spring (April and May) and autumn (October), which were not abundant in other seasons. In addition, similarities were found between summer (September) and winter (February). In these two months, polychaete sp. 3 and harpacticoid sp. 1 were abundant. Similarities were also observed in autumn (November) and winter (March), which was probably because of the high abundance of bivalve molluscs (*Littorinimorpha* sp. 1) and harpacticoid copepods (Harpacticoida spp. 3–4), which were not abundant in other seasons (Supplementary Table S2). Moreover, consecutive months (March and April) were separated because of community compositional differences. Harpacticidae sp. 1 was dominant in March (winter), but cyclopoid nauplii sp. 1 was dominant in April (spring). Notably, these occurred in different seasons (April and March) in relation to general sea water temperature changes in temperate Japan. It is probable that environmental differences, e.g. water temperature, in these two months affects zooplankton composition between these months.

We recorded a noticeable seasonal difference in OTU numbers. Lowest numbers of OTUs were found in December (autumn). Some environmental characteristics of Sado Island in late autumn, such as cool weather, high water transparency, low wave height, and low water level occurred in relation to seasonal changes of water volume of the TWC (Fukudome et al. 2010). These may affect the zooplankton community of the coast in late autumn.

Habitat characteristics influence the plankton community. Crustacean nauplii and bivalve veligers

were abundant. Ecological features of the sampling site included shallow depth, regular inflow of freshwater and snow-melt water in winter from the Tassha River, and low wave action due to artificial barriers near the beach, the presence of seaweeds, and rocky shores that are surrounded by mountains on three sides. These features could make this site a stable spawning and nursery for many aquatic organisms. In fact, meroplankton were one of the major groups and strongly influenced numbers of OTUs in this study.

The Shannon diversity index was highest in March (4.2 in winter) and lowest in April (1.5 in spring). In March, some OTUs were collected that were not observed in other months, such as Oithonidae sp. 4, Calanoid nauplius sp. 3, *Pseudoblennius* sp. 1 and flatworm (spp. 3–5) (Supplementary Table S2). On the other hand, some OTUs that were dominant at some seasons, such as Phyllostauridae sp. 1, Polychaeta sp. 3, Harpacticoida sp. 9 and Harpacticidae sp. 1, were not observed in April (Supplementary Table S2). There were some fluctuations among months in the same season, but those were relatively minor. For example, the diversity index was high in September (4.1 in summer) and somewhat low in July (3.4 in summer) (Fig. 2C). Various zooplankton (molluscs, copepods, annelids, foraminiferans and radiolarians) were observed and those were reflected in the high diversity index in summer. In addition, several unique OTUs, copepods (*Oncaea* sp. 1, *Oncaea* sp. 1, Calanoida sp. 13), radiolarians (Phyllostauridae sp. 1), foraminiferans (*Rotaliida* sp. 2), Chaetognaths (*Sagittidae* sp. 5), annelid worms (*Syllidae* sp. 1), Ostracoids (*Ostracoda* sp. 2), and nauplii (Calanoid nauplius sp. 2) also contributed to higher diversity in summer. High species diversity of warm water foraminiferans and radiolarians during summer, which have been reported previously (Abe et al. 1984; Matsuoka et al. 2001, 2002), may be related to the strength of the TWC around Sado Island.

Zooplankton abundance along the coast was high in spring due to the large number of cyclopoid nauplii

(sp. 1) and veliger (sp. 1). Arima et al. (2014) reported that copepod nauplii dominated during late winter and spring in Ishikari Bay, in the northern part of the Sea of Japan. In the present study, chlorophyll-*a* levels were high in spring and summer (Table 1). These increase in response to increasing nutrient loads because nutrient levels are directly correlated with sunlight intensity in relation to depth (Maradhy et al. 2022). Thus, the dominance of cyclopoid nauplii (sp. 1) and veliger (sp. 1) from winter to spring in this study likely reflects zooplankton and bivalve reproduction, initiated by phytoplankton blooms.

Characteristics of major zooplankton groups

Some distributional characteristics of zooplankton were observed along the coast of Sado Island. Regarding specific groups of copepods, harpacticoid copepods were an important component of the zooplankton community in winter due to their high abundance. This group is distributed in brackish, shallow, and algae-covered coastal waters (Biancalana et al. 2020). Both soft and hard bottom substrates as well as algae fronds provide micro-habitats for harpacticoid copepods and other crustaceans such as amphipods and isopods (Arroye et al. 2006). During winter, seaweed (*Sargassum*) was commonly observed on the coast at Tassha during sampling. Copepods tend to be abundant in winter when foraging activity of fish is lower (Choi and Kim 2021). Environmental characteristics such as snow-melt from the Tassha River on the sea-surface, seaweed, and the low consumption and metabolic activity of fish would provide suitable habitat for harpacticoid copepods.

Several meroplankton also showed seasonality in their distributions. Veliger (sp. 1) was abundant in spring when a lot of juvenile/adult gastropod snails (*Littorinimorpha* sp. 1) were observed (Table 2) and they were a major group of the summer zooplankton community on the coast. Veligers in spring were probably the earlier stage of molluscs found in summer. Arima et al. (2014) reported a similar distribution of

snails during summer and autumn in Ishikari Bay in the northern Sea of Japan.

Conclusions

Seasonal changes in the abundance, diversity and composition of zooplankton occurred along the coast of Sado Island. A total of 113 zooplankton OTUs were found, with OTUs being most numerous in late winter. Zooplankton abundance was greatest during spring. Environments in the bay, including benthic habitats (rocky substrates and seaweed) used by species such as gastropods, likely influence observed differences in zooplankton composition and abundances throughout the year. These results have improved our understanding of zooplankton diversity and community composition along Sado Island.

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