東海大学大学院令和 4 年度博士論文

Ecological studies on marine cladocerans in Suruga Bay, Japan: mass occurrence in offshore waters, formation mechanisms, and roles in the offshore food-web

（駿河湾における海産枝角類の生態学的研究：
沖合域における大量出現とその形成メカニズム
および食物網における役割）

指導　西川　淳　教授

東海大学大学院生物科学研究科

生物科学専攻

剣持　瑛行

Contents

Chapter 1. Population dynamics of marine cladocerans in offshore Suruga Bay, Japan

1-1. Introduction

Cladocerans are small crustaceans belonging to the class Branchiopoda. Four orders are recognized: Anomopoda, Ctenopoda, Onychopoda, and Haplopoda (Forró et al. 2008). More than 620 species of cladocerans have been reported globally, and are mostly freshwater species (Forró et al. 2008). In contrast to the high species diversity in freshwater species, only 8 species are known from marine habitats (Onbé 1999).

Based on previous molecular studies and fossil records, cladocerans are considered to be an ancient crustacean group (e.g. Van Damme and Kotov 2016). Marine cladocerans belong to two orders, Onychopoda and Ctenopoda. Most species of onychopods are considered to originated from the Ponto-Caspian region, such as Baltic, Caspian, and Aral Seas, and expanded their distributions in the world ocean during the Pliocene–Pleistocene (Durbin et al. 2008). *Penilia avirostris*, which only marine species belongs to the onychopoda, is considered to more recent oceanic expansion than Podonids (Durbin et al. 2008).

Marine cladocerans are known as members of coastal zooplankton, and are distributed widely from tropical to arctic waters. In coastal regions, they sometimes comprise substantial portions of mesozooplankton communities in certain seasons (Tang et al. 1995; Onbé et al.

1996b; Marazzo and Valentin 2000). Nevertheless, despite their high abundance, there have been relatively few studies on marine cladocerans, compared to other crustacean plankton, such as copepods. In particular, there are only a few studies on marine cladocerans which conducted for over a five year-period. According to Onbé (1974), based on near two year's survey, five species of marine cladocerans appeared in the Seto Inland Sea from spring to autumn every year. On the other hand, Terbiyik Kurt and Polat (2014) reported that the seasonal pattern of the cladoceran population varied remarkably with changing environmental parameters, such as salinity. These evidence suggest the importance of studying the interannual variation to understand the seasonal patterns of occurrence in cladoceran population.

Cladocerans usually have two reproductive modes: parthenogenesis and gamogenesis (Egloff et al. 1997). Although cladocerans employ parthenogenetic reproduction during favorable conditions, they produce resting eggs gamogenetically in response to environmental changes and survive unfavorable conditions as resting eggs that sink to the bottom (Egloff et al. 1997). Thus, their habitats are usually restricted to shallow coastal areas, since their populations are initiated annually by the hatching of resting eggs. On the other hand, relatively high numbers of cladocerans have also been found in offshore waters in various parts of the oceans (Wiborg 1955; Della Croce and Venugopal 1972; Longhurst and Seibert 1972; Kim and Onbé 1995). Some of these populations are transported sporadically from coastal areas (Egloff et al.

1997). However, the frequency of occurrences and the fates of offshore populations are mostly unknown.

While some mesozooplankton in offshore Suruga Bay, such as chaetognaths (e.g. Nagasawa and Marumo 1972), euphausiids (e.g. Hirota et al. 1984), and decapods (e.g. Omori 1983; Bishop et al. 1989) have been studied, little is known about cladocerans. In addition, most studies in other Japanese waters have been conducted in shallow coastal waters, where they are abundant (Saito and Hattori 2000; Komazawa and Endo 2002; Onbé and Kumagai 2019).

The aim of this chapter is to examine the species composition and seasonal abundances of cladocerans in offshore Suruga Bay based on the investigations over a 5-year period. In addition, to examine whether cladocerans reproduce in offshore Suruga Bay, reproduction parameters were analyzed on a dominant species, *Penilia avirostris*.

1-2. Materials and Methods

1-2-1. Study site

Suruga Bay is located in Shizuoka Prefecture in central Japan, and opens southward into the Pacific Ocean (Fig. 1-1). The bay possesses a deep canyon, called the Suruga Trough, in the center of the bay with a maximum depth of ca. 2450 m. The main stream of Kuroshio Current, which is a warm current, flows northeastward off the mouth of Suruga Bay. The branch

of the Kuroshio Current sometimes flows into the bay, and affects its surface currents and hydrography (Inaba 1984). In contrast to the oceanic condition in offshore Suruga Bay, a large amount of freshwater flows into the bay from large rivers, such as the Fuji, Abe, and Oi Rivers (Tanaka et al. 2009). That freshwater mixes with surface waters of the bay, and forms a water mass known as "coastal water" (Nakamura 1982). Coastal water is characterized by low temperature, low salinity, and low transparency, and it expands from the inner region along the western coastal area of the bay.

1-2-2. Sampling design and data analysis

Zooplankton sampling was conducted monthly at a fixed station, SR1 (35˚03'20"N, 138˚41'00"E, depth: ca. 1000 m, Fig. 1-1), located in offshore Suruga Bay, from 21 June 2014 to 7 December 2019, during research cruises of the R/V Hokuto of Tokai University. An ORI net (Omori et al. 1965) with 335 μm mesh was used for mesozooplankton collection. While micro-sized zooplankton could pass through the mesh size used in this study, and this may cause an underestimation of micro cladoceran abundances, most meso-sized adult cladocerans were assumed to collect effectively with this mesh size. The net was hauled obliquely with a maximum wire-length of 200 m. A flowmeter (Rigosha & Co., Ltd., Japan) was equipped at the mouth of the net to estimate the filtered volume (m^3) of seawater. Sampling depths of the net

were recorded with a data logger (Compact-TD, JFE Advantech Co., Ltd., Japan). In this study, the average of maximum sampling-depth of the net was 116 ± 12.6 m.

Vertical profiles of water temperature and salinity were recorded with a CTD system (SBE 19Plus, Sea-Bird Electronics, USA). Water temperature and salinity values at 1 m intervals were used for analysis. Water samples from 8 layers (0, 5, 10, 20, 30, 50, 75, 100 m) were collected using Niskin bottles for chlorophyll *a* analysis. In the laboratory, water samples were filtered with a GF/F filter (GE Healthcare, USA), and extracted with N, N-dimethylformamide (Suzuki and Ishimaru 1990). Then, chlorophyll *a* concentrations were determined by fluorometry (Welschmeyer 1994) (Trilogy Laboratory Fluorometer, Turner Designs, Inc., USA).

After collection, zooplankton samples were preserved in 10% buffered formalin-seawater. Fifteen mesozooplankton taxa shown in Table 1-1 were sorted, identified, and counted under a dissecting microscope. And, their abundances (individuals m^{-3}) were estimated. Cladocerans were then identified to species, following Onbé (1997). In addition, specimens of *Penilia avirostris* Dana, 1849 were randomly sorted to analyze body length, reproductive stages, and brood sizes. Body length was measured from the tip of the head to the base of the caudal seta (Onbé 1999). The reproductive stage of each individual (parthenogenetic, females without embryos, and gamogenetic individuals) was identified based on Onbé (1974). Brood pouches of

parthenogenetic females were dissected to determine their brood sizes (number of embryos). Analyses of *Pe. avirostris* were carried out with samples obtained from 2015 to 2019.

Community structure of offshore cladocerans was analyzed based on abundances of each cladoceran species. Group average clustering was conducted based on Bray-Curtis similarity index, which was calculated from log (x + 1)-transformed abundances. Community structure analysis was performed with PRIMER 6 (PRIMER-E Ltd., UK).

The Kruskal-Wallis test with Dunn's post-hoc test was employed to assess inter-annual differences in environmental parameters and abundances of cladocerans from 2015 to 2019. The relationships between cladoceran abundance and hydrographical parameters were evaluated with Spearman's rank correlation. Since marine cladocerans are mostly distributed in the upper 100 m (Onbé 1974; Kim and Onbé 1995; Egloff et al. 1997), hydrographical conditions in shallow depth layers may affect cladoceran abundance. Thus, mean temperature and salinity in the 0–30 m, 0–50 m, and 0–100 m layers were calculated and tested. Analysis of variance (ANOVA) with Tukey-Kramer HSD post-hoc test was used to examine temporal variation in body size of *Pe. avirostris*. Since most of the data on brood size of *Pe. avirostris* did not follow a normal distribution, the Kruskal-Wallis test with Dunn's post-hoc test was applied for monthly differences in brood size. All statistical analyses were performed using JMP 13 (SAS Institute Inc., Cary, NC, USA). The contour graphs of temporal changes in temperature,

salinity, and chlorophyll *a* concentration in the 0–100 m water-column were drawn with Ocean Data View (Schlitzer 2019).

1-3. Results

1-3-1. Hydrography

Vertical profiles of temperature, salinity, and chlorophyll *a* concentrations in the 0–100 m water column from June 2014 to December 2019 were shown in Figure 1-2. Surface temperatures and salinities (at 1 m) varied from 12.8 to 28.1 °C and 28.5 to 34.4, respectively. While there were no inter-annual differences in the mean surface temperatures, mean surface salinities recorded in 2017 were higher than 2015 and 2018 (Kruskal-Wallis test: $n = 42$, $h = 14.5$, $df = 4$, $p < 0.01$; Dunn's test: $p < 0.05$). Vertical distributions of temperature and salinity showed seasonal variations typical to those in mid-latitude waters: higher temperatures and lower salinities were recorded, and seasonal thermoclines were formed from July to September. From November to March, temperatures were relatively low, and vertical mixing was observed in the upper 100 m. While chlorophyll *a* concentrations occasionally showed higher values, especially during winter, no clear seasonal patterns were observed. There were no inter-annual differences in the mean temperatures (Kruskal-Wallis test, 0–30 m: $n = 60$, $h = 2.0$, $df = 4$, $p > 0.1$; 0–50m: $n = 60$, $h = 2.8$, $df = 4$, $p > 0.1$; 0–100 m: $n = 60$, $h = 3.7$, $df = 4$, $p > 0.1$) and

salinities (Kruskal-Wallis test, 0–30 m: $n = 60$, $h = 3.4$, $df = 4$, $p > 0.1$; 0–50 m: $n = 60$, $h = 3.9$, $df = 4$, $p > 0.1$; 0–100 m: $n = 60$, $h = 4.0$, $df = 4$, $p > 0.1$), maximum chlorophyll *a* values (Kruskal-Wallis test, $n = 60$, $h = 6.2$, $df = 4$, $p > 0.1$), or accumulated chlorophyll *a* concentrations (Kruskal-Wallis test, $n = 60$, $h = 8.1$, $df = 4$, $p > 0.1$).

1-3-2. Seasonal abundance of cladocerans

At the offshore station, cladocerans appeared in all research years (Fig. 1-3). During winter, their abundances were relatively low, and they increased remarkably from June to August. After September, population densities dropped, and they completely disappeared from the water column in January. Although the timings of appearance and disappearance of cladocerans were slightly different between years, they appeared in high abundances (>10 inds. m^{-3}) during spring and summer every year. There were no significant differences in annual median abundances between years (Kruskal-Wallis test, $n = 59$, $h = 2.0$, $df = 4$, $p = 0.63$).

When cladocerans occurred at their maximum abundances, they constituted major portions of the offshore mesozooplankton community (Fig. 1-4), i.e., 23–87% of total zooplankton abundance. Except for 2015 and 2019, cladocerans were the dominant mesozooplankton taxa in the offshore area, exceeding even copepods.

Seven species of cladocerans were observed during the study: *Penilia avirostris*,

Pseudevadne tergestina Claus, 1877, *Evadne nordmann*i Lovén, 1836, *Evadne spinifera* P. E. Müller, 1867, *Pleopis schmackeri* (Poppe, 1889), *Pleopis polyphemoides* (Leuckart, 1859), and *Podon leuckartii* (G.O. Sars, 1862) (Table 1-2). *Penilia avirostris* was the dominant species every year, followed by *Ps. tergestina*, *E. spinifera*, and *E. nordmanni*. Contrastingly, *Pl. polyphemoides* and *Po. leuckartii* occurred only at low densities (< 0.1 individuals m^{-3}) throughout the investigation periods. Three of the dominant species, *E. nordmanni*, *Pe. avirostris*, and *Ps. tergestina*, showed clear seasonal transitions: *E. nordmanni* dominated from February to April, while *Pe. avirostris* and/or *Ps. tergestina* dominated from June to September (Fig. 1-5a). *Evadne spinifera* and *Pl. schmackeri* occurred at relatively low densities during the summer season, from May to August (Fig. 1-5b). There were no significant differences in annual median abundances of the five cladoceran species, except for *Pl. polyphemoides* and *Po. leuckartii* from 2015 to 2019 (Kruskal-Wallis test, *Pe. avirostris*: $n = 59$, $h = 1.5$, $df = 4$, $p > 0.1$; *Ps. tergestina*: $n = 59$, $h = 3.4$, $df = 4$, $p > 0.1$; *E. nordmanni*: $n = 59$, $h = 1.1$, $df = 4$, $p > 0.1$; *E. spinifera*: $n = 59$, $h = 0.7$, $df = 4$, $p > 0.1$; *Pl. schmackeri*: $n = 59$, $h = 0.3$, $df = 4$, $p > 0.1$).

As the result of cluster analysis, offshore cladoceran communities were divided into three groups based on the 45% Bray-Curtis similarity index (Fig. 1-6): group C1 was dominated by *Pe. avirostris* and/or *Ps. tergestina*, and was composed of the communities appear in summer season (from June to August). Group C2 was dominated by *E. spinifera* and/or *Ps. tergestina*.

Group C3 was constituted by spring-season communities (April and May), when *E. nordmanni* appeared.

Relationships between total cladoceran abundances and six environmental parameters were examined (Table 1-3). While there were no significant correlations between the abundances in four of the five dominant species and environmental parameters, the abundance of *Pe. avirostris* showed positive correlations with surface temperatures (at 1 m) and mean temperatures in the upper 30 m (Spearman's rank correlation test, $\rho = 0.59$, $p < 0.01$, and $\rho = 0.38$, $p < 0.05$, respectively). On the other hand, negative correlations were detected between *Pe. avirostris* and accumulated chlorophyll *a* concentrations in each water column layer (Spearman's rank correlation test, 0–30m: $\rho = -0.41$, $p = 0.04$; 0–50m: $\rho = -0.45$, $p = 0.02$; 0–100 m: $\rho = -0.44$, $p = 0.02$). Total cladoceran abundance exhibited a positive correlation only with surface temperatures (at 1 m) (Spearman's rank correlation test, $\rho = 0.41$, $p < 0.01$).

1-3-3. Reproduction of *Penilia avirostris*

Body length and reproductive parameters of a total of 811 individuals of dominant cladocerans, *Pe. avirostris* were analyzed (Fig. 1-7). Body length varied from 0.23 to 1.20 mm, and was significantly larger in June or July than other months in 2015 (ANOVA: $n = 131$, $f = 7.3$, $df = 2$, $p < 0.01$; Tukey-Kramer HSD test: $p < 0.01$), 2017 (ANOVA: $n = 200$, $f = 22.2$, $df =$

3, $p < 0.01$; Tukey-Kramer HSD test: $p < 0.01$), and 2019 (ANOVA: $n = 200$, $f = 24.9$, $df = 3$, $p < 0.01$; Tukey-Kramer HSD test, $p < 0.05$). Brood size was also larger in June or July than other months in 2015 (Kruskal-Wallis test: $n = 95$, $h = 32.0$, $df = 2$, $p < 0.01$; Dunn's test, $p < 0.01$), 2017 (Kruskal-Wallis test: $n = 115$, $h = 53.5$, $df = 3$, $p < 0.01$; Dunn's test, $p < 0.01$), 2018 (Kruskal-Wallis test: $n = 94$, $h = 70.7$, $df = 2$, $p < 0.01$; Dunn's test, $p < 0.01$), and 2019 (Kruskal-Wallis test: $n = 102$, $h = 35.0$, $df = 3$, $p < 0.01$; Dunn's test, $p < 0.01$). Parthenogenetic individuals accounted for 34–96% of reproductive stages during the research period. Gamogenetic individuals, including females with resting eggs and males, were few in number, comprising 2–18% of the population.

1-4. Discussion

In order to clarify seasonal and interannual variabilities in offshore cladoceran communities, field survey was conducted for over five years in offshore Suruga Bay. Cladocerans appeared and dominated the offshore mesozooplankton communities every summer. Occurrence patterns of all species were basically similar each year. These results suggest that summer outbreaks of cladocerans and their dominance in the offshore area of Suruga Bay are regular events, rather than a sporadic phenomenon.

Copepods usually dominate offshore mesozooplankton communities (Hirakawa et al.

1992; Kobari et al. 2008; Minowa et al. 2011). According to Onbé and Ikeda (1995), who reported seasonal patterns of marine cladocerans in offshore Toyama Bay in the Sea of Japan, cladocerans comprised 30.5% of all zooplankton in terms of abundance. In this study, cladocerans accounted for 87% of total mesozooplankton abundance, exceeding copepods, when they occurred at maximum abundance, even offshore. These findings suggest that the occurrences of cladocerans in offshore Suruga Bay are a unique phenomenon in terms of their periodicity and high abundance.

Seven cladoceran species identified in this study have been reported previously in Japanese waters (Onbé 1997). Among them, *Penilia avirostris* was the most common species in Suruga Bay. This species appears during the warm-water season in temperate water (Onbé 1968; Lipej et al. 1997; Viñas et al. 2007). As some previous studies reported (Atienza et al. 2008; Miyashita et al. 2010; Kane 2013), this study also observed a positive correlation between *Pe. avirostris* abundance and water temperature. On the other hand, negative correlations between *Penilia* abundances and chlorophyll *a* concentrations are probably due to the sporadic high chlorophyll *a* concentrations observed during the cold-water season from November to March, when the abundance of this species is low (Table 1-3, Fig. 1-2).

Parthenogenetic females dominated the reproductive state of *Pe. avirostris* throughout the study period (Fig. 1-7), suggesting that they reproduced parthenogenetically in the offshore

area. It is known that the fecundity of marine cladocerans is higher during the initial phase of population growth (Müllin and Onbé 1992; Egloff et al. 1997; Põllupüü et al. 2010). Higher brood sizes when *Penilia* first appeared in June or July observed in this study is consistent with those studies. In this study, gamogenetic individuals comprised a relatively small portion (~18%), which is not accordance with Onbé (1974) reporting that gamogenetic individuals comprised 49.7% of all reproductive individuals in the Seto Inland Sea. Although it is difficult to clarify the reason of this discrepancy only from this study, the sampling intervals might not detect the peak abundance of gamogenetic individuals.

Sinking of resting eggs is considered an adaptation to avoid unfavorable conditions for cladocerans (Egloff et al. 1997). Resting eggs released in offshore Suruga Bay are assumed to sink rapidly to the deep-seafloor, where they would remain in sea floor sediments unless they were transported upward by a strong upwelling. Because hatching of resting eggs is related to changing environmental factors, such as salinity, temperature, and photoperiod (Egloff et al. 1997; Vandekerkhove et al. 2005; Sopanen 2008), resting eggs would be unable to hatch on the deep seafloor, where it is dark and cold. Therefore, it seems unlikely that resting eggs released by offshore populations in Suruga Bay could contribute to seed populations for the next season.

In contrast to the periodic population explosions of the dominant species, two podonid cladocerans, *Pleopis polyphemoides* and *Podon leuckartii*, appeared only occasionally in small

numbers during the study period. In Seto Inland Sea, these podonids occurred in high numbers in shallow water (Onbé 1974). While these micro-sized cladocerans might pass through the mesh of the plankton net (335 μm) used in this study, relatively low numbers of occurrences were also recorded in Norpac-net samples with 100 μm mesh size towed during the same sampling periods as in this sampling (see chapter 2). Thus, these species are considered to occur rarely in offshore area, rather than an underestimation due to the sampling method.

Occurrence patterns of cladocerans were slightly different between years. For example, while *Pseudevadne tergestina* appeared from April to December in 2015, they occurred from May to October in 2016 (Fig. 1-5). Whereas *Pe. avirostris*, *Ps. tergestina* and *Evadne spinifera* mainly appeared and dominated during the warm-water season from June to September, very few individuals of those species were found from October to December in 2014, 2015, and 2017. However, there were no significant interannual variations in environmental factors, such as surface temperature (at 1 m), mean temperature and salinity of the water column, maximum chlorophyll *a* concentrations and accumulated chlorophyll *a* concentrations, throughout the study period. Thus, the cause of the discrepancy in the period of occurrence in each species remains unclear. Since offshore cladoceran populations are considered to originate from coastal waters, variation in the population dynamics of each species in coastal areas might affect the timing of the appearance of the offshore population.

Some studies have reported the appearance of marine cladocerans in oceanic waters (e.g. Kim and Onbé 1995). Egloff et al. (1997) indicated that those occurrences resulted from transportation by surface currents from coastal waters. In Suruga Bay, previous studies indicated that cyclonic current flow in inner and mouth part of Suruga Bay (Inaba 1981; Inaba 1984). Summer abundances of the offshore cladoceran population may be related with these surface currents in Suruga Bay. The hydrographic features in the bay imply another possibility with the offshore population. The mechanism of forming offshore cladoceran population will be examined in the Chapter 2.

Chapter 2. Formation mechanisms of offshore cladoceran community

2-1. Introduction

As documented in the chapter 1, marine cladocerans appear abundantly every spring-summer season, and comprise a major portion of the pelagic mesozooplankton community in offshore Suruga Bay. Since cladoceran populations are usually initiated by the hatch of resting eggs on coastal bottom sediments, offshore cladoceran populations are thought to originate from coastal areas of the bay. However, it is uncertain the origin of offshore cladoceran populations, and the mechanisms of the formation of these regular offshore communities.

Particle-tracking methods to estimate transport processes of aquatic organisms have been applied to marine animals, such as jellyfish (Moon et al. 2010), squid (Onitsuka et al. 2010), and fish eggs and larvae (Nishikawa et al. 2013). Since the distribution of planktonic animals is strongly affected by the direction and magnitude of ocean currents, their transport by surface currents can be reproduced by particle-tracking experiments using ocean models. Using such methods, some studies have been conducted. As planktonic animals, marine cladocerans are usually distributed in shallow coastal waters. However, some studies have reported that cladocerans appear in oceanic regions (Wiborg 1955; Longhurst and Seibert 1972; Della Croce and Angelino 1987), and those occurrences are thought to have resulted from transport by

surface currents (Egloff et al. 1997).

In order to clarify mechanisms by which offshore cladoceran populations form in Suruga Bay, we conducted both field samplings and particle-tracking experiments to compare species composition and occurrence patterns between coastal and offshore areas of the bay, and to illuminate seasonal changes in transportation of cladoceran populations.

2-2. Materials and Methods

2-2-1. Sampling

Zooplankton sampling was conducted in offshore and coastal areas of Suruga Bay from April 2018 to March 2019. Sampling in offshore areas was carried out at stations SR1 (35˚03'20" N, 138˚41'00" E, depth: ca. 1000 m) and SR3 (34˚53'00" N, 138˚38'30" E, depth: ca. 1600 m) (Fig. 2-1b), located in the inner and central parts of the bay, respectively, during the research cruises of R/V Hokuto of Tokai University. Zooplankton samples were collected using a Norpac net (Motoda 1957), which has a 0.45-m mouth diameter and a 100-μm mesh size. The net was hauled vertically, from 100 m depth to the surface. A flowmeter (Rigosha & Co., Ltd., Japan) was attached to the mouth of the net to estimate filtered volume (m^3). Vertical profiles of temperature and salinity from 100 m depth to the surface were recorded with a CTD (SBE 19plus, Sea-Bird Electronics, USA). At offshore stations, water samples were collected using

Niskin bottles from 8 layers at 0, 5, 10, 20, 30, 50, 75, and 100 m depth, for chlorophyll *a* analysis. Each water sample was filtered using a GF/F filter (GE Healthcare, USA), and chlorophyll *a* was extracted in *N, N*-dimethylformamide (Suzuki and Ishimaru 1990). Chlorophyll *a* concentrations were determined by fluorometry (Welschmeyer 1994) (Trilogy Laboratory Fluorometer, Turner Designs, Inc., USA).

Sampling in coastal areas was performed almost twice a month during the same period at two stations, C1 (34˚59'30" N, 138˚30'39" E, depth: ca. 3 m) and C2 (35˚00'02" N, 138˚29'50" E, depth: ca. 8 m), located in the innermost and central parts of Orido Bay, an inner part of Suruga Bay, respectively (Fig. 2-1c). A plankton net (0.3-m mouth diameter, 100-μm mesh size) was used for zooplankton collection, and was hauled vertically from near the bottom to the surface. Filtered volume (X; m^3) was calculated using following equation:

$$X = M \times (D - N)$$

Where M is net mouth area (m^2), D is bottom depth (m), and N is length of the plankton net (m). Bottom depth was measured using a rope before sampling. Filtration efficiency was assumed to be 100% due to shorter towing distance. At stations C1 and C2, hauls of the plankton net were performed 10 and 5 times, respectively. Vertical profiles of temperature and salinity from the upper bottom to the surface were recorded using a portable multi-item water quality meter (WQC-24, DKK-TOA Corporation, Japan).

Zooplankton samples from both areas were preserved immediately in 5% buffered formalin seawater after collection. In the laboratory, cladocerans were sorted and counted under a dissecting microscope, to calculate their abundances (individuals m^{-3}). Cladoceran species were identified following Onbé (1997). In order to compare the coastal and offshore environments of the bay, data from two coastal stations and two offshore stations were grouped as a coastal area and an offshore area, respectively. The Mann-Whitney *U*-test was employed to assess differences in hydrographic conditions between coastal and offshore areas. Statistical analyses were performed using JMP 13 (SAS Institute Inc., Cary, NC, USA). Contour graphs of hydrographical parameters were drawn with Ocean Data View (Schlitzer 2019).

2-2-2. Model configuration

In the present study, the triply nested ocean model developed by Kuroda et al. (2013) was used to simulate oceanographic conditions in Suruga Bay. The three-component models are based on the Regional Ocean Modeling System (ROMS), which is a free-surface, terrain-following, S-coordinate, primitive equation ocean model (Haidvogel et al. 2008). The vertical and horizontal grid points are defined by S-coordinates (Song and Haidvogel 1994) and spherical coordinates, respectively. The three models have grid sizes of 1/2°, 1/10°, and 1/50°, respectively, and are connected via one-way nesting. The 1/2°, 1/10°, and 1/50° models cover

the entire North Pacific, the western North Pacific, and the vicinity of Japan, respectively. The 1/2° and 1/10° models are also embedded in an operational ocean forecast system called "FRA-ROMS" This system reproduces realistic basin-scale and mesoscale variations using a data assimilation (Kuroda et al. 2017).

The 1/50° model domain is shown in Fig. 2-1a and covers all of the coastal regions around Japan. The vertical resolution has 21 levels. The thickness of the uppermost layer changes depending on depth and is within 10 m inside the bay. For tracer and momentum advection, third-order upstream-biased and fourth-order centered schemes are applied for horizontal and vertical directions, respectively. These configurations of the 1/50° model are basically the same as those in Kuroda et al. (2014) and Takahashi et al. (2017), in which spin-up experiments were conducted by using climatological forcings with a one-year cycle, but the present study conducted hindcast experiments. Heat and momentum fluxes at the sea surface were estimated on the basis of 3-hourly mean net shortwave and downward longwave radiation from the Japan Meteorological Agency Climate Data Assimilation System (JCDAS) (Onogi et al. 2007) and hourly meteorological data (wind, relative humidity, air temperature, precipitation, and atmospheric pressure) were acquired from the Grid Point Value–Mesoscale Model (GPV/MSM) (Saito et al. 2006). Daily mean discharges of water from all water systems were estimated and introduced to the 1/50° model with the same method used by Kuroda et al.

(2018). Lateral boundary conditions were derived from the daily mean re-analysis data of the 1/10° FRA-ROMS.

The initial value of the 1/50° model was derived from the daily mean reanalysis data of the 1/10° FRA-ROMS, and the model was integrated from January 2007 to December 2014 with time step intervals of 2.4 s for baroclinic component and of 0.04 s for barotropic component. We analyzed climatological monthly mean surface salinity and flow fields in the model output from January 2008 to December 2014, which were also used to perform particle-tracking experiments.

2-2-3. Particle-tracking experiments

We performed particle-tracking using the Euler-Lagrangian method (Awaji et al. 1980). The position of each particle was determined by solving the following equation:

$$\frac{d\boldsymbol{X}}{dt} = \boldsymbol{u}(\boldsymbol{X}, t)$$

with the initial value of $\boldsymbol{X}_0$, where $\boldsymbol{X}$ is the particle position, t is time and $\boldsymbol{u}$ is the climatological monthly mean surface current. The particles were tracked in the surface layer using a fourth-order Runge-Kutta scheme, and it was based only on the surface flow field in the climatological monthly mean, which was assumed to be steady state. The initial distribution of the particles $\boldsymbol{X}_0$ is shown in Fig. 2-1b, where particles are deployed at grids along the coastal line inside the bay.

In total, 106 particles were released, and tracking was performed for 30 days.

Since marine cladocerans are planktonic animals with little swimming ability, their horizontal distribution was expected to be largely determined by surface currents. Although some species undergo diel vertical migrations (Egloff et al. 1997), their habitats are concentrated near the surface (Onbé and Ikeda 1995). Therefore, vertical migration or settling of individual cladocerans was not considered in this experiment. Random walks were not included because we would simply simulate the influence of surface current and its seasonal variations on cladoceran communities. The simulation and particle tracking experiments were performed by Dr. Daisuke Takahashi of Tokai University.

2-3. Results

2-3-1. Hydrography

Water temperature showed typical seasonal fluctuations. Relatively higher temperatures were recorded from July to September, and lower temperatures from January to March. At offshore stations, a seasonal thermocline developed in the upper 10–80 m during the summer, and vertical mixing was detected during winter (Fig. 2-2a). In contrast, temperatures were vertically homogeneous throughout the study period at coastal stations, due to shallow water depth (Fig. 2-3a). Surface temperatures of offshore and coastal stations varied from

15.6°C to 27.7°C and from 11.8°C to 29.4°C, respectively. While relatively lower salinities were observed at surface waters from July to November in offshore stations, no clear seasonal patterns were detected at coastal stations (Fig. 2-2b, Fig. 2-3b). Surface salinities of offshore and coastal stations varied from 29.9 to 34.4 and from 20.3 to 34.2, respectively. While no differences in surface temperatures were detected between offshore and coastal stations (Mann-Whitney *U*-test, $p = 0.47$), surface salinities recorded at coastal stations were significantly lower than those at offshore stations (Mann-Whitney *U*-test, $p < 0.01$). Seasonal variations in chlorophyll *a* concentration at offshore stations are shown in Fig. 2-2c. Integrated concentrations of chlorophyll *a* in the 0–100-m water column at offshore stations SR1 and SR3 ranged from 32.6 to 170.6 and 29.7 to 114 mg m^{-2}, respectively. Higher chlorophyll *a* values (> 2 µg L^{-1}) were recorded in May and June at station SR1, and in April and May at station SR3. There were no significant differences in integrated chlorophyll *a* concentration between stations SR1 and SR3 (Mann-Whitney *U*-test, $p = 0.85$).

2-3-2. Validation of the model

Figure 2-4 shows the horizontal distribution of the climatological monthly mean surface salinity during each month. Seasonal variation in the surface salinity was evident inside the bay, i.e. less saline water (< 34), which was always found off the mouths of the Fuji River

and the Kano River through the year, spread along the coastal area from the innermost to the western parts of the bay from March to June, and was then distributed over the entire bay from June to October. Such seasonality in surface salinity field has been reported from direct observations (Nakamura 1982). In addition, we compared surface salinity at offshore sampling stations between field observation result and simulation result. Although salinity data recorded in the field observations were more variable, seasonal variations were largely consistent with the simulation results: salinity tended to be lower from summer to autumn, and seasonal changes were more variable in SR1 than in SR3 (Fig. 2-2, Fig. 2-5). Mean surface salinity in the station SR1 were relatively lower than SR3. Thus, typical seasonal variation in the surface salinity fields inside the bay was successfully simulated using the nested 1/50° model.

2-3-3. Population dynamics of cladocerans

Seasonal abundance of cladocerans based on the five-year sampling at station SR1 was described in chapter 1. In this study, field sampling in both coastal and offshore areas were conducted to compare cladoceran population dynamics. While the ORI net with 335 μm mesh was used for five-year sampling in the chapter 1, a plankton net with finer mesh size (100 μm mesh), that covers size ranges of all cladoceran species, including neonates was used in this survey.

At coastal stations, cladocerans occurred throughout the year except for November and December (Fig. 2-6). Maximum abundances, recorded in June, were 4347.4 and 653.3 individuals m^{-3} at stations C1 and C2, respectively. On the other hand, cladocerans appeared only from April to September at offshore stations, and total abundances were highest in July (537.5 and 16.1 inds. m^{-3} at stations SR1 and SR3, respectively).

Seven species of marine cladocerans were identified: *Penilia avirostris*, *Pseudevadne tergestina*, *Evadne nordmanni*, *Evadne spinifera*, *Pleopis polyphemoides*, *Pleopis schmackeri*, and *Podon leuckartii*. While these seven species were found in the coastal stations, *Po. leuckartii* did not appear at offshore stations.

At the coastal stations, *Po. leuckartii* and *E. nordmanni* were dominant from April to May and January to March (Fig. 2-6). *Pseudevadne tergestina* and *Pe. avirostris* appeared in abundance from July to August. *Pleopis schmackeri* appeared at relatively low densities from May to August, and *E. spinifera* appeared only in June. *Pleopis polyphemoides* was dominant from June to July, and showed the greatest abundance in the coastal cladoceran community (Fig. 2-6, Table 2-1). These seasonal patterns were largely similar between two coastal stations.

At offshore stations, occurrence patterns of *Pe. avirostris*, *Ps. tergestina*, *E. spinifera*, and *Pl. schmackeri* were basically similar to those in coastal areas (Fig. 2-6). However, three species (*E. nordmanni*, *Pl. polyphemoides*, and *Po. leuckartii*) exhibited distinctly different

patterns. Although *E. nordmanni* appeared in coastal areas from April to May and from February to March, it appeared in offshore areas from April to May. While *Pl. polyphemoides* appeared at coastal stations in high numbers (> 100 inds. m^{-3}), only a few individuals were collected at offshore stations (< 1 inds. m^{-3}) (Table 2-1). *Podon leuckartii* occurred in coastal stations from April to June and from January to March, and sometimes occurred at high density (> 100 inds. m^{-3}). However, they did not appear at offshore stations during the same period.

2-3-4. Particle-tracking experiments

Results of the particle-tracking experiments in each month are shown in Fig. 2-7. In January, particles released along the west coast of the mouth of the bay mostly flowed out of the bay until Day 15. On the other hand, particles placed along the east coast of the bay remained near their release sites during the month. Similar dispersal patterns were also observed in west and east coast of the bay, in February, November and December. From March to August, most particles remained inside the bay throughout the month. Particles released along the west coast dispersed northeastward, and were transported to the east coast or offshore of the inner bay. During this period, particles distributed offshore in the inner bay moved anticlockwise, and accumulated remarkably offshore in the inner bay. In September, those accumulations were not observed. Almost all of the particles traveled along the east coast, and left the bay on Day 30. In

October, most particles left the coast, and moved westward, and then remained at the mouth of the bay.

In this simulation, 106 particles were released, and remarkable accumulations in offshore inner bay were observed only for a certain period of the year. In order to compare the magnitude of accumulations each month, particles that reached the inner bay on Day 30 were counted (Fig. 2-8). As a result, while few particles (0–3.8% of all released particles) reached the offshore inner bay from September to February, more than 30% of the particles were concentrated in the inner part of the bay from March to August (Fig. 2-8a). Especially from June to August, more than half the particles (50–57.5%) accumulated in the offshore inner bay.

2-4. Discussion

In the particle-tracking experiments, particles showed different behavior each month. From March to August, released particles were transported to the inner part of the bay, and accumulated remarkably (Fig. 2-7). However, those accumulations were not observed in other months. When particles accumulated in the offshore inner bay, they were transported in a counterclockwise manner. Surface circulation currents have been reported in the inner bay and the mouth of Suruga Bay (Nakamura 1982). While the direction of the surface circulation in the mouth of the bay is affected by variation in the Kuroshio Current (Inaba 1984), a

counterclockwise current usually predominates in the inner bay (Inaba 1981). Furthermore, circulation current in the inner part of the bay is known to vary seasonally (Katsumata et al. 2018; Katsumata et al. 2019). Katsumata et al. (2018) reported that northward flow (inflow into the bay) tends to be stronger from April to June, while southward flow (outflow from the bay) tends to be strong from September to December. This seasonal pattern coincided with behavior of particles observed in the particle tracking experiments in this study. Particle transport and accumulations observed in this study seemed to reflect these surface currents, unique to the Bay.

Kuroshio Current alternate two types of the paths: large meander path and non-large meander path (Kawabe 1985). Large meander (LM) is known to affect pacific cast of Japan, including Suruga Bay. During this study, Kuroshio taken large meandered path after summer of 2017 (Sugimoto et al. 2020). However, cladocerans showed similar occurrence pattern throughout the research period. Thus, influence of LM on the cladoceran community during the study period was considered to be limited. The period from June to August when particles tended to accumulate in the inner bay coincided with the time during at which marine cladocerans became most abundant at offshore stations (Fig. 2-8). The offshore area, where particles accumulated markedly in the numerical experiments, was almost same as the location of offshore sampling station SR1. On the other hand, the accumulation was not observed near station SR3, in central part of the bay. In addition, while seasonal patterns of offshore

cladoceran populations were similar between stations SR1 and SR3, abundances of cladocerans recorded at SR1 were much higher than at SR3 (Table 2-1). The result of particle tracking experiments was consistent with the difference of cladoceran abundance between the station SR1 and SR3. According to Tanaka et al. (2011), who studied transport processes of eggs and larvae of *Lucensosergia lucens*, known as "Sakura Shrimp", by numerical experiments, the surface circulation forms a retention area in the inner bay where eggs and larvae of *L. lucens* can remain for a long time. Circulation of the inner bay is at least 100 m deep (Katsumata et al. 2019), and this includes the vertical distribution range of marine cladocerans (Onbé and Ikeda 1995). These evidence suggest that circulation of the inner bay transports inshore cladoceran populations to offshore from June to August in Suruga Bay.

Figure 2-9 shows temporal variations of the number of particles distributed at offshore area of inner Suruga Bay. Throughout the year, some particles (1–15 of 106 particles) were distributed inner bay (box region) by 3 days after released. Müllin and Onbé (1992) reported that range of the time from birth to the first reproduction of *Pe. avirostris* is 3.4–5.9 days. This suggests that marine cladocerans, which in the phase of parthenogenetic reproduction, can be transported from coastal area to offshore inner bay. Indeed, parthenogenetic females dominated offshore *Penilia* population (chapter 1). From March to August, when remarkable accumulations of the particles were observed, relatively high number of the particles (16–22

particles, more than 30% of the particles counted in the day 30) were distributed at inner bay by the day 10. Once the particles were accumulated in inner bay, most of the particles did not move out from the area. Although the integration period is rather long compared with their generation times (it corresponds to the sampling intervals), since particles reach the offshore area within 10 days and the number of particles flowing to the offshore area increases over time during the mass occurrence period, it is inferred that physical transports and accumulation are one of the cause of mass occurrence in offshore Suruga Bay.

Some previous studies reported their oceanic appearances. Wiborg (1955) reported that *E. nordmanni* appears in the offshore Norwegian Sea, far from the coast. In the northeastern Pacific and western Japan Sea, *Pe. avirostris* has been found in a wide area of offshore waters (Kim and Onbé 1995). These offshore occurrences were considered to be due to advective transport by surface currents (Egloff et al. 1997), and their occurrence were rather sporadic. Periodic mass occurrences in offshore Suruga Bay due to regional surface currents seems to be a unique phenomenon observed in the bay.

River discharges from large rivers may contribute to form cladoceran population in offshore Suruga Bay. The coastal water originated from the river water, which is a water mass of Suruga Bay, is distributed mainly upper 20 m in coastal area (Nakamura 1982). During summer season, amount of river waters flow into the bay are increase (Tanaka et al. 2009), and

the distribution of the coastal water originated from the river water extends more offshore (Nakamura 1982). Indeed, relatively lower salinity was observed in the offshore stations during summer season (Fig. 2-4). In addition, as the result of particle tracking experiments, released particles were accumulated at inner bay from spring to summer, but any accumulations were not detected when the simulation performed without river discharge (data not shown). Cladocerans usually distribute within shallow layers (Onbé 1974; Egloff et al. 1997), and are distribute upper 50 m even in deep offshore region (Onbé and Ikeda 1995). Those evidences suggest that possible importance of river discharges in the transport and accumulation of cladocerans.

Particle accumulations during cladoceran-abundant season in the numerical experiments implies that transport due to a unique surface current system in Suruga Bay contributes to formation of the periodic mass occurrence of cladocerans in offshore Suruga Bay. On the other hand, the difference in species composition between coastal and offshore cladocerans suggesting that not all cladoceran species were transported and/or survived in the offshore area. During this study, *Penilia avirostris* appeared at much higher abundance in offshore areas than in coastal waters (Table 2-1). This species is known to be a unique filter feeder among marine cladocerans (Onbé 1997; Onbé 1999; Atienza et al. 2006). It has fine filtering appendages to feed on pico-, nano- and microplankton, enabling them to thrive in oligotrophic environments (Paffenhöfer and Orcutt 1986; Turner et al. 1988; Lipej et al. 1997;

Katechakis and Stibor 2004; Atienza et al. 2006). During this study, chlorophyll *a* concentrations at offshore stations were mostly less than 3 µg L^{-1} (Fig. 2-4). While chlorophyll *a* concentrations were not analyzed during coastal surveys, the chlorophyll data obtained at offshore stations in this study were much lower than those in previous reports conducted in coastal Suruga Bay (Shimada et al. 1995; Mishra et al. 2020). Unique feeding characteristics of *Pe. avirostris* may be adaptive to survive in the relatively low chlorophyll concentrations in offshore Suruga Bay.

In contrast, while *Pleopis polyphemoides* and *Podon leuckartii* occurred abundantly in coastal areas, they rarely occurred in offshore areas, as also documented in chapter 2. *Pl. polyphemoides* has been reported from neritic waters (Bosch and Taylor 1968; Onbé 1999; Marazzo and Louis Valentin 2003a). In the northeast Baltic Sea, they appeared abundantly in regions where average salinity was less than 5 (Põllupüü et al. 2010). In this study, average surface salinities of coastal stations were much lower than those at offshore stations, where they might be influenced by freshwater input from Tomoe River (Fig. 2-1c). Hence, it is inferred that *Pl. polyphemoides* occurred abundantly at coastal stations due in part to their preference for low salinity, whereas they couldn't reproduce and/or survive in high-salinity offshore waters. *Po. leuckartii* is also known from neritic waters (Viñas et al. 2007; Souza et al. 2011; Kodama et al. 2021), and is considered a cold-water species (Onbé 1997; Viñas et al. 2007). In this study, they

appeared only at coastal stations from January to June, and their peak abundance was observed in March (Fig. 2-6). From February to March, when *Po. leuckartii* appeared abundantly (> 100 inds. m^{-3}), surface temperatures recorded in the coastal area (11.9–17.0°C) were relatively lower than offshore area (15.6–18.1°C). When *Po. leuckartii* occurred in coastal areas, *Evadne nordmanni* also appeared. However, only *E. nordmanni* appeared in offshore areas from April to May. *Podon leuckartii* is considered a stenohaline and stenothermic species (Viñas et al. 2007). Offshore area temperature and salinity might not be favorable for survival and/or reproduction of *Po. leuckartii*. Therefore, even under conditions that cause coastal cladocerans to be transported and to accumulate in offshore Suruga Bay, *Pl. polyphemoides* and *Po. leuckartii* could not be a component of the offshore cladoceran community.

Predation pressure is considered an important factor affecting cladoceran abundance (Marazzo and Valentin 2003; Atienza et al. 2008; Oghenekaro and Chigbu 2019). Cladocerans have been found in the gut contents of pelagic fish larvae collected in open ocean, such as Pacific saury (Morita and Arima 2022), billfish (Llopiz and Cowen 2008), and Pacific bluefin tuna (Kodama et al. 2017). These studies indicates that cladocerans are preyed upon by carnivorous animals not only in coastal waters, but also in offshore waters. According to Kodama et al. (2020), cladocerans belonging to Podonidae were found from the gut contents of larvae of Pacific bluefin tuna, while *Pe. avirostris* (Sididae) was rare despite being abundant in

the water column. A similar feeding pattern was also reported in larvae of red mullet (Sabatés 2015). This evidence suggests that predation pressure by fish may differ on podonids and *Pe. avirostris*. Although predation pressure on cladocerans were not investigated in this study, these top-down controls might affect the *Penilia*-dominated offshore population in Suruga Bay.

In conclusion, results of this study suggest that offshore cladoceran populations were formed by a combination of transport of coastal populations by unique surface circulation currents and biological characteristics of the species to survive in relatively oligotrophic and high-salinity offshore environments. Cladocerans serve an important function as secondary producers in marine ecosystems (Turner et al. 1988; Sabatés et al. 2015). Identifying prey-predator interactions and trophic dynamics of the cladoceran species in offshore food-web will provide clues to understand the ecological role of them in Suruga Bay, which exhibits unique and rich fishery resources, such as Sakura shrimp and Shirasu (fish larvae composed of *Engraulis japonicus*, *Sardinops melanostictus*, *Etrumeus teres*).

Chapter 3. Roles of marine cladocerans in the offshore food-web: An approach using stable isotope and metabarcoding diet analyses

3-1. Introduction

From tropical to temperate waters, marine cladocerans comprise a substantial portion of coastal mesozooplankton. According to Onbé (1974), cladocerans appear in extremely high numbers, exceeding 10,000 inds. m^{-3}, in shallow waters of the Seto Inland Sea, Japan, during the warm water season. Because of their high abundance, cladocerans are thought to play important roles in coastal food-webs and microbial loops (Onbé 1974; Turner et al. 1988; Lipej et al. 1997). On the other hand, little information has accumulated on the ecological role of cladocerans in offshore waters.

To assess food-web structures in aquatic ecosystem, stable isotope analysis of nitrogen and carbon, $\delta^{15}N$ (^{15}N /^{14}N) and $\delta^{13}C$ (^{13}C /^{12}C), have been widely used. In marine environments, $\delta^{15}N$ and $\delta^{13}C$ values of many organisms are empirically known to increase by 3.4‰ and <1‰ at each trophic level, respectively (Minagawa and Wada 1984; Post 2002; McCutchan et al. 2003). Since the $\delta^{15}N$ value indicates trophic positions of organisms, reflecting assimilated prey, it is used to estimate prey-predator interactions in food-webs (Minagawa and Wada 1984). $\delta^{13}C$ value shows limited isotopic fractionation between trophic levels, and indicates the carbon

sources of consumers (France 1995; Post 2002). Many studies have reported food-web structure using this method (Stowasser et al. 2012; Kürten et al. 2013; Kozak et al. 2020). However, among them, only a few studies have focused on marine cladocerans, especially those in offshore populations.

As for marine cladocerans, the feeding mode of *Penilia* is largely different from those of other species in the family Podonidae (see Table 1-2). *Penilia avirostris* is a filter feeder (Atienza et al. 2006), and mainly grazes on phytoplankton using filtering appendages (Turner et al. 1988). Podonids have a large compound eye and appendages with spines to catch and hold target prey (Nival and Ravera 1979). They locate micro-sized prey visually. However, subsequent studies have suggested that podonids do not prey on animals (Kim et al. 1989), and are raptorial herbivores (Jagger et al. 1988).

Studies on feeding habits of marine cladocerans have been conducted by various methods, such as microscopic analysis of gut contents (Kim et al. 1989), gut pigment analysis (Wong et al. 2006), and rearing experiments (Atienza et al. 2006). However, each method has its own limitations. Morphological identification of gut contents is often difficult due to digestion, and rearing experiments are time consuming, and culturing cladocerans is challenging. Recent advances in molecular techniques have enabled more comprehensive and detailed analysis of gut contents in zooplankton. DNA metabarcoding of gut contents has been

used to investigate feeding habits of mesozooplankton, such as copepods (e.g. Hirai et al. 2018), and is also used for investigation of prey-predator interactions of marine planktonic food-webs (e.g. Kobari et al. 2021). However, there are few molecular studies on feeding habits of cladocerans.

As described in previous chapters, cladocerans appear abundantly every spring-summer season in offshore waters of Suruga Bay. Although cladoceran populations may be important to offshore food-webs due to their high abundance, there is little information about their feeding habits and ecological roles in offshore Suruga Bay. The objective of this chapter is to clarify the trophic position of marine cladocerans in the offshore planktonic food web using stable isotope analysis. In particular, feeding habits of two dominant cladoceran species, *Penilia avirostris* and *Pseudevadne tergestina*, were studied. This chapter reports the first study on feeding habits of these species using DNA metabarcoding.

3-2. Materials and Methods

3-2-1. Sampling

Zooplankton samples were collected at stations SR1 and SR3, from January 2017 to December 2019 (Fig. 2-1) by oblique tows of an ORI net (mesh size: 335 μm, mouth diameter of the net: 1.6 m) (Omori et al. 1965). After collection, samples were divided into two aliquots:

one was fixed with 10% buffered formalin seawater for community structure analysis, and the other was frozen at -80°C for stable isotope analysis. Details of sampling methods and sorted taxonomic groups are listed in Table S1.

For DNA metabarcoding analysis of gut contents, cladoceran samples were collected at stations SR1 and SR3 in July 2021 (Fig. 2-1) by surface towing of a Norpac net (0.45 m mouth diameter and 335 μm mesh) (Motoda 1957). Two cladocerans species, *Penilia avirostris* and *Pseudevadne tergestina* were sorted and preserved immediately in 99% ethanol. Surface seawater samples were also collected at the same time using a bucket to examine compositions of organisms in the seawater. Water samples (1–2 L) were filtered through Sterivex GP filters (0.22 μm pore size Sterivex filter units, Merck Millipore, USA) using a peristaltic pump. Filter units were stored at -80°C until further analysis.

3-2-2. Community structure analysis

To clarify temporal variation in offshore mesozooplankton community, zooplankton samples obtained at station SR1 from 2017 to 2019 were sorted and counted for 19 taxonomic groups, and their abundances (inds. m^{-3}) were calculated. In addition, cluster analysis using the Bray-Curtis similarity index based on log(x+1)-transformed abundances was used to characterize community structure of offshore mesozooplankton. Clustering was performed

using PRIMER6 (PRIMER-E, UK).

3-2-3. Measurement of stable isotope ratios

Frozen zooplankton samples were thawed and sorted under a dissecting microscope. Sorted individuals were tallied by month in order to obtain at least 0.5 mg dry weight, which was minimum amount needed to measure stable isotope ratios. Sorted samples were lyophilized, and then milled using a mortar and pestle. Each powdered sample was divided into two subsamples for $\delta^{15}N$ and $\delta^{13}C$ measurements, respectively. Since the $\delta^{13}C$ value of lipids in the body is significantly lower than that of protein (DeNiro and Epstein 1977), lipids in $\delta^{13}C$-measurement subsamples were removed to avoid bias. Chloroform/ethanol (2:1, v:v) was added to powdered samples. After 5 min, samples were centrifuged and supernatants were discarded. These steps were performed twice, followed by drying at 60°C for at least 12 h. Acid treatment was performed both $\delta^{15}N$ and $\delta^{13}C$-measurement samples to remove inorganic carbonate. Several drops of diluted hydrochloric acid (0.1N HCl) were added to samples, which were then dried at 60°C for at least 12 h. After these treatments, each sample was weighed and placed in tin capsules. Measurements of isotope ratios were conducted using a mass spectrometer connected to an element analyzer (Delta Plus XP with Flash EA 1112, Thermo Electron Co. Ltd., USA, in Kitasato University or Delta V with Flash 2000, Thermo Scientific, USA in

Isotope Research Institute, Co., Ltd.).

$\delta^{15}N$ and $\delta^{13}C$ values were calculated using the following equation:

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

where X is a stable isotope (^{15}N or ^{13}C), and R is the ratio of the heavier isotope to the lighter isotope ($^{15}N/^{14}N$ or $^{13}C/^{12}C$). Vienna Pee Dee Belemnite and atmospheric nitrogen were used as standards for carbon and nitrogen, respectively.

3-2-4. Metabarcoding

Ten individuals of *Penilia* and *Pseudevadne* were randomly selected from all ethanol-fixed specimens. Cladocerans were dissected, and whole guts were taken under the dissecting microscope. Gut samples were transferred into 30 µL of 5% Chelex buffer (Bio-Rad Laboratories, USA), and homogenized for 1 minute using disposable pestles. After homogenization, samples were incubated at 95°C for 20 min to extract DNA. DNA extraction of particles/plankton collected in Sterivex filters from environmental water were performed with a DNeasy Plant Mini Kit (QIAGEN, Germany), according to the manufacturer's instructions, with a slight modification for Sterivex samples at the beginning of the procedure. Specifically, the lysis buffer was directly injected into the Sterivex unit and after a 10-min incubation at 65°C with rotation, lysate was removed from the unit using a disposable syringe and transferred to a

tube.

For eukaryotes, the V9 region of 18S rRNA gene was amplified using universal primers 1389F (TTGTACACACCGCCC) and 1510R (CCTTCYGCAGGTTCACCTAC) (Amaral-Zettler et al. 2009). When first PCR of 18S, peptide nucleic acid (PNA) was added to block amplification of cladoceran DNA. The first PCR amplification was performed in a 25-μL reaction volume containing 6.5 μL of distilled water, 1.5μL of ×10 buffer, 1.5μL of dNTP, 0.9 μL of $MgSO_4$, 0.9 μL of each primer (5 μM), 1.5 μL of PNA, 0.3 μL of KOD polymerase, and 1 μL of template DNA. Thermal cycling was performed as follows: 94°C for 2 min, followed by 30 cycles of 98°C for 10 s, 56°C annealing for 30 s, 68°C for 1 min, and final extension step of 7 min at 68°C.

For prokaryotes, the V4–V5 region of 16S rRNA gene was amplified using universal primers 515FB (GTGYCAGCMGCCGCGGTAA) and 926R (CCGYCAATTYMTTTRAGTTT) (Parada et al. 2016). The first PCR amplification was performed with 8 μL of distilled water, 1.5μL of ×10 buffer, 1.5μL of dNTP, 0.9 μL of $MgSO_4$, 0.9 μL of each primer (515FB and 926R) (5 μM), 0.3 μL of KOD, and 1 μL of template DNA. PCR was performed as follows: 94°C for 2 min, followed by 30 cycles of 98°C for 10 s, 56°C annealing for 30 s, 68°C for 1 min, and final extension step of 7 min at 68°C. In the first PCR step of 18S and 16S, each sample was assayed in triplicate, and equal amounts of each were

combined in the following step (second PCR).

The second PCR amplification of 18S and 16S was performed in a 14.1-μL reaction volume containing 8 μL of distilled water, 1.5μL of ×10 buffer, 1.5μL of dNTP, 0.9 μL of $MgSO_4$, 0.9 μL of each index adapter (5 μM), 0.3 μL of KOD, and 1 μL of template DNA. In this step, adapters and dual-index barcodes (Nextera XT Index Kit v2, Illumina, USA) were used to attach index sequences. Thermal cycling conditions were 94°C for 2 min, followed by 8 cycles of 98°C for 10 s, 56°C annealing for 30 s, 68°C for 1 min, and a final extension of 7 min at 68°C. Final PCR products of all samples were purified using AMPure XP (Beckman Coulter Inc., USA). Sequencing for 18S and 16S analysis was performed using MiSeq Reagent Kit V2 and V3 (Illumina, Inc., USA), respectively, on an Illumina MiSeq (Illumina, Inc., USA).

Quality filtering of raw data was performed using Trimmomatic (Bolger et al. 2014). Further bioinformatic analyses were performed using MOTHUR (Schloss et al. 2009). Paired-end reads were merged, and primer sites were eliminated based on 1 and 3 mismatch for F and R primers, respectively. Criteria for further quality filtering were follows: no ambiguous bases, 100–200 bp and >400 bp for 18S and 16S, respectively, and 6 and 8 of maximum homopolymers for 18S and 16S, respectively. Alignment of sequence reads was performed using the SILVA 132 databases (Quast et al. 2013). Chimeras were removed using the dataset, UCHIME (Edgar et al. 2011). The V9_PR2 reference database (Guillou et al. 2013) was used to

classify sequence reads into taxonomic groups. In this study, taxonomic groups “Metazoa”, “Streptophyta”, and “Fungi” were removed to avoid sequence reads due to contamination. Obviously abnormal sequence data were removed before further analysis. Operational taxonomic units (OTUs) were clustered with a 99% similarity threshold of sequence reads. Dominant OTUs were defined when the occurrence rate was more than 30% and the proportion of the OTU in the total sequence reads was more than 1%. BLAST of the National Center for Biotechnology Information (NCBI) database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to identify dominant OTUs. Dominant OTUs with >95% query coverage and identity were identified at the family level, and others were identified at the phylum level. Taxonomic names of prokaryotes were based on taxonomy database of NCBI (https://www.ncbi.nlm.nih.gov/taxonomy). In order to visualize similarities of gut-content composition among individuals, nonmetric multidimensional scaling (NMDS) was performed, based on the OTU composition of each individual. Analyses and data plots were demonstrated using the Bray-Curtis similarity index with the function “metaMDS” of the R package “vegan” (R Core Team 2021).

3-3. Results

3-3-1. Structure of the offshore mesozooplankton community

Figure 3-1 shows seasonal variations of mesozooplankton composition in the 0–100 m water column at the station SR1 during the study period. Copepods represented 16.7–96.9% of total zooplankton abundance, and dominated offshore mesozooplankton communities in most months. Cladocerans were abundant from spring to summer, and accounted for 0–75.8% of total zooplankton abundance. Abundance of cladocerans exceeded that of copepods in June and July 2017 and July 2018.

As a result of community structure analysis, offshore mesozooplankton communities were divided into two groups based on the 70% Bray-Curtis similarity index (Fig. 3-2). One was the copepod and/or cladoceran-dominated group, which appeared from April to August (Community A), and the other was the copepod-dominated group that appeared mainly in autumn and winter (Community B).

3-3-2. Stable isotope analysis

Stable isotope compositions of 13 zooplankton groups obtained in this study are shown in Table 3-1. Mean ± SD values of zooplankton $\delta^{13}C$ and $\delta^{15}N$ ranged from –20.61 ± 1.26% (*Pe. avirostris*) to –14.40 ± 2.44% (thecosomes) and from 3.95 ± 0.80% (*Pe. avirostris*)

to 9.45 ± 0.66% (mysids), respectively. Copepods belong to the families Euchaetidae and Candaciidae, mysids (mostly deep-sea species *Boreomysis* sp.), amphipods, and chaetognaths showed relatively higher $\delta^{15}N$ values (> 8‰). On the other hand, the $\delta^{15}N$ values of cladocerans, calanoid copepods, and thecosome pteropods were relatively lower than those of other zooplankton (< 6‰). Thecosome pteropods showed much higher $\delta^{13}C$ values with relatively low $\delta^{15}N$ values (ca. 4.4‰).

Figure 3-3 shows stable isotope maps of zooplankton communities A and B, which were defined by the cluster analysis (Fig. 3-2). The mean $\delta^{15}N$ value of *Penilia* (3.95 ± 0.80‰) was the lowest among the mesozooplankton measured (Fig. 3-3a). *Pseudevadne* also showed lower $\delta^{15}N$ values (5.79 ± 1.28‰) than most other plankton, but higher than *Penilia* and thecosomes.

The $\delta^{15}N$ value of each zooplankton taxon was compared between communities A and B (Table 3-2). Those values did not differ between the communities (Kruskal-Wallis test, $p >$ 0.06 or 0.1) for any taxon.

3-3-3. Metabarcoding analysis

In the 18S analysis for eukaryotes, sequence data from 39 cladoceran individuals (19 *Penilia* and 20 *Pseudevadne*) and 2 water samples (stations SR1 and SR3) were obtained. After

quality filtering was performed, 267542 and 16792 sequence reads were obtained from cladoceran gut contents and water samples, respectively. Fig. 3-4 shows taxonomic compositions of organisms detected from cladoceran gut samples. Taxonomic compositions of eukaryotes were largely similar between *Penilia* and *Pseudevadne*: Dinoflagellata, Bacillariophyta, Radiolaria, and Chlorophyta were major components (Fig. 3-4a), and occur frequently (Table 3-3). On the other hand, Ciliophora, Haptophyta, and Discoba showed lower proportions, and those taxa occurred at higher rates in *Penilia* than in *Pseudevadne*. Dominant OTUs in the guts of *Penilia* and *Pseudevadne* are shown in Table 3-4. All dominant OTUs in *Pseudevadne* were also dominant in *Penilia*, except *Cladococcus* (Radiozoa). Unidentified Metamonada, unidentified Cercozoa, and Bryozoa were dominant OTUs in the guts of both cladoceran species. Taxonomic compositions of eukaryotes in seawater differed between the stations SR1 and SR3. Whereas Dinoflagellata were dominant at station SR1, Bacillariophyta (diatoms) dominated at SR3. Plot of the NMDS analysis based on 18S OTUs data was shown in Fig. 3-5. Although there was an overlap between *Penilia* and *Pseudevadne*, data plots of *Penilia* individuals were more concentrated than those of *Pseudevadne*, indicating that *Penilia* showed greater similarity in gut contents among individuals.

In the 16S analysis for prokaryotes, 33 individual cladocerans (20 *Penilia* and 11 *Pseudevadne*) and 2 water samples were analyzed. 176 and 3971 sequences were obtained from

gut and water samples, respectively. In the 16S analysis, only 1–16 sequence reads per gut sample were obtained. Because this is too small a number to provide detailed information about prokaryotes in cladoceran guts, differences in prokaryote composition were roughly examined. Proteobacteria and actinobacteria comprised > 30% in both *Penilia* and *Pseudevadne* (Table 3-5). In contrast, Planctomycetota, Bacteroidota, and cyanobacteria occurred only in the guts of *Penilia*. Taxonomic compositions of water samples were similar between stations.

3-4. Discussion

Results of the stable isotope analysis suggest that cladocerans were at relatively low trophic levels in the offshore mesozooplankton community. These results are consistent with previous studies that indicated lower $\delta^{15}N$ values of cladocerans compared with other zooplankton in temperate and subtropical regions (Nagata et al. 2015; Uzundumlu and Büyükateş 2019). Because of lower $\delta^{15}N$ values, periodic mass occurrence of cladocerans might affect trophic structures of offshore zooplankton, especially carnivores. However, there were no significant differences in the $\delta^{15}N$ values of major zooplankton groups between the two communities, cladoceran-dominated and non-dominated (Table 3-2). These results suggest that seasonal high abundance of cladocerans in offshore Suruga Bay does not remarkably alter the mesozooplankton food-web structures.

The $\delta^{13}C$ value indicates primary carbon sources. France (1995) reported that ^{13}C is more enriched in inshore benthic food-webs than pelagic food-webs. As indicated in chapter 2, cladoceran populations occurring in offshore Suruga Bay are thought to be transported from coastal areas to offshore areas. Although this study did not investigate the food web structure of coastal zooplankton, if cladocerans are simply transported from coast to offshore without feeding, $\delta^{13}C$ values of cladocerans are expected to differ from those of other plankton in offshore areas (Hansen et al. 2012). However, positions of *Penilia* and *Pseudevadne* in the scatter plot occur in a straight line of major zooplankton groups, i.e. $\delta^{15}N/\delta^{13}C$ in cladoceran species were similar to those of other zooplankton (Fig. 3-3a). These results suggest that cladocerans were a component of the offshore zooplankton food-web during spring-summer. The short generation time of cladocerans may accelerate replacement of assimilated organic matter, which originated in prey items from coastal to offshore waters. In this study, $\delta^{13}C$ values of thecosome pteropods were largely different from those of other zooplankton groups. Since pteropods members of the oceanic zooplankton group (Parra-Flores and Gasca 2009), they may be transported from the outer part of Suruga Bay, or they may have unique physiological systems for C isotopic fractionation.

Mean $\delta^{15}N$ values of the two dominant cladocerans were different. *Pseudevadne* (5.79 ± 1.29‰) showed a mean $\delta^{15}N$ value similar to those of Calanidae copepods (5.75 ± 0.91‰),

mainly the omnivorous *Calanus sinicus*, suggesting that it occupies a similar trophic position. On the other hand, the mean $\delta^{15}N$ value of *Penilia* (3.95 ± 0.80‰) was the lowest among the zooplankton measured. They are considered secondary producers, feeding mainly on phytoplankton, as suggested in former studies (Kim et al. 1989).

Gut content analysis using metabarcoding techniques revealed food habits of the two dominant cladocerans. In the 18S analysis targeting eukaryote prey, there is no substantial difference in taxonomic composition of gut samples of *Penilia* and *Pseudevadne*. In addition, most of the dominant OTUs overlapped in these species. Previous studies on feeding habits of marine cladocerans indicated that both *Penilia* and podonids, including *Pseudevadne*, feed mainly on diatoms, dinoflagellates, and ciliates (Onbé 1974; Kim et al. 1989; Atienza et al. 2006). According to Kim et al. (1989), who analyzed gut contents using scanning electron microscopy, centric diatoms were the major items detected from both *Penilia* and podonids, and there was no substantial difference in diets of these cladocerans, consistent with results of the 18S analysis in this study. On the other hand, NMDS plots of *Penilia* was more concentrated than those of *Pseudevadne*. This means that prey composition varies more among individuals in *Pseudevadne* than in *Penilia*. This may reflect filter feeding of *Penilia* (Onbé 1997) and raptorial feeding of *Pseudevadne* (Jagger et al. 1988).

In contrast to the similarity of eukaryote composition in the guts of *Penilia* and

Pseudevadne, results of the 16S analysis were rather different between the two species. Some taxonomic groups, such as Planctomycetota and Bacteroidota, are found only in guts of *Penilia*. There has been a debate as to whether *Penilia* feeds on bacterioplankton. While some studies suggest that *Penilia* does feed on bacteria (Paffenhöfer and Orcutt 1986; Lipej et al. 1997), other studies claim that they cannot ingest such small organisms (Turner et al. 1988). Atienza et al. (2007) conducted rearing experiments and showed that *Penilia* does not feed on bacterioplankton, including heterotrophic bacteria and *Synechococcus*. However, most of the heterotrophic bacterial groups detected in the guts of *Penilia* in this study (Planctomycetota, Bacteroidota) are described as particle-associated bacteria in marine environments (Crespo et al. 2013; Bizic-Ionescu et al. 2015; Milici et al. 2017). Thus, these bacteria are assumed to be passively consumed by *Penilia*, rather than actively consumed as free-living bacteria.

Cyanobacteria were also detected only from the gut contents of *Penilia*, with a frequency of 35% (Table 3-5). Lipej et al. (1997) also reported cyanobacteria in the guts of *Penilia*. Cyanobacteria found in this study comprised mainly *Synechococcus* sp. *Synechococcus* is a unicellular photosynthetic prokaryote distributed in open oceans (Johnson and Sieburth 1979; Waterbury et al. 1979). In Suruga Bay, *Synechococcus* appears abundantly during summer–autumn, rather than winter–spring (Sohrin et al. 2011). Thus, it is highly possible that offshore *Pe. avirostris*, occurring abundantly every summer, feeds on *Synechococcus*.

Synechococcus is likely too small for a visual predator like *Pseudevadne*. That *Penilia* feeds on a primary producer, *Synechococcus* sp., is consistent with the stable isotope analysis showing that the trophic level of *Penilia* is lower than that of *Pseudevadne*. Moreover, feeding on such small organisms allows *Penilia* to survive in oligotrophic offshore environments. Periodic mass-occurrences of the species may affect the microbial loop in the surface layer of offshore Suruga Bay during summer season.

Chapter 4. General discussions

4-1. Marine cladocerans in Suruga Bay–their high species richness and abundance

In this study, all seven species of marine cladocerans recorded from Japanese waters (Onbé 1997) were found in both coastal and offshore areas of Suruga Bay. Species lists in the north Pacific and Arctic regions are summarized in Table 4-1. *Evadne nordmanni* and *Podon leuckartii*, are known to distributed to not only temperate regions, but also cold-water areas, such as Barents and Bering Seas (Onbé et al. 1996a; Saigusa et al. 2000). On the other hand, in tropical regions, warm-water species, *Penilia avirostris* and *Pseudevadne tergestina*, usually dominate cladoceran communities (Tang et al. 1995; Li et al. 2021). Species richness seems to be higher in subtropical and subarctic regions than in arctic and tropical regions. Suruga Bay has one of the highest species diversities of marine cladocerans in Japan, hosting both warm-water and cold-water species. The bay is characterized by the high number of species that occur seasonally and regularly in offshore areas. Maximum abundances recorded in coastal and offshore areas are comparable to or higher than those recorded in other places in Japan (e.g. Onbé and Ikeda 1995).

4-2. Feeding impact and role of cladocerans in offshore Suruga Bay

In this study, *Penilia avirostris* showed the highest abundance among the cladocerans in offshore Suruga Bay. Since this species sometimes appears in extremely high numbers in tropical to subtropical coastal waters, many ecological studies have examined growth rates, feeding habits, and reproductive parameters (Marazzo and Valentin 2003b).

Feeding impact of zooplankton can be expressed using ingestion rates (IR) and clearance rates (CR). According to Wong et al. (1992), who studied gut pigments (chlorophyll *a*; chl *a*) of *Pe. avirostris*, the mean IR of *Pe. avirostris* during the warm-water season was 43.1 ng chl *a* ind^{-1} day^{-1}. In this study, in the water-column at depths of 0–200 m, the integrated chlorophyll *a* concentration was 22.5–222.5 mg chl *a* m^{-2} and *Pe. avirostris* abundance ranged from 0.6–161037.7 inds. m^{-2}. Applying the mean IR value from Wong et al. (1992), *Pe. avirostris* daily grazed on < 0.01–18% (1.57%, average) of the standing phytoplankton crop, in terms of chlorophyll *a* in offshore Suruga Bay. Wong et al. (1992) reported that *Pe. avirostris* ingested only 1% of total amount of chlorophyll *a* (from 228.4 to 435.3 mg m^{-3}), and this was consistent with that study. In offshore Suruga Bay, food resources of cladocerans are abundant, and their grazing impact on primary production appears to be limited.

According to previous studies, mean CRs of *Pe. avirostris* ranged from 2.2–25.5 mL ind^{-1} day^{-1} (Table 4-2). However, copepods sometimes show much higher CRs than cladocerans.

For example, Li et al. (2004) indicated that the CR of a calanoid copepod, *Calanus sinicus*, often dominates those of other copepods in Suruga Bay, which ranged from 202 to 1066 mL ind^{-1} day^{-1}. Although cladocerans occur in high numbers in offshore Suruga Bay, their feeding may have a limited impact on offshore food webs, compared with those of copepods.

Metabarcoding diet analysis in Chapter 3 confirmed that *Pe. avirostris* consumes cyanobacteria (*Synechococcus*) in offshore waters of Suruga Bay. *Synechococcus* sp. sometimes comprise a major portion of primary production in oligotrophic waters (Iturriaga and Marra 1988), and have important role in primary production and the carbon cycle in marine environments (Johnson and Sieburth 1979; Li 1994). Freshwater cladocerans feed on cyanobacteria, and are important suppliers of cyanobacterial carbon to the freshwater food-web (Tõnno et al. 2016). In this study, the feeding impact of cladocerans on primary production does not appear substantial. However, in marine ecosystems, cladocerans may link nano-sized cyanobacteria and higher trophic predators by feeding.

4-3. Secondary production rate of *Penilia avirostris*

In Chapter 1, specimens of *Pe. avirostris* were measured in a study of reproductive parameters. Uye (1982) reported length-weight relationships of various marine zooplankton, and indicated that carbon biomass (*C*; µg) of *Pe. avirostris* can be calculated from total length

(*TL*; μm) using the following equation:

$$\log C = 4.51 \log TL - 12.74$$

The daily secondary production rate (*P*; μg C ind^{-1} d^{-1}) can be calculated using the following equation (Uye 1997),

$$P = C \times g$$

where *g* is growth rate of zooplankton (d^{-1}). According to Atienza et al. (2007), the growth rate of *Pe. avirostris* ranged from 0.10 to 0.24 d^{-1}. Applying the above equations, the daily secondary production rate of offshore population of *Pe. avirostris* was estimated from 0.02 to 114.9 μg C m^{-3} d^{-1}. According to Ara and Hiromi (2006), who studied production of copepods in Sagami Bay, the mean copepod secondary production rate was 780 μg C m^{-3} d^{-1}. Secondary production rates of copepods reported in other temperate waters, were much higher than that of *Pe. avirostris*, recorded in this study (Table 4-3). While *Pe. avirostris* sometimes shows high densities and comprises major portions of zooplankton communities, secondary production seems to be lower than that of copepods. This may be due in part to their smaller body size and lower C content.

4-4. Predators of cladocerans

Various carnivores prey upon marine cladocerans (Table 4-4), which have been found in the guts of various fish larvae, including commercially important species such as Pacific bluefin tuna (Kodama et al. 2020). Predation on marine cladocerans is reported not only in coastal regions, but also in open oceans. Morita and Arima (2022) found *Pseudevadne tergestina* in the guts of Japanese sardine, collected in open ocean. This suggests that cladocerans in oceans waters may be important prey for pelagic fish.

Suruga Bay is known to have rich fishery resources. Larvae of anchovies, sardines, and round herring, called "Shirasu", is one of the commercially important in the bay. These larvae are transported from outside to the eastern part of the bay, and the main fishing grounds occur on the shelf in the inner and western parts of Suruga Bay (Nakamura 1982). In Suruga Bay, the fishing season for Shirasu is from March to January of the following year, with a peak during the spring and summer, coinciding with cladoceran abundance. Larvae are mainly distributed in the upper 30 m, and this depth coincides with the vertical distribution of cladocerans. Although this study did not investigate predation by these fish larvae on cladocerans, some studies have reported cladocerans in the guts of anchovy larvae (Mitani 1988; Morote et al. 2010; Okazaki et al. 2019). Moreover, Uotani (1985) indicated that *Evadne* comprises one of the main prey for anchovy larvae in Suruga Bay. The foregoing evidence

suggests that cladocerans are an important food for fish larvae in offshore Suruga Bay, and may support the high biomass of commercially important fish-larvae.

4-5. Possible changes in cladoceran communities due to climate change

Warming of seawater due to climate change is changing the distributions of marine animals (e.g. Constable et al. 2014), including cladocerans. Some studies suggest possible northward expansion of cladoceran distributions due to the temperature increase (Johns et al. 2005; Atienza et al. 2016). In the North Sea, *Pe. avirostris* appeared abundantly corresponded with unusual warm water temperatures in 1999, and they has become one of the component of local zooplankton community since that climatological event (Johns et al. 2005). Onbé et al. (1996a) found a small number of *Pe. avirostris* in the Bering Sea, and this occurrence was thought to reflect transportation by northward currents. Although some *Penilia* in coastal waters are probably transported northward by ocean currents, they have not established resident populations in cold-water regions. However, increasing water temperature may enable warm-water species to expand their distributions northward. On the other hand, Atienza et al. (2016) reported that a 2˚C increase in water temperature due to a heat wave caused a decrease in the abundance of *Pe. avirostris* in the northwest Mediterranean Sea. Seawater warming may not only promote northward habitat expansion, but may also cause population collapses in tropical

and subtropical waters. Effects of climate change on cladoceran populations need to be clarified with long-term research.

Some studies suggested that global warming has weakened the Kuroshio Current (Wang et al. 2016; Liu et al. 2021). Oceanic water originating with the Kuroshio Current, flows into Suruga Bay (Nakamura 1982), and variation in its flow affects hydrographic conditions and surface currents of the bay (Iwata et al. 2005; Toyoda et al. 2021). Aoyama et al. (2008) suggested that water temperatures in Kuroshio Current in South China Sea have increased rapidly for the past three decades due to global warming, and zooplankton biomass in these areas decreased. Water temperature is one of the main factors controlling distributions of marine cladocerans (Onbé 1974; Marazzo and Valentin 2003; Miyashita et al. 2010), and population dynamics of offshore cladocerans will need to be evaluated to understand the effect of climate change on Suruga Bay ecosystems.

4-6. Future perspective in marine cladoceran studies

Ecological study on marine cladocerans have been delayed compared with those of freshwater species. This study provided information on marine cladocerans, especially those in offshore regions, which have been overlooked in previous studies.

In Suruga Bay, cladocerans occurred abundantly offshore due to transportation and

accumulation by cyclonic currents. Cladocerans are sometimes found in oceanic area in Kuroshio and adjacent regions (e.g. Kim and Onbé 1995), and are preyed upon by pelagic fish larvae (Morita and Arima 2022). The Kuroshio Current flows along the Pacific coast of Japan, is known to transport various animals including fish eggs and larvae (e.g. Kon et al. 2006). If cladocerans are able to reproduce in the Kuroshio, they may contribute to development of pelagic fish in that region. In addition, warm eddies form along the Kuroshio's path, enhancing primary production due to upwelling of nutrients (Kimura et al. 1997). Although the geographic scale is much different, cladocerans may form populations within eddies of the Kuroshio like those we observed in Suruga Bay. Further investigations are needed to clarify the distributions and niches of marine cladocerans, especially along the Kuroshio path.

One reason for the dearth of studies on marine cladocerans is the difficulty of rearing in the laboratory. Since freshwater species are rather easy to culture, various experiments on physiology (Olmstead and LeBlanc 2000), feeding habits (DeMott 1982) and behavioral ecology (Nikitin and Latypova 2014) have been conducted. Establishing a cultivation method for marine cladocerans would provide detailed biological information of marine species, such as growth, feeding, and responses to changing environments.

Molecular studies on freshwater cladocerans, especially *Daphnia*, have been extensively carried out (Crease 1999, Colbourne et al. 2011). In contrast, genetic information on

marine cladocerans is very limited. Despite worldwide distribution, there are few studies on population genetics of marine species (Bockmann et al. 2018). Scarcity of genetic information may be one reason for fewer molecular studies on marine cladocerans. Accumulating genetic information is important for further studies on evolution, phylogenesis and adaptive radiation of marine species.

Marine cladocerans are thought to have originated in freshwater environments. Although they are currently distributed throughout the world's oceans, detailed distributional information is scare. Podonids originated in the Ponto-Capian region (Egloff et al. 1997), and their speciation is related to the hydrogeologic history of the region (Cristescu and Hebert 2002). On the other hand, the origin of *Penilia* is still unknown. Further studies are required to clarify the evolutionary history of marine cladocerans. This information may also provide clues about ancient geographic changes in their original localities, enabling predictions of further radiations during climate change.

Summary

1) Marine cladocerans are known as members of coastal zooplankton, and few studies were focused on the population in offshore waters. This study investigated the population dynamics of cladocerans in offshore area of Suruga Bay from June 2014 to December 2019. As the result, all seven species previously reported in the Japanese waters were identified. Cladocerans appeared at high abundance from spring to summer in all years and were dominant in the offshore mesozooplankton community. These results suggested that mass occurrence of cladocerans in offshore area is regularly event rather than sporadic phenomenon.

2) To clarify the mechanisms of forming offshore populations, field samplings and numerical experiments using particle-tracking methods were conducted. Cladocerans showed different occurrence patterns in coastal and offshore areas. Particle-tracking experiments demonstrated that particles released from the coast of Suruga Bay were transported to and accumulated at the inner offshore region of the Bay only from March to August which is corresponded to the periods of mass occurrence of certain period. These results suggest that a combination of transport of coastal populations by surface circulation currents and biological characteristics of the species to survive in oligotrophic and high-salinity offshore environments contribute to form periodic mass occurrences of cladocerans in offshore

Suruga Bay.

3) To elucidate how the observed offshore populations of cladocerans affect the offshore food-web, two methods: stable isotope ratio analysis and metabarcoding analysis on gut contents were carried out. $\delta^{15}N$ values of cladocerans *Pe. avirostris* (4.0±1.4‰) and *Ps. tergestina* (5.8±1.3‰) indicating that they are in the lower trophic level of the zooplankton community. 18S metabarcoding analysis suggested that *Penilia* and *Pseudevadne* have similar prey composition in terms of eukaryotes prey, feeding mainly on diatoms, dinoflagellates, and radiolarians. On the other hand, 16S analysis, which focused mainly on prokaryotes, showed that planctomycetota and cyanobacteria were detected only from *Penilia*, indicating that feeding habits were differed between the two species.

4) The clearance and production rate of cladocerans in offshore Suruga Bay were lower than those of copepods, suggesting that the effects of feeding and secondary production by them are limited. On the other hand, cladocerans have been found in the guts of various fish larvae, suggesting that they may be an important initial prey for fish. The increasing water temperature and weakening of the Kuroshio Current due to global climate change may weaken zooplankton production in the Kuroshio Basin and affect the formation of cladoceran populations in the offshore area.

Acknowledgments

I would like to express my gratitude to my advisor, Professor Jun Nishikawa (Graduate School of Bioscience, Tokai University) for his thoughtful guidance and continuous support. I am also particularly grateful to Professor Atsushi Fukui, Professor Tsukasa Murayama, Professor Takashi Yoshikawa (Graduate School of Bioscience, Tokai University), and Professor Shinji Shimode (Graduate School of Environment and Information Sciences, Yokohama National University) for constructive suggestions and variable comments. I am very grateful to Captain Iwao Hashigaya and Boatswain Hidetsugu Sakata of R/V Hokuto of Tokai University for their support on samplings. I would like to express my appreciation to Associate Professor Hiroyuki Matsuura (School of Marine Science and Technology, Tokai University), Associate Professor Rumi Sohrin (Faculty of Science, Shizuoka University), Lecturer Yumiko Obayashi (Center for Marine Environmental Studies, Ehime University), for helpful advice on samplings and data analysis. I especially would like to express my appreciation to Associate Professor Daisuke Takahashi (School of Marine Science and Technology, Tokai University) for performing simulation analysis and helpful suggestions. I would like to express my appreciation to Assistant Professor Junya Hirai (Atmosphere and Ocean Research Institute, University of Tokyo), for his elaborates guidance on the molecular experiment and analysis. I also thank to all members of the laboratory for their support and encouragement.

References

Amaral-Zettler LA, McCliment EA, Ducklow HW, Huse SM (2009) A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. PLoS One 4: e6372 doi 10.1371/journal.pone.0006372

Aoyama M, Goto H, Kamiya H, Kaneko I, Kawae S, Kodama H, Konishi Y, Kusumoto K-I, Miura H, Moriyama E, Murakami K, Nakano T, Nozaki F, Sasano D, Shimizu T, Suzuki H, Takatsuki Y, Toriyama A (2008) Marine biogeochemical response to a rapid warming in the main stream of the Kuroshio in the western North Pacific. Fisheries Oceanography 17: 206–218 doi 10.1111/j.1365-2419.2008.00473.x

Ara K, Hiromi J (2006) Temporal variability in primary and copepod production in Sagami Bay, Japan. Journal of Plankton Research 29: i85-i96 doi 10.1093/plankt/fbl069

Atienza D, Calbet A, Saiz E, Lopes RM (2007) Ecological success of the cladoceran *Penilia avirostris* in the marine environment: feeding performance, gross growth efficiencies and life history. Marine Biology 151: 1385–1396 doi 10.1007/s00227-006-0578-8

Atienza D, Sabatés A, Isari S, Saiz E, Calbet A (2016) Environmental boundaries of marine cladoceran distributions in the NW Mediterranean: Implications for their expansion under global warming. Journal of Marine Systems 164: 30–41 doi

10.1016/j.jmarsys.2016.08.003

Atienza D, Saiz E, Calbet A (2006) Feeding ecology of the marine cladoceran *Penilia avirostris*: natural diet, prey selectivity and daily ration. Marine Ecology Progress Series 315: 211–220

Atienza D, Saiz E, Skovgaard A, Trepat I, Calbet A (2008) Life history and population dynamics of the marine cladoceran *Penilia avirostris* (Branchiopoda: Cladocera) in the Catalan Sea (NW Mediterranean). Journal of Plankton Research 30: 345–357 doi 10.1093/plankt/fbm109

Bishop GH, Omori M, Muranaka F (1989) Temporal and spatial variations in the spawning activity of the micronektonic shrimp, *Sergia lucens* (Hansen) in Suruga Bay, Japan. Journal of the Oceanographical Society of Japan 45: 243–259

Bizic-Ionescu M, Zeder M, Ionescu D, Orlic S, Fuchs BM, Grossart HP, Amann R (2015) Comparison of bacterial communities on limnic versus coastal marine particles reveals profound differences in colonization. Environmental Microbiology 17: 3500–3514 doi 10.1111/1462-2920.12466

Böckmann S, Seidler M, Schubert H, Kube S (2018) Population genetics of two allopatric (North Sea and Baltic Sea) populations of *Evadne nordmanni* (Podonidae): Similarities and differences. International Review of Hydrobiology 103: 54–62 doi

10.1002/iroh.201701930

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30: 2114–2120 doi 10.1093/bioinformatics/btu170

Bosch HF, Taylor WR (1968) Marine Cladocerans in the Chesapeake Bay Estuary. Crustaceana 15: 161–164

Colbourne JK, Pfrender ME, Gilbert D, Thomas WK, Tucker A, Oakley TH, Tokishita S, Aerts A, Arnold GJ, Basu MK, Bauer DJ, Cáceres CE, Carmel L, Casola C, Choi JH, Detter JC, Dong Q, Dusheyko S, Eads BD, Fröhlich T, Geiler-Samerotte KA, Gerlach D, Hatcher P, Jogdeo S, Krijgsveld J, Kriventseva EV, Kültz D, Laforsch C, Lindquist E, Lopez J, Manak JR, Muller J, Pangilinan J, Patwardhan RP, Pitluck S, Pritham EJ, Rechtsteiner A, Rho M, Rogozin IB, Sakarya O, Salamov A, Schaack S, Shapiro H, Shiga Y, Skalitzky C, Smith Z, Souvorov A, Sung W, Tang Z, Tsuchiya D, Tu H, Vos H, Wang M, Wolf YI, Yamagata H, Yamada T, Ye Y, Shaw JR, Andrews J, Crease TJ, Tang H, Lucas SM, Robertson HM, Bork P, Koonin EV, Zdobnov EM, Grigoriev IV, Lynch M, Boore JL (2011) The ecoresponsive genome of *Daphnia pulex*. Science 331: 555–561 doi 10.1126/science.1197761

Constable AJ, Melbourne-Thomas J, Corney SP, Arrigo KR, Barbraud C, Barnes DK, Bindoff NL, Boyd PW, Brandt A, Costa DP, Davidson AT, Ducklow HW, Emmerson L,

Fukuchi M, Gutt J, Hindell MA, Hofmann EE, Hosie GW, Iida T, Jacob S, Johnston NM, Kawaguchi S, Kokubun N, Koubbi P, Lea MA, Makhado A, Massom RA, Meiners K, Meredith MP, Murphy EJ, Nicol S, Reid K, Richerson K, Riddle MJ, Rintoul SR, Smith WO, Jr., Southwell C, Stark JS, Sumner M, Swadling KM, Takahashi KT, Trathan PN, Welsford DC, Weimerskirch H, Westwood KJ, Wienecke BC, Wolf-Gladrow D, Wright SW, Xavier JC, Ziegler P (2014) Climate change and Southern Ocean ecosystems I: how changes in physical habitats directly affect marine biota. Global Change Biology 20: 3004–3025 doi 10.1111/gcb.12623

Crease TJ (1999) The complete sequence of the mitochondrial genome of *Daphnia pulex* (Cladocera: Crustacea). Gene 233: 89–99 doi 10.1016/s0378-1119(99)00151-1

Crespo BG, Pommier T, Fernandez-Gomez B, Pedros-Alio C (2013) Taxonomic composition of the particle-attached and free-living bacterial assemblages in the Northwest Mediterranean Sea analyzed by pyrosequencing of the 16S rRNA. MicrobiologyOpen 2: 541–552 doi 10.1002/mbo3.92

Cristescu MEA, Hebert PDN (2002) Phylogeny and adaptive radiation in the Onychopoda (Crustacea, Cladocera): evidence from multiple gene sequences. Journal of Evolutionary Biology 15: 838–849 doi 10.1046/j.1420-9101.2002.00466.x

Della Croce N, Angelino N (1987) Marine cladocera in the Gulf of Mexico and the Caribbean

Sea. Cahiers de Biologie Marine 28: 263–268

Della Croce N, Venugopal P (1972) Distribution of marine cladocerans in the Indian Ocean. Marine Biology 15: 132–138

DeMott WR (1982) Feeding selectivities and relative ingestion rates of *Daphnia* and *Bosmina* 1. Limnology and Oceanography 27: 518–527 doi 10.4319/lo.1982.27.3.0518

DeNiro MJ, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197: 261–263 doi doi:10.1126/science.327543

Durbin A, Hebert PDN, Cristescu MEA (2008) Comparative phylogeography of marine cladocerans. Marine Biology 155: 1–10 doi 10.1007/s00227-008-0996-x

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27: 2194–2200 doi 10.1093/bioinformatics/btr381

Egloff DA, Fofonoff AW, Onbé T (1997) Reproductive biology of marine cladocerans. Advances in Marine Biology 31: 79–167

Forró L, Korovchinsky NM, Kotov AA, Petrusek A (2008) Global diversity of cladocerans (Cladocera; Crustacea) in freshwater. Hydrobiologia 595: 177–184 doi 10.1007/s10750-007-9013-5

France RL (1995) Carbon-13 enrichment in benthic compared to planktonic algae: foodweb

implications. Marine Ecology Progress Series 124: 307–312 doi 10.3354/meps124307

Guillou L, Bachar D, Audic S, Bass D, Berney C, Bittner L, Boutte C, Burgaud G, de Vargas C, Decelle J, Del Campo J, Dolan JR, Dunthorn M, Edvardsen B, Holzmann M, Kooistra WH, Lara E, Le Bescot N, Logares R, Mahe F, Massana R, Montresor M, Morard R, Not F, Pawlowski J, Probert I, Sauvadet AL, Siano R, Stoeck T, Vaulot D, Zimmermann P, Christen R (2013) The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. Nucleic Acids Res 41: D597–604 doi 10.1093/nar/gks1160

Hansen JH, Hedeholm RB, Sünksen K, Christensen JT, Grønkjær P (2012) Spatial variability of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios in an Arctic marine food web. Marine Ecology Progress Series 467: 47–59 doi 10.3354/meps09945

Hirai J, Hamamoto Y, Honda D, Hidaka K (2018) Possible aplanochytrid (Labyrinthulea) prey detected using 18S metagenetic diet analysis in the key copepod species *Calanus sinicus* in the coastal waters of the subtropical western North Pacific. Plankton and Benthos Research 13: 75–82 doi 10.3800/pbr.13.75

Hirakawa K, Imamura A, Ikeda T (1992) Seasonal variability in abundance and composition of zooplankton in Toyama Bay, Southern Japan Sea. Bulletin of the Japan Sea National Fisheries Research Institute: 1–15

Hirota Y, Nemoto T, Marumo R (1984) Vertical distribution of larvae of *Euphausia nana* and *E. similis* (Crustacea: Euphausiacea) in Sagami Bay and Suruga Bay, Central Japan. Marine Biology 81: 131–137

Inaba H (1981) Circulation pattern and current variations with respect to tidal frequency in the sea near the head of Suruga Bay. Journal of the Oceanographical Society of Japan 37: 149–159

Inaba H (1984) Current variation in the sea near the mouth of Suruga Bay. Journal of the Oceanographical Society of Japan 40: 193–198

Iturriaga R, Marra J (1988) Temporal and spatial variability of chroococcoid cyanobacteria Synechococcus spp. specific growth rates and their contribution to primary production in the Sargasso Sea. Marine Ecology Progress Series 44: 175–181

Iwata T, Shinomura Y, Natori Y, Igarashi Y, Sohrin R, Suzuki Y (2005) Relationship between salinity and nutrients in the subsurface layer in the Suruga Bay. Journal of Oceanography 61: 721–732 doi 10.1007/s10872-005-0079-2

Jagger RA, Kimmerer WJ, Jenkins GP (1988) Food of the cladoceran *Podon intermedius* in a marine embayment. Marine Ecology Progress Series 433: 245–250

Johns DG, Edwards M, Greve W, Sjohn AWG (2005) Increasing prevalence of the marine cladoceran Penilia avirostris (Dana, 1852) in the North Sea. Helgoland Marine Research

59: 214–218 doi 10.1007/s10152-005-0221-y

Johnson PW, Sieburth JM (1979) Chroococcoid cyanobacteria in the sea: A ubiquitous and diverse phototrophic biomass. Limnology and Oceanography 24: 928–935 doi 10.4319/lo.1979.24.5.0928

Kürten B, Painting SJ, Struck U, Polunin NVC, Middelburg JJ (2013) Tracking seasonal changes in North Sea zooplankton trophic dynamics using stable isotopes. Biogeochemistry 113: 167–187 doi 10.1007/s10533-011-9630-y

Kane J (2013) Long-term abundance and distribution trends of the cladoceran *Penilia avirostris* in the US northeast shelf ecosystem. Journal of Northwest Atlantic Fishery Science 45: 1–10 doi 10.2960/J.v45.m682

Katechakis A, Stibor H (2004) Feeding selectivities of the marine cladocerans *Penilia avirostris*, *Podon intermedius* and *Evadne nordmanni*. Marine Biology 145:529–539 doi 10.1007/s00227-004-1347-1

Katsumata T, Niki M, Tanaka A, Tan H, Takashima K, Takahashi D, Fukuda G (2019) The current of the inner part of Suruga Bay in 2017 –The current observation by using Acoustic Doppler Current Profiler (ADCP) mounted on the Suruga-wan Ferry –. Bulletin of institute of oceanic research and development, Tokai University 41: 45–53

Katsumata T, Tanaka A, Tan H, Niki M (2018) The current of the inner part of Suruga Bay in

2015: The current observation by using mounted Acoustic Doppler Current Profiler (ADCP) on the Suruga-wan Ferry. Bulletin of institute of oceanic research and development, Tokai University 39: 27–35

Kawabe M (1985) Sea level variations at the Izu Islands and typical stable paths of the Kuroshio. Journal of the Oceanographical Society of Japan 41: 307–326 doi 10.1007/BF02109238

Kim SW, Onbé T (1995) Distribution and zoogeography of the marine cladoceran *Penilia avirostris* in the northwestern Pacific. Bulletin of Plankton Society of Japan 42: 19–28

Kim SW, Onbé T, Yoon YH (1989) Feeding habits of marine cladocerans in the Inland Sea of Japan. Marine Biology 100: 313–318 doi 10.1007/BF00391145

Kimura S, Kasai A, Nakata H, Sugimoto T, Simpson JH, Cheok JVS (1997) Biological productivity of meso-scale eddies caused by frontal disturbances in the Kuroshio. ICES Journal of Marine Science 54: 179–192 doi 10.1006/jmsc.1996.0209

Kobari T, Moku M, Takahashi K (2008) Seasonal appearance of expatriated boreal copepods in the Oyashio–Kuroshio mixed region. ICES Journal of Marine Science 65: 469–476

Kobari T, Tokumo Y, Sato I, Kume G, Hirai J (2021) Metabarcoding analysis of trophic sources and linkages in the plankton community of the Kuroshio and neighboring waters. Scientific Reports 11: 23265 doi 10.1038/s41598-021-02083-8

Kodama T, Hirai J, Tamura S, Takahashi T, Tanaka Y, Ishihara T, Tawa A, Morimoto H, Ohshimo S (2017) Diet composition and feeding habits of larval Pacific bluefin tuna *Thunnus orientalis* in the Sea of Japan: integrated morphological and metagenetic analysis. Marine Ecology Progress Series 583: 211–226 doi 10.3354/meps12341

Kodama T, Ohshimo S, Tanaka H, Ashida H, Kameda T, Tanabe T, Okazaki M, Ono T, Tanaka Y (2021) Abundance and habitats of marine cladocerans in the Sea of Japan over two decades. Progress in Oceanography 194 doi 10.1016/j.pocean.2021.102561

Kodama T, Hirai J, Tawa A, Ishihara T, Ohshimo S (2020) Feeding habits of the Pacific Bluefin tuna (*Thunnus orientalis*) larvae in two nursery grounds based on morphological and metagenomic analyses. Deep Sea Research Part II: Topical Studies in Oceanography 175 doi 10.1016/j.dsr2.2020.104745

Komazawa H, Endo Y (2002) Experimental studies on hatching conditions of the resting eggs of marine cladocerans and their seasonal variation in Onagawa Bay. Tohoku Journal of Agricultural Research 52: 57–85

Kon I, Yatsu A, Nishida H, Noto M, Mori K (2006) Estimation of hatching period and transport processes of juvenile jack mackerel (*Trachurus japonicus*) and young Japanese common squid (*Todarodes pacificus*) distributing in the Kuroshio-Oyashio Transition

Zone. Bulletin of the Japanese Society of Fisheries Oceanography 70: 229–239

Kozak ER, Franco-Gordo C, Godínez-Domínguez E, Suárez-Morales E, Ambriz-Arreola I (2020) Seasonal variability of stable isotope values and niche size in tropical calanoid copepods and zooplankton size fractions. Marine Biology 167 doi 10.1007/s00227-020-3653-7

Li C, Sun S, Wang R, Wang X (2004) Feeding and respiration rates of a planktonic copepod (*Calanus sinicus*) oversummering in Yellow Sea cold bottom waters. Marine Biology 145: 149–157 doi 10.1007/s00227-004-1306-x

Li K, Ma J, Huang L, Tan Y, Song X (2021) Environmental Drivers of Temporal and Spatial Fluctuations of Mesozooplankton Community in Daya Bay, Northern South China Sea. Journal of Ocean University of China 20: 1013–1026 doi 10.1007/s11802-021-4602-x

Li WKW (1994) Primary production of prochlorophytes, cyanobacteria, and eucaryotic ultraphytoplankton: Measurements from flow cytometric sorting. Limnology and Oceanography 39: 169–175 doi 10.4319/lo.1994.39.1.0169

Lipej L, Mozetic P, Turk V, Malej A (1997) The trophic role of the marine cladoceran *Penilia avirostris* in the Gulf of Trieste. Hydrobiologia 360: 197–203

Liu ZJ, Zhu XH, Nakamura H, Nishina A, Wang M, Zheng H (2021) Comprehensive observational features for the Kuroshio transport decreasing trend during a recent global

warming hiatus. Geophysical Research Letters 48 doi 10.1029/2021gl094169

Llopiz JK, Cowen RK (2008) Precocious, selective and successful feeding of larval billfishes in the oceanic Straits of Florida. Marine Ecology Progress Series 358: 231–244 doi 10.3354/meps07320

Longhurst AR, Seibert DLR (1972) Oceanic distribution of *Evadne* in the Eastern Pacific (Cladocera). Crustaceana 22: 239–248

Müllin M, Onbé T (1992) Diel reproduction and vertical distributions of the marine cladocerans, *Evadne tergestina* and *Penilia avirostris*, in contrasting coastal environments. Journal of Plankton Research 14: 41–59 doi 10.1093/plankt/14.1.41

Marazzo A, Valentin JL (2000) Daily variation of marine cladoceran densities in a tropical bay - Brazil. Hydrobiologia 428: 205–208

Marazzo A, Valentin JL (2003a) Population parameters of *Pleopis polyphemoides* (Crustacea, Cladocera) in a tropical bay. Estuarine, Coastal and Shelf Science 57: 1015–1021 doi 10.1016/s0272-7714(03)00007-6

Marazzo A, Valentin JL (2003b) *Penilia avirostris* (Crustacea, Ctenopoda) in a tropical bay: variations in density and aspects of reproduction. Acta Oecologica 24: S251–S257 doi 10.1016/s1146-609x(03)00019-5

McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable

isotope ratios of carbon, nitrogen, and sulfur. Oikos 102: 378–390 doi 10.1034/j.1600-0706.2003.12098.x

Milici M, Vital M, Tomasch J, Badewien TH, Giebel H-A, Plumeier I, Wang H, Pieper DH, Wagner-Döbler I, Simon M (2017) Diversity and community composition of particle-associated and free-living bacteria in mesopelagic and bathypelagic Southern Ocean water masses: Evidence of dispersal limitation in the Bransfield Strait. Limnology and Oceanography 62: 1080–1095 doi 10.1002/lno.10487

Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta 48: 1135–1140

Minowa M, Kobari T, Akamatsu H, Ichikawa T, Fukuda R, Higashi M (2011) Seasonal changes in abundance, biomass and depth distribution of mesozooplankton community in Kagoshima Bay. Bulletin of the Japanese Society of Fisheries Oceanography 75: 71–81

Mishra RK, Senga Y, Nakata K, Mishra S, Sahu BK (2020) Spatio-temporal variation of Prochlorococcus and phytoplankton community between Shimizu coast and Suruga bay, Northwest Pacific Ocean. Regional Studies in Marine Science 33 doi 10.1016/j.rsma.2019.100890

Miyashita LK, Pompeu M, Gaeta SA, Lopes RM (2010) Seasonal contrasts in abundance and

reproductive parameters of *Penilia avirostris* (Cladocera, Ctenopoda) in a coastal subtropical area. Marine Biology 157: 2511–2519 doi 10.1007/s00227-010-1515-4

Moon J-H, Pang I-C, Yang J-Y, Yoon WD (2010) Behavior of the giant jellyfish *Nemopilema nomurai* in the East China Sea and East/Japan Sea during the summer of 2005: A numerical model approach using a particle-tracking experiment. Journal of Marine Systems 80: 101–114 doi 10.1016/j.jmarsys.2009.10.015

Morita K, Arima D (2022) Specific feding habits of Pacific saury *Cololabis saira* caught off Japan in the western North Pacific from August to September 2020. Nippon Suisan Gakkaishi 88: 168–170 doi 10.2331/suisan.21-00045

Morote E, Olivar MP, Villate F, Uriarte I (2010) A comparison of anchovy (Engraulis encrasicolus) and sardine (Sardina pilchardus) larvae feeding in the Northwest Mediterranean: influence of prey availability and ontogeny. ICES Journal of Marine Science 67: 897–908 doi 10.1093/icesjms/fsp302

Motoda S (1957) North Pacific standard net. Information bulletin on planktology in Japan 4: 13–15

Nagasawa S, Marumo R (1972) Feeding of a pelagic chaetognath, *Sagitta nagae* Alvariño in Suruga Bay, Central Japan. Journal of the Oceanographical Society of Japan 28: 181–186

Nagata RM, Moreira MZ, Pimentel CR, Morandini AC (2015) Food web characterization based on $\delta^{15}N$ and $\delta^{13}C$ reveals isotopic niche partitioning between fish and jellyfish in a relatively pristine ecosystem. Marine Ecology Progress Series 519: 13–27 doi 10.3354/meps11071

Nakamura Y (1982) Oceanographic feature of Suruga Bay from view point of fisheries oceanography. Bulletin of the Shizuoka Prefectural Fisheries Experiment Station 17: 1–153

Nikitin O, Latypova V (2014) Behavioral response of *Daphnia magna* (Crustacea, Cladocera) to low concentration of microcystin2: 85–92

Nishikawa H, Yasuda I, Itoh S, Komatsu K, Sasaki H, Sasai Y, Oozeki Y (2013) Transport and survival of Japanese sardine (*Sardinops melanostictus*) eggs and larvae via particle-tracking experiments. Fisheries Oceanography 22: 509–522 doi 10.1111/fog.12041

Nival S, Ravera S (1979) Morphological study of the appendages of the marine cladoceran Evadne spinifera Muller by means of the scanning electron microscope. Journal of Plankton Research 1: 207–213 doi 10.1093/plankt/1.3.207

Oghenekaro EU, Chigbu P (2019) Population dynamics and life history of marine cladocera in the Maryland Coastal Bays, U.S.A. Journal of Coastal Research 35: 1225–1236

Okazaki Y, Tadokoro K, Kubota H, Kamimura Y, Hidaka K (2019) Dietary overlap and optimal

prey environments of larval and juvenile sardine and anchovy in the mixed water region of the western North Pacific. Marine Ecology Progress Series 630: 149–160

Olmstead AW, LeBlanc GA (2000) Effects of endocrine-active chemicals on the development of sex characteristics of *Daphnia magna*. Environmental Toxicology and Chemistry 19: 2107–2113 doi 10.1002/etc.5620190821

Omori M (1983) Abundance assessment of micronektonic sergestid shrimp in the ocean. Biological Oceanography 2: 199–210 doi 10.1080/01965581.1983.10749458

Omori M, Marumo R, Aizawa Y (1965) A 160-cm opening-closing plankton net. Journal of the Oceanographical Society of Japan 21: 245–252

Onbé T (1968) Studies on the marine cladocerans—I. A biological note on *Penilia*. Journal of Faculty of Fisheries and Animal Husbandry, Hiroshima University 7: 269–279

Onbé T (1974) Studies on the ecology of marine cladocerans. Journal of the Faculty of Fisheries and Animal Husbandry, Hiroshima University 13: 88–179

Onbé T (1997) Cladocera. In: Chihara M, Murano M (eds) An illustrated guide to marine plankton in Japan. Tokai University Press, Tokyo, pp 609–624

Onbé T (1999) Ctenopoda and Onychopoda (=Cladocera). In: Boltovskoy D (ed) South Atlantic Zooplankton. Brachuys Publishers, Leiden, pp 797–811

Onbé T, A. T, Fukuchi M, Hattori H, Sasaki H, Matsuda O (1996a) Distribution of marine

cladocerans in the Northern Bering Sea and the Chukchi Sea. Proceedings of the NIPR Symposium on Polar Biology 9: 141–154

Onbé T, Ikeda T (1995) Marine cladocerans in Toyama Bay, southern Japan Sea: seasonal occurrence and day-night vertical distributions. Journal of Plankton Research 17: 595–609

Onbé T, Kumagai H (2019) Summer occurrence of marine cladocerans in Uragami Inlet, central Honshu, Japan. Aquaculture science 67: 319–332

Onbé T, Terazaki M, Nagasawa S (1996b) Summer distribution of marine cladocerans in Otsuchi Bay, northeastern Honshu, Japan. Bulletin of Plankton Society of Japan 43: 121–131

Onitsuka G, Hirose N, Miyahara K, Ota T, Hatayama J, Mitsunaga Y, Goto T (2010) Numerical simulation of the migration and distribution of diamond squid (*Thysanoteuthis rhombus*) in the southwest Sea of Japan. Fisheries Oceanography 19: 63–75 doi 10.1111/j.1365-2419.2009.00528.x

Paffenhöfer G-A, Orcutt JD (1986) Feeding, growth and food conversion of the marine cladoceran *Penilia avirostris*. Journal of Plankton Research 8: 741–754 doi 10.1093/plankt/8.4.741

Parada AE, Needham DM, Fuhrman JA (2016) Every base matters: assessing small subunit

rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology 18: 1403–1414 doi 10.1111/1462-2920.13023

Parra-Flores A, Gasca R (2009) Distribution of pteropods (Mollusca: Gastropoda: Thecosomata) in surface waters (0-100 m) of the Western Caribbean Sea (winter, 2007). Revista de Biología Marina y Oceanografía 44: 647–662

Põllupüü M, Simm M, Ojaveer H (2010) Life history and population dynamics of the marine cladoceran *Pleopis polyphemoides* (Leuckart) (Cladocera, Crustacea) in a shallow temperate Pärnu Bay (Baltic Sea). Journal of Plankton Research 32: 1459–1469 doi 10.1093/plankt/fbq063

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83: 703–718

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glockner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research 41: D590–596 doi 10.1093/nar/gks1219

R Core Team (2021) A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Sabatés A, Zaragoza N, Raya V (2015) Distribution and feeding dynamics of larval red mullet

(*Mullus barbatus*) in the NW Mediterranean: the important role of cladocera. Journal of Plankton Research 37: 820–833 doi 10.1093/plankt/fbv040

Saigusa M, Oishi K, Ikumoto A, Iwasaki H, Terajima M (2000) Emergence patterns of small subtidal arthropods in relation to day/night, tidal, and surface/bottom factors: Investigations in the Boreal Sea, Japan (Akkeshi, Hokkaido). Journal of Oceanography 56: 295–310 doi 10.1023/A:1011103700784

Saito H, Hattori H (2000) Diel vertical migration of the marine cladoceran *Podon leuckarti*: Variations with reproductive stage. Journal of Oceanography 56: 153–160

Schlitzer R (2019) Ocean Data View. https://odv.awi.de/

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75: 7537–7541 doi 10.1128/AEM.01541-09

Shimada A, Nishijima M, Murayama T (1995) Seasonal appearance of *Prochlorococcus* in Suruga Bay, Japan in 1992–1993. Journal of Oceanography 51: 289–300

Sohrin R, Isaji M, Obara Y, Agostini S, Suzuki Y, Hiroe Y, Ichikawa T, Hidaka K (2011) Distribution of *Synechococcus* in the dark ocean. Aquatic Microbial Ecology 64: 1–14

doi 10.3354/ame01508

Sopanen S (2008) The effect of temperature on the development and hatching of resting eggs of non-indigenous predatory cladoceran Cercopagis pengoi in the Gulf of Finland, Baltic Sea. Marine Biology 154: 99–108 doi 10.1007/s00227-008-0903-5

Souza CS, Mafalda P, Sallés S, Ramirez T, Cortés D, Garcia A, Mercado J, Vargas-Yañez M (2011) Seasonal changes in the distribution and abundance of marine cladocerans of the Northwest Alboran Sea (Western Mediterranean), Spain. Braz arch biol technol 54: 543–550

Stowasser G, Atkinson A, McGill RAR, Phillips RA, Collins MA, Pond DW (2012) Food web dynamics in the Scotia Sea in summer: A stable isotope study. Deep Sea Research Part II: Topical Studies in Oceanography 59–60: 208–221 doi 10.1016/j.dsr2.2011.08.004

Sugimoto S, Qiu B, Kojima A (2020) Marked coastal warming off Tokai attributable to Kuroshio large meander. Journal of Oceanography 76: 141–154 doi 10.1007/s10872-019-00531-8

Suzuki R, Ishimaru T (1990) An improved method for the determination of phytoplankton chlorophyll using N, N-dimethylformamide. Journal of the Oceanographical Society of Japan 46: 190–194 doi 10.1007/BF02125580

Tanaka K, Komatsu T, Michida Y, Saitoh S-I (2011) A numerical study on the transport of eggs

and larvae of *Sergia lucens* in Suruga Bay, Japan. Fisheries Oceanography 20: 206–218 doi 10.1111/j.1365-2419.2011.00580.x

Tanaka K, Michida Y, Komatsu T, Ishigami K (2009) Spreading of river water in Suruga Bay. Journal of Oceanography 65: 165–177

Tang KW, Chen QC, Wong CK (1995) Distribution and biology of marine cladocerans in the coastal waters of southern China. Hydrobiologia 307: 99–107 doi 10.1007/bf00032001

Terbiyik Kurt T, Polat Sm (2014) Characterization of seasonal and inter-annual changes in the abundance of species of marine Cladocera on the Turkish coast of the northeastern Levantine Basin. Crustaceana 87: 769–783 doi 10.1163/15685403-00003325

Tõnno I, Agasild H, Kõiv T, Freiberg R, Nõges P, Nõges T (2016) Algal diet of small-bodied crustacean zooplankton in a cyanobacteria-dominated eutrophic lake. PLOS ONE 11: e0154526 doi 10.1371/journal.pone.0154526

Toyoda T, Sakamoto K, Usui N, Hirose N, Tanaka K, Katsumata T, Takahashi D, Niki M, Kutsuwada K, Miyama T, Nakano H, Urakawa LS, Komatsu KK, Kawakami Y, Yamanaka G (2021) Surface-layer circulations in Suruga Bay induced by intrusions of Kuroshio branch water. Frontiers in Marine Science 8 doi 10.3389/fmars.2021.721500

Turner JT, Tester PA, Ferguson RL (1988) The marine cladoceran *Penilia avirostris* and the "microbial loop" of pelagic food webs. Limnology and Oceanography 32: 245–255

Uotani I (1985) The relation between feeding mode and feeding habit of the anchovy larvae. Bulletin of the Japanese Society of Scientific Fisheries 51(7) 1057–1065 doi 10.2331/suisan.51.1057

Uye S (1982) Length-weight relationships of important zooplankton from the Inland Sea of Japan. Journal of the Oceanographical Society of Japan 38: 149–158 doi 10.1007/BF02110286

Uye S (1997) Zooplankton. Bulletin on Coastal Oceanography 34: 199–207

Uzundumlu S, Büyükateş Y (2019) Stable isotope compositions of dominant zooplankton species in relation to environmental factors in the Dardanelles. Archives of Biological Sciences 71: 177–185 doi 10.2298/abs181003059u

Van Damme K, Kotov AA (2016) The fossil record of the Cladocera (Crustacea: Branchiopoda): Evidence and hypotheses. Earth-Science Reviews 163: 162–189 doi 10.1016/j.earscirev.2016.10.009

Vandekerkhove J, Declerck S, Brendonck LUC, Conde-Porcuna JM, Jeppesen E, Meester LD (2005) Hatching of cladoceran resting eggs: temperature and photoperiod. Freshwater Biology 50: 96–104 doi 10.1111/j.1365-2427.2004.01312.x

Viñas MD, Ramírez FC, Santos BA, Marrari M (2007) Spatial and temporal distribution patterns of Cladocera in the Argentine Sea. Hydrobiologia 594: 59–68 doi

10.1007/s10750-007-9077-2

Wang Y-L, Wu C-R, Chao S-Y (2016) Warming and weakening trends of the Kuroshio during 1993-2013. Geophysical Research Letters 43: 9200–9207 doi 10.1002/2016gl069432

Waterbury JB, Watson ST, Guillard RRL, Brand LE (1979) Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium. Nature 277: 293–294 doi 10.1038/277293a0

Welschmeyer NA (1994) Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll b and pheopigments. Limnology and Oceanography 39: 1985–1992

Wiborg KF (1955) Zooplankton in relation to hydrography in the Norwegian Sea. Report no Norwegian Fishery and Marine Investigations 11: 1–66

Wong CK, Chan ALC, Tang KW (1992) Natural ingestion rates and grazing impact of the marine cladoceran Penilia avirostris Dana in Tolo Harbour, Hong Kong. Journal of Plankton Research 14: 1757–1765 doi 10.1093/plankt/14.12.1757

Wong CK, Liu X-J, Siu YY, Hwang J-S (2006) Study of selective feeding in the marine cladoceran *Penilia avirostris* by HPLC pigment analysis. Journal of Experimental Marine Biology and Ecology 331: 21–32 doi 10.1016/j.jembe.2005.09.019

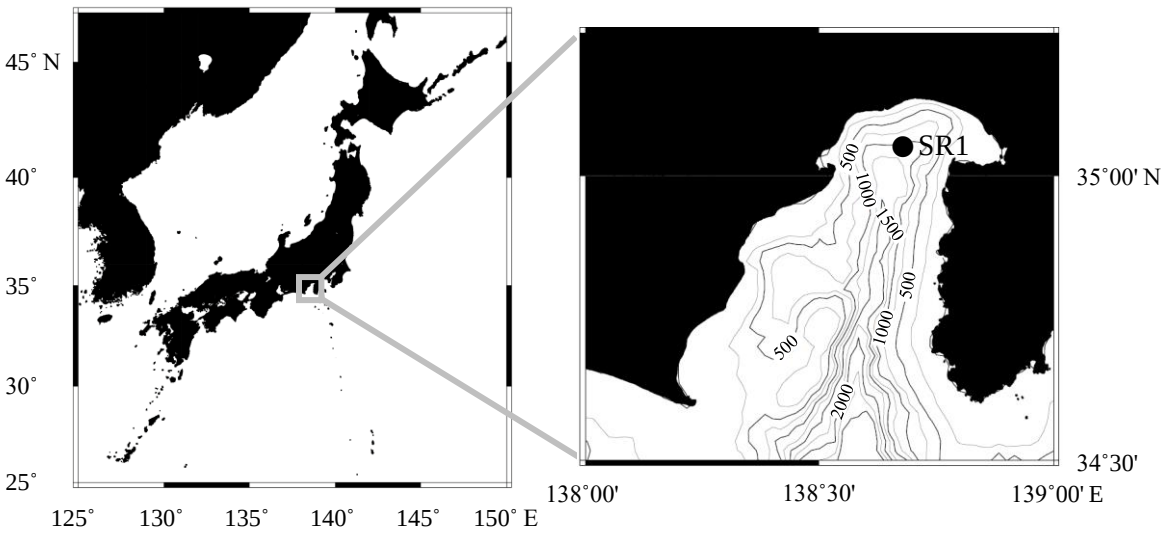

Fig. 1-1 Location of the sampling station, SR1 in the offshore area of Suruga Bay, Japan. Station SR1 is about 8 km away from the nearest coast.

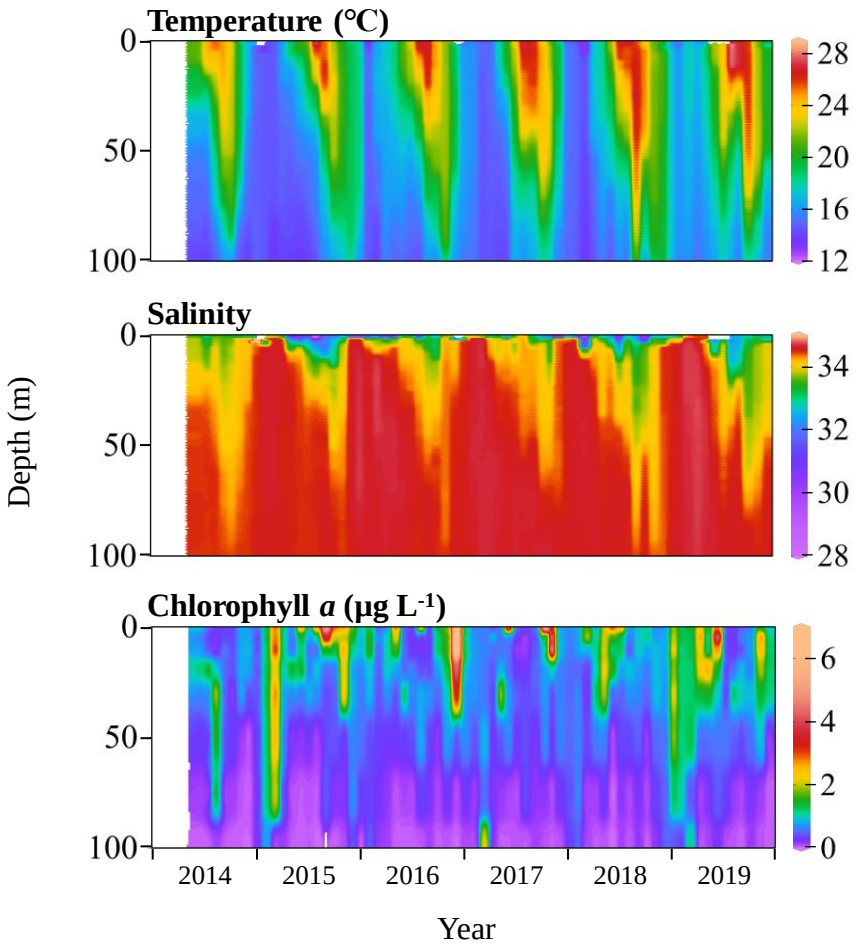

Fig. 1-2 Seasonal and vertical variations of temperature, salinity, and chlorophyll *a* concentrations at station SR1 in Suruga Bay from June 2014 to December 2019.

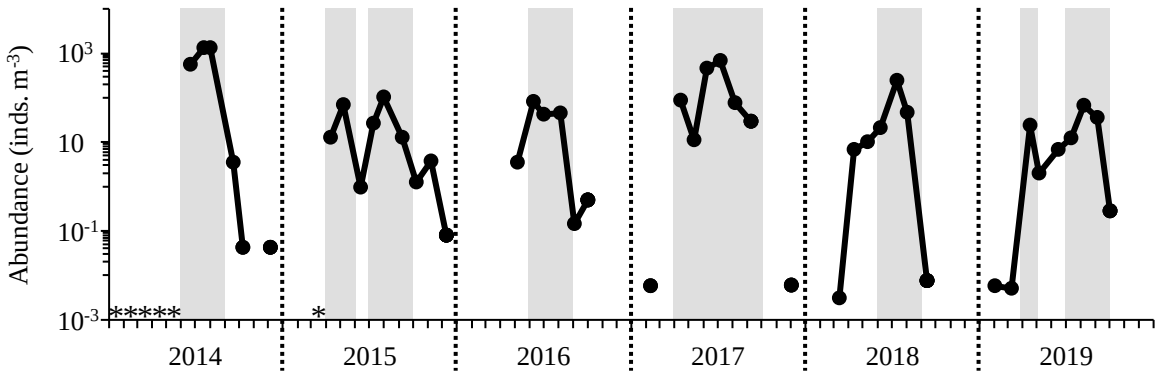

Fig. 1-3 Seasonal changes in the abundance of marine cladocerans at station SR1 from June 2014 to December 2019. Gray bars indicate when total cladoceran abundances exceeded 10 individuals m^{-3}. Asterisks indicate no data.

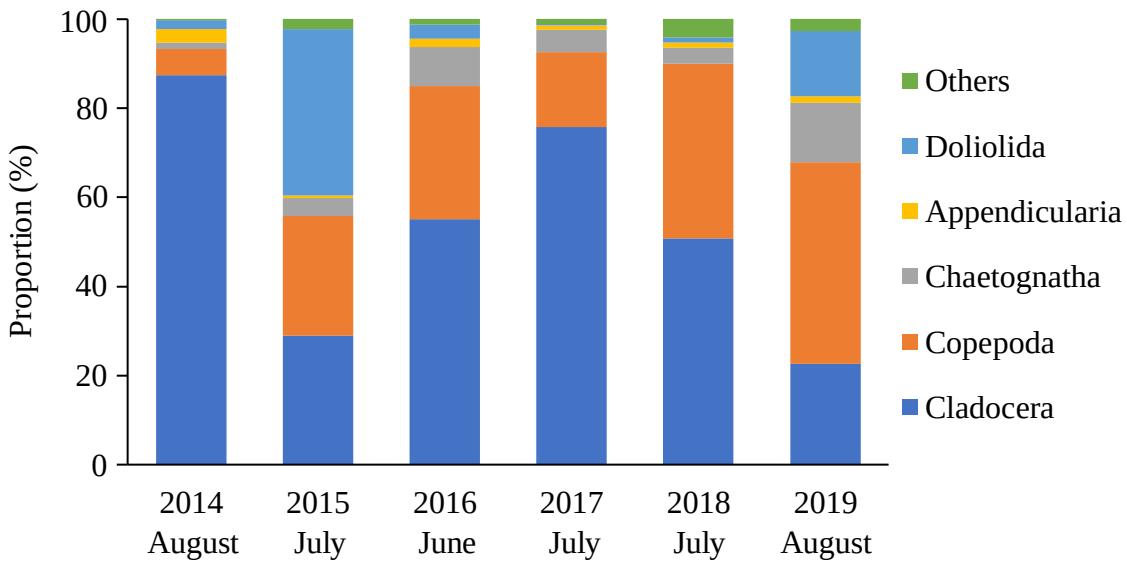

Fig. 1-4 Mesozooplankton composition at the station SR1 in months when cladocerans exhibited maximum abundance in each year.

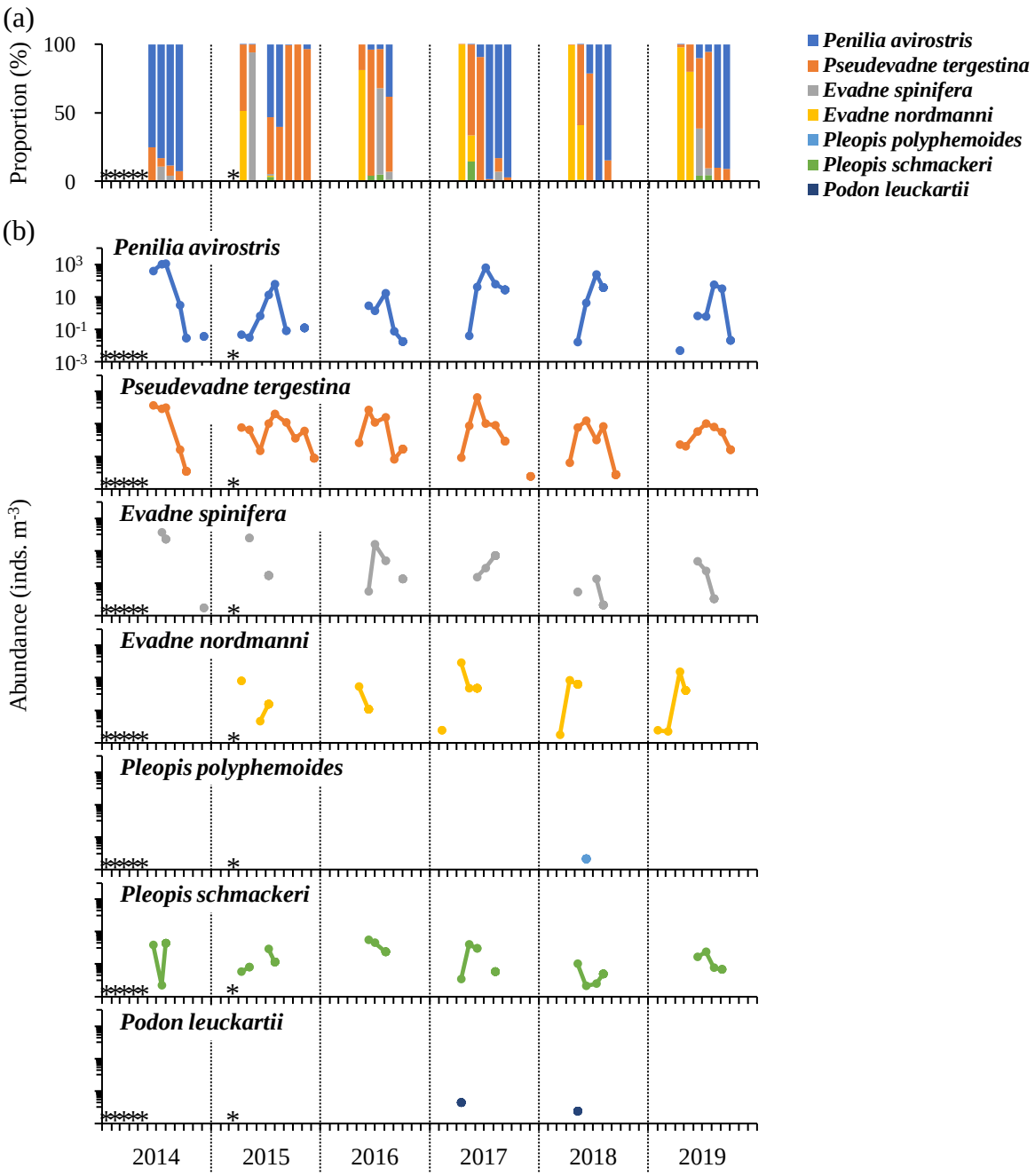

Fig. 1-5 Species composition of cladocerans (a), and year-to-year variation of abundance in each species (b). Species compositions are shown when total abundances exceeded 1 individual m^{-3}. Asterisks indicate no data.

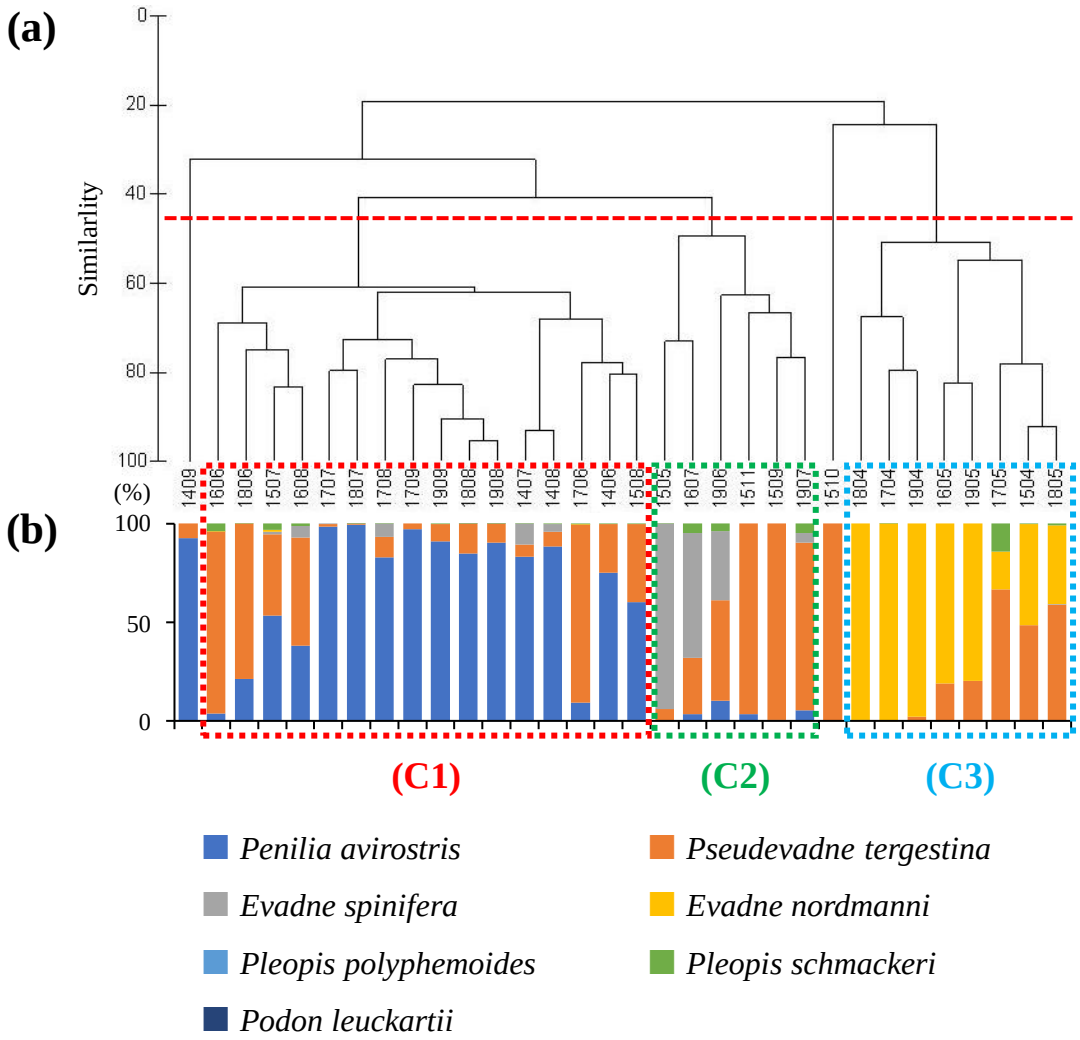

Fig. 1-6 (a) Dendrogram shows the classification of the cladoceran communities based on the Bray-Curtis similarity index between the months. First two and last two digits indicate samling year and month, respectively ("1409" indicates "September 2014"). As the result of clustering, cladoceran communities were divided into three seasonal connumities, C1, C2, and C3 (see text in detail). (b) Bar graphs indicate species compositions of cladocerans in the 0–100 m water column.

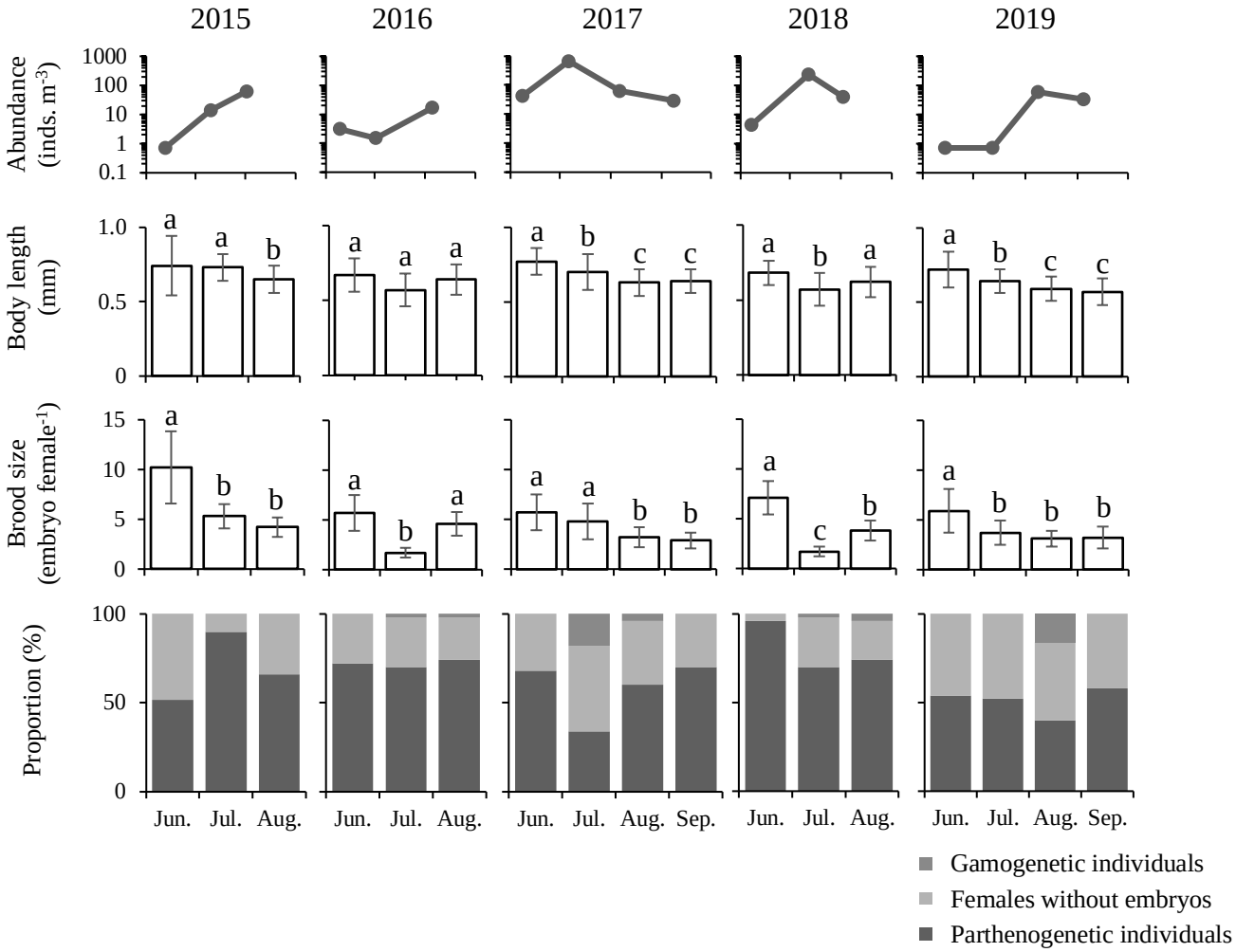

Fig. 1-7 *Penilia avirostris.* Abundance (inds. m^{-3}), body length (mm, average ± SD), brood size of parthenogenetic females (average ± SD), and reproductive stage composition from 2015 to 2019. Data are shown when their abundances were more than 0.1 individuals m^{-3}. Different letters indicate significant differences with other letters (Body length: ANOVA with Tukey-Kramer HSD test, $p < 0.01$, Brood size: Kruskal-Wallis test with Dunn's post hoc test, $p < 0.01$).

Table. 1-1 List of zooplankton groups counted in this study.

Hydrozoa	Amphipoda
Chaetognatha	Euphausiacea
Polychaeta	Decapoda
Heteropoda	Appendicularia
Thecosomata	Doliolida
Cladocera	Salpida
Ostracoda	Pices
Copepoda	

Table 1-2. List of cladoceran species and their maximum abundances observed in this study. Abbreviation of genus used in this study are shown in parenthesis.

Order	Family	Species (Abbreviation of genus)	Maximum abundance (inds. m^{-3})	Month/Year
Ctenopoda	Sididae	*Penilia avirostris* Dana, 1849 (Pe)	1184.4	August 2014
Onychopoda	Podonidae	*Pseudevadne tergestina* Claus, 1877 (Ps)	414.6	June 2017
		Evadne nordmanni Lovén, 1836 (E)	86.7	April 2017
		Evadne spinifera P.E. Müller, 1867 (E)	138.5	July 2014
		Pleopis schmackeri (Poppe, 1889) (Pl)	3.2	July 2016
		Pleopis polyphemoides (Leuckart, 1859) (Pl)	< 0.1	June 2018
		Podon leuckartii (G. O. Sars, 1862) (Po)	< 0.1	April 2017

Table 1-3. Spearman's rank correlation coefficients (ρ) for abundances of each cladoceran species with six environmental factors. Asterisks indicate significant correlations (* : $p < 0.05$, ** : $p < 0.01$). Mean temperature and salinity were calculated using the values at 1 m intervals. See text for details.

	Surface temperature (˚C)	Surface salinity	Mean temperature (˚C)			Mean salinity			Maximum chl. *a* ($\mu g\ L^{-1}$)	Chl. *a* ($\mu g\ m^{-2}$)		
			0–30 m	0–50 m	0–100 m	0–30 m	0–50 m	0–100 m		0–30 m	0–50 m	0–100 m
Penilia avirostris	0.59**	-0.19	0.38*	0.29	0.2	-0.15	-0.11	-0.1	-0.17	-0.41*	-0.45*	-0.44*
Pseudevadne tergestina	0.254	-0.01	0.06	-0.01	-0.1	-0.08	-0.01	-0.02	0.23	-0.07	0.01	0.04
Evadne nodmanni	-0.01	0.2	0.003	0.02	-0.03	-0.17	0.18	0.11	0.25	0.04	0.15	0.17
Evadne spinifera	0.03	-0.03	-0.26	-0.24	-0.18	0.38	0.35	0.36	0.07	-0.02	0.03	0.01
Pleopis schmackeri	-0.09	0.05	-0.17	-0.17	-0.11	0.09	0.19	0.17	-0.01	-0.20	-0.02	0.09
Total cladocerans	0.43**	-0.02	0.25	0.18	0.06	-0.17	-0.11	-0.12	0.1	-0.22	-0.15	-0.17

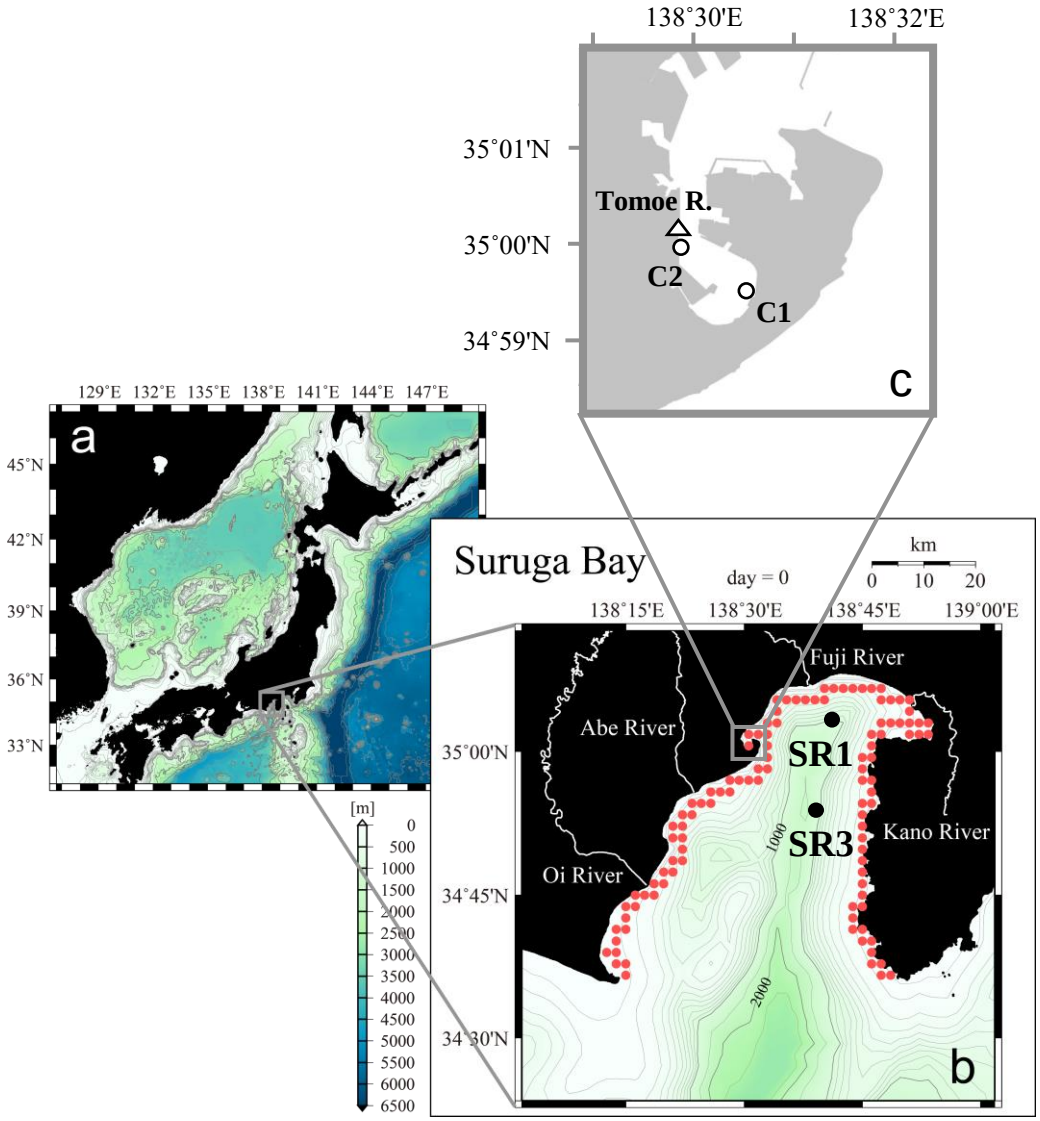

Fig. 2-1 (a) Domain of the nested 1/50° model and its bottom topography. (b) Locations of sampling stations in offshore Suruga Bay, and initial distribution of particles in particle-tracking experiments, which is assumed to be steady state. Red circles in (b) indicate the particles distributed in the surface layer at the initial time. (c) Locations of sampling stations in Orido Bay. White triangle indicates the mouth of Tomoe River.

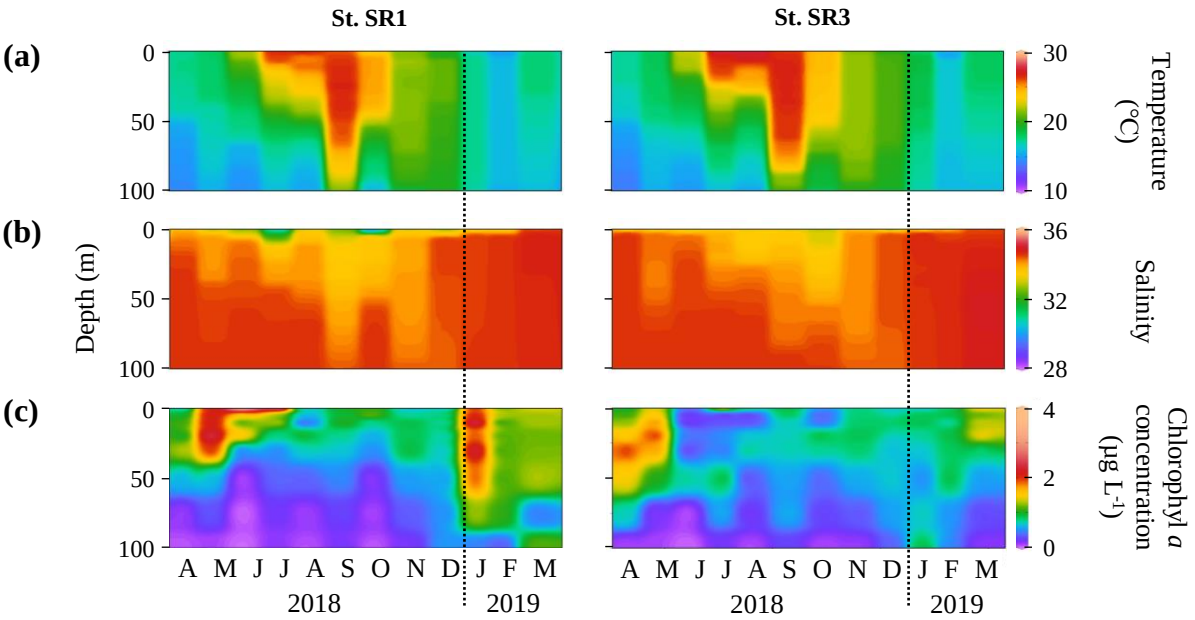

Fig. 2-2 Seasonal variations in vertical distributions of (a) temperature (˚C), (b) salinity, and (c) chlorophyll *a* concentrations (μg L^{-1}) in the upper 100 m at stations SR1 and SR3 during the study.

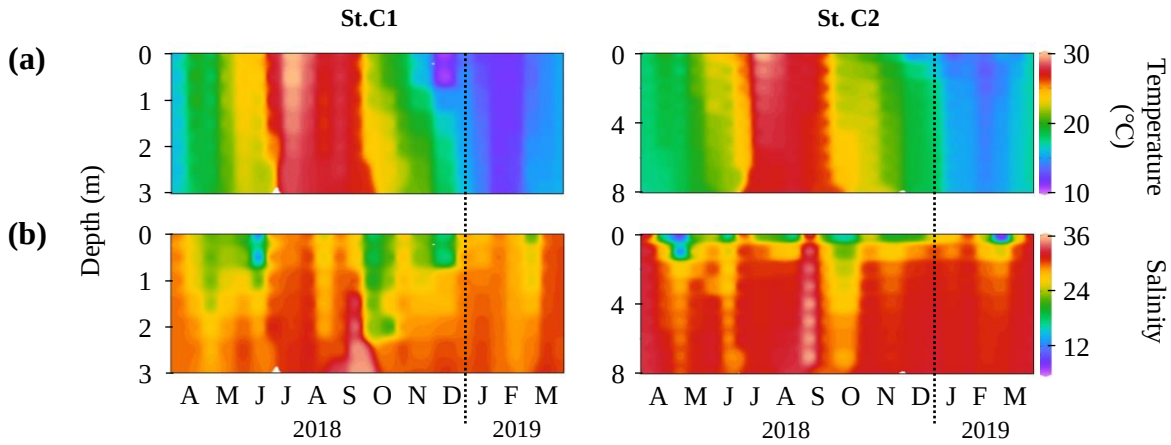

Fig. 2-3 Seasonal variations in vertical distributions of (a) temperature (℃) and (b) salinity at stations C1 and C2 during the study.

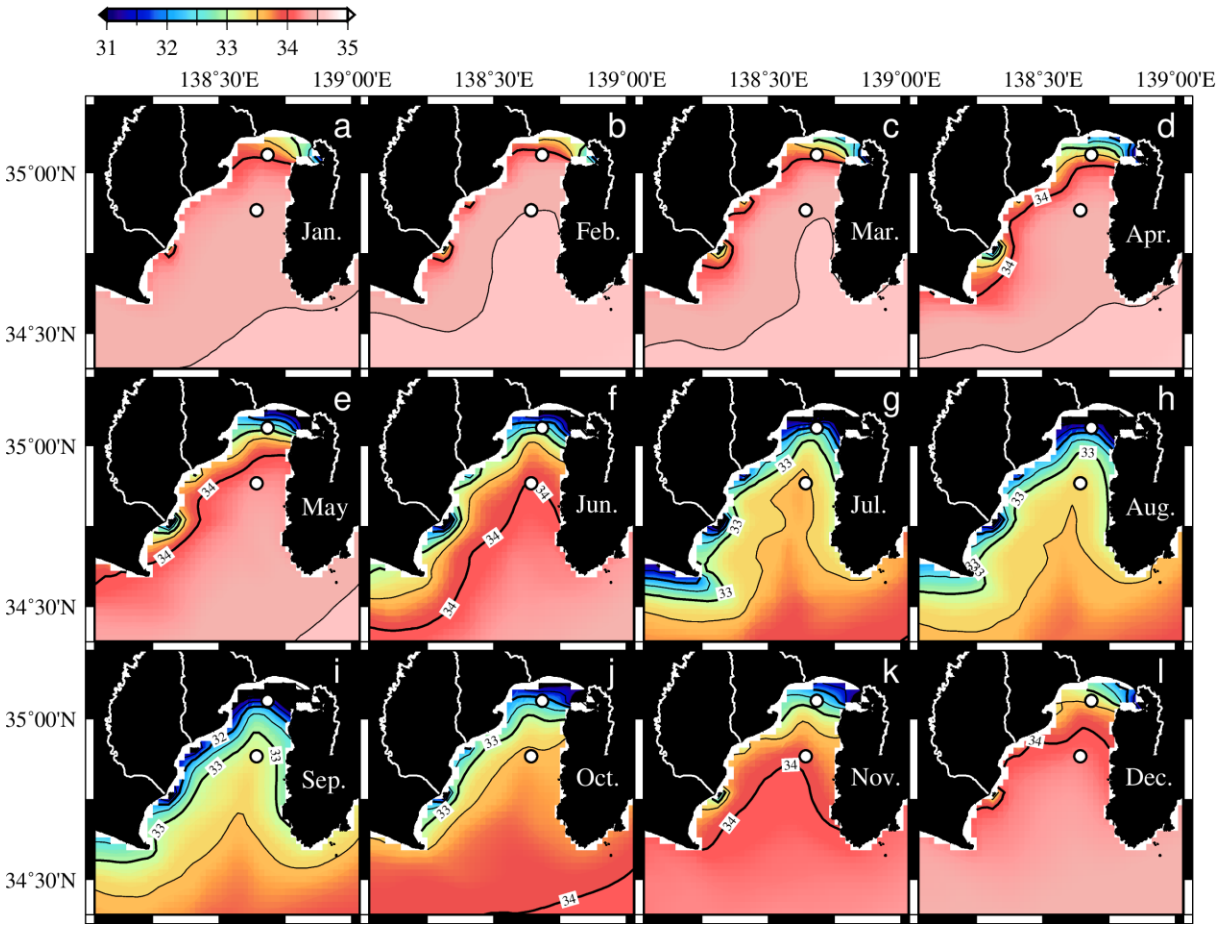

Fig. 2-4 Horizontal distribution of climatological monthly mean salinities at each month in the model output. Contour intervals are 0.5. Open circles at inner and central part of the bay were indicate the location of the offshore station SR1 and SR3, respectively.

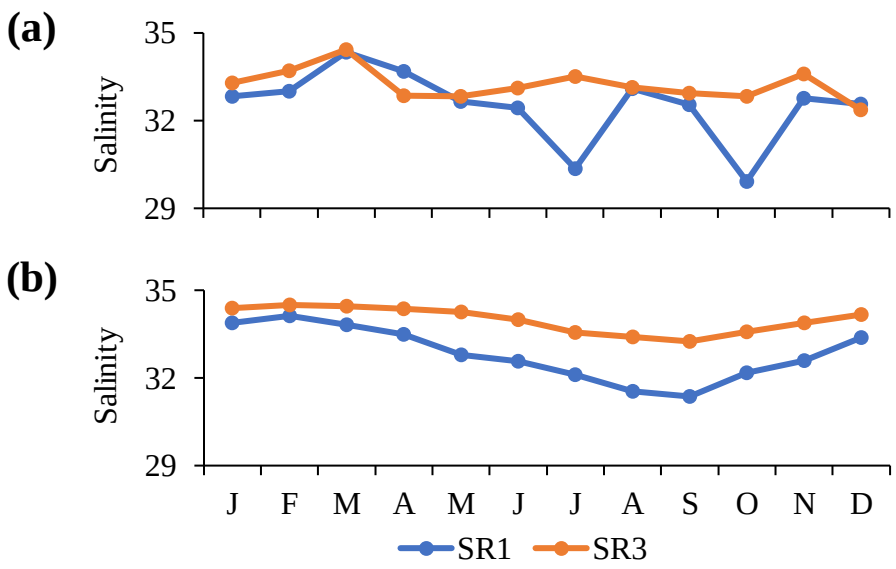

Fig. 2-5 Seasonal variations of surface salinities at stations SR1 and SR3: (a) the values recorded by field observations during the study, and (b) those reproduced by the simulation. See text for detail.

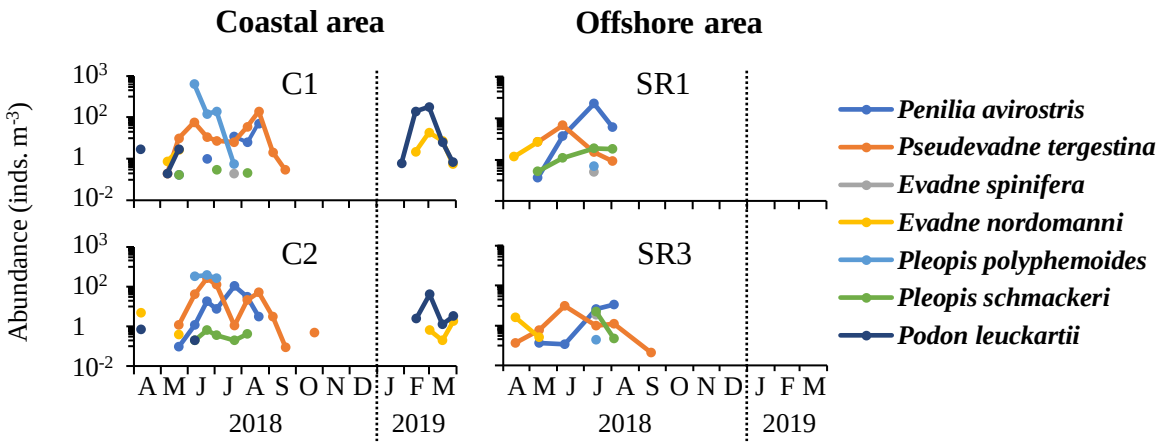

Fig. 2-6 Seasonal variations in abundances of seven marine cladocerans at each sampling station from April 2018 to March 2019.

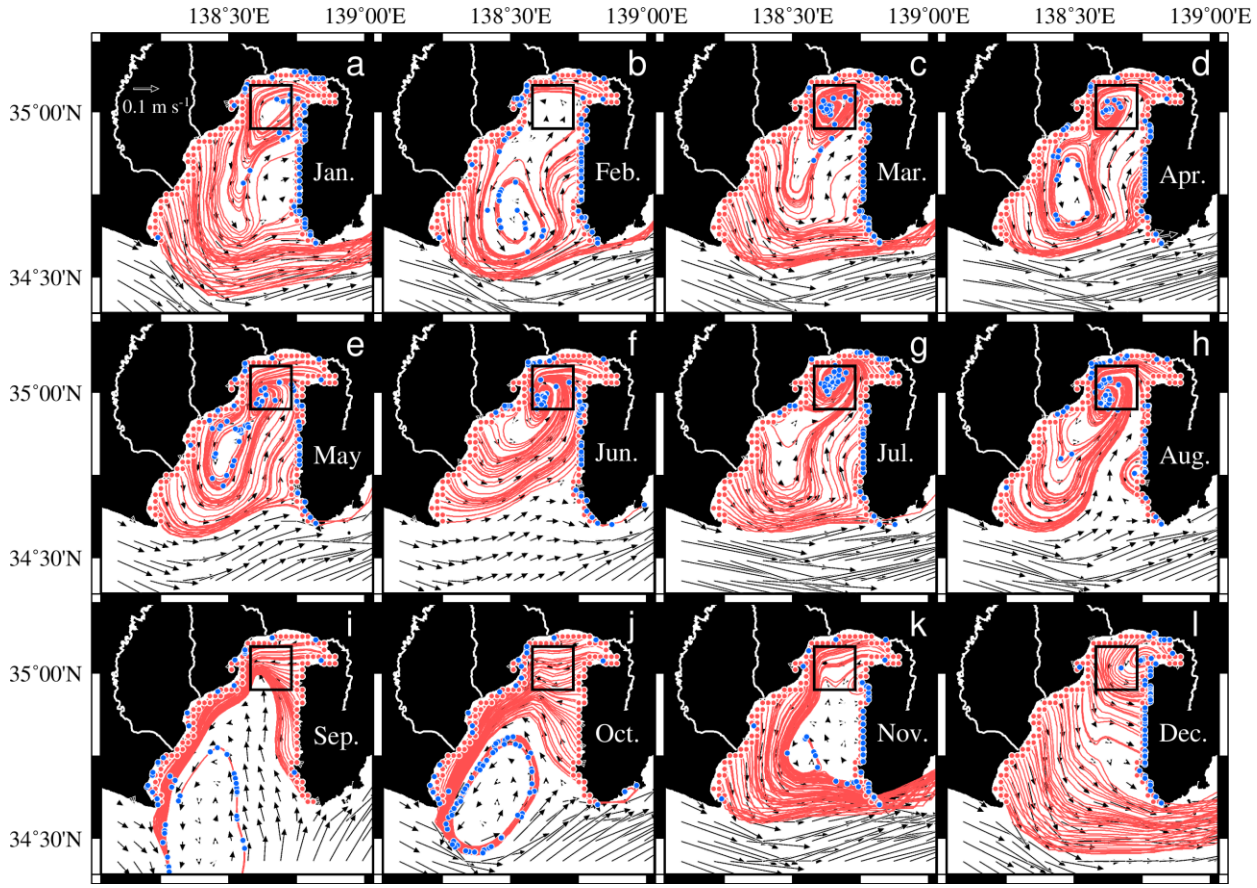

Fig. 2-7 Results of the particle-tracking experiment in each month in Suruga Bay. Red particles and red lines indicate initial positions of particles and the trajectory line, respectively. Blue particles indicate the position 30 days after release. Arrows indicate magnitudes and directions of surface flow field. Each month, particles that reached the offshore area of the inner bay (shown in squares) were counted.

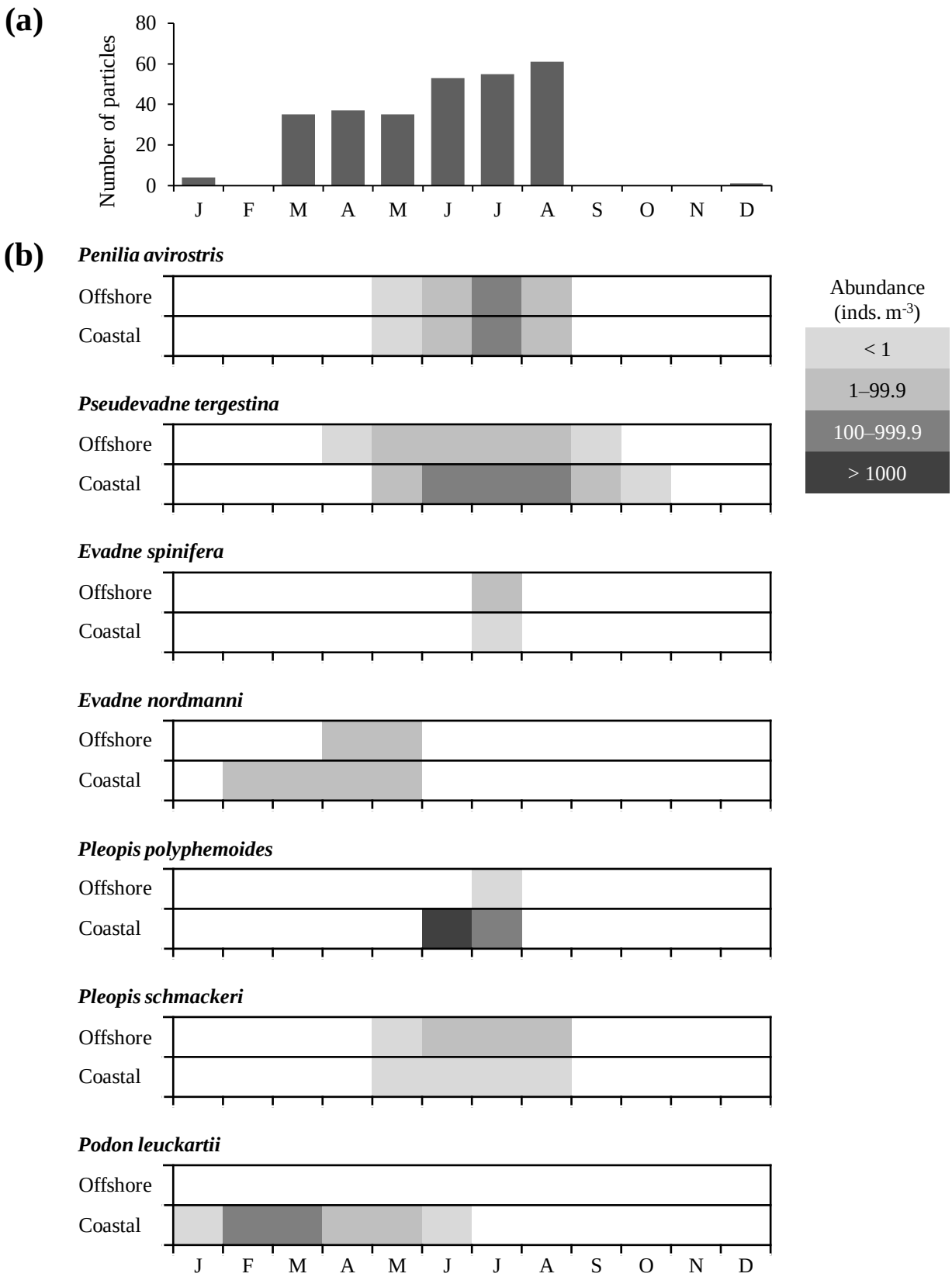

Fig. 2-8 (a) Numbers of particles distributed in inner Suruga Bay on Day 30 in the particle-tracking experiment. (b) Schematic diagram of seasonal occurrence patterns of cladocerans in offshore and coastal Suruga Bay. Graduations of color indicate abundance levels of cladoceran species.

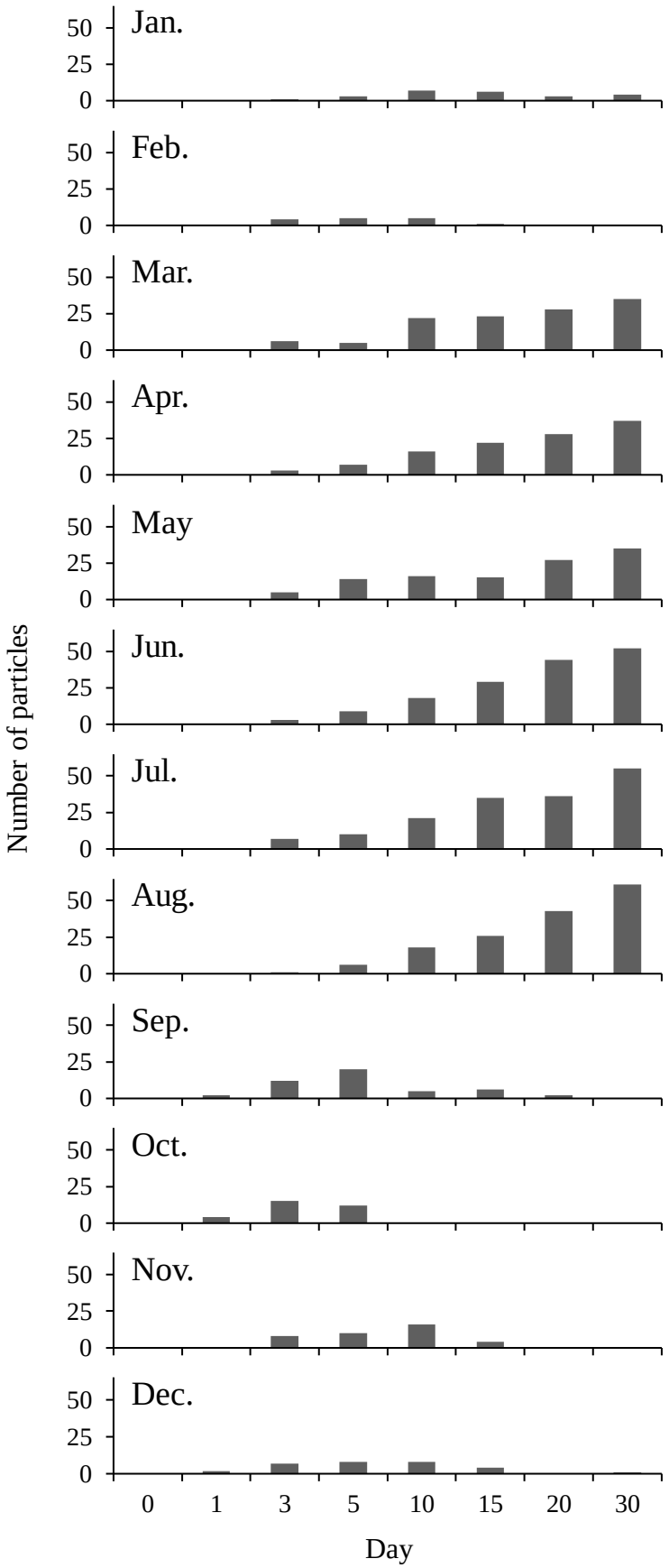

Fig. 2-9 Number of particles distributed in inner Suruga Bay on day 0, 1, 3, 5, 10, 15, 20, and 30 in the particle tracking experiment.

Table. 2-1. Maximum abundances (individuals m^{-3}) of cladocerans recorded in Orido Bay (coastal area) and Suruga Bay (offshore area) from April 2018 to March 2019

	Orido Bay		Suruga Bay	
	C1	C2	SR1	SR3
Penilia avirostris	52.4	105.2	531.0	10.9
Pseudevadne tergestina	193.8	250.2	46.3	9.5
Evadne spinifera	0.2	-	0.2	3.5
Evadne nordomanni	18.9	4.6	7.4	2.6
Pleopis polyphemoides	4288.7	384.9	0.5	0.2
Pleopis schmackeri	0.3	0.6	3.5	4.9
Podon leuckartii	321.0	40.3	-	-

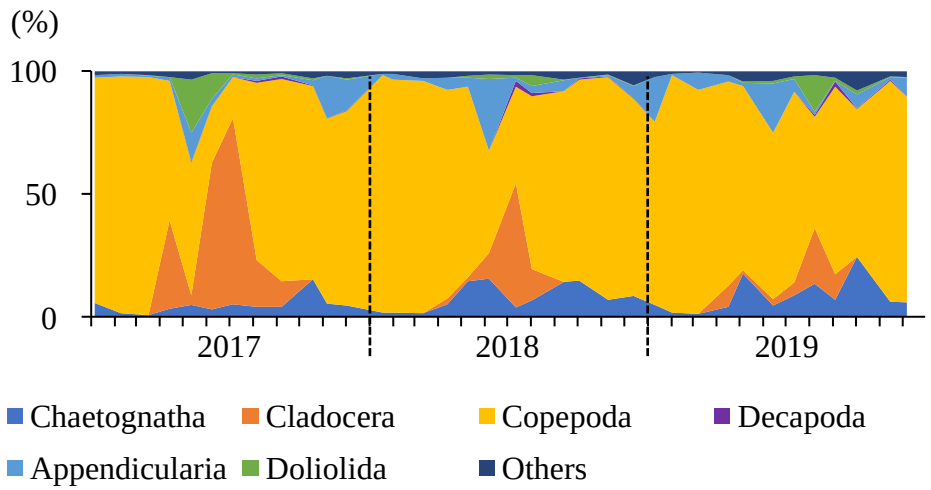

Fig. 3-1 Year-to-year variability of mesozooplankton composition at station SR1.

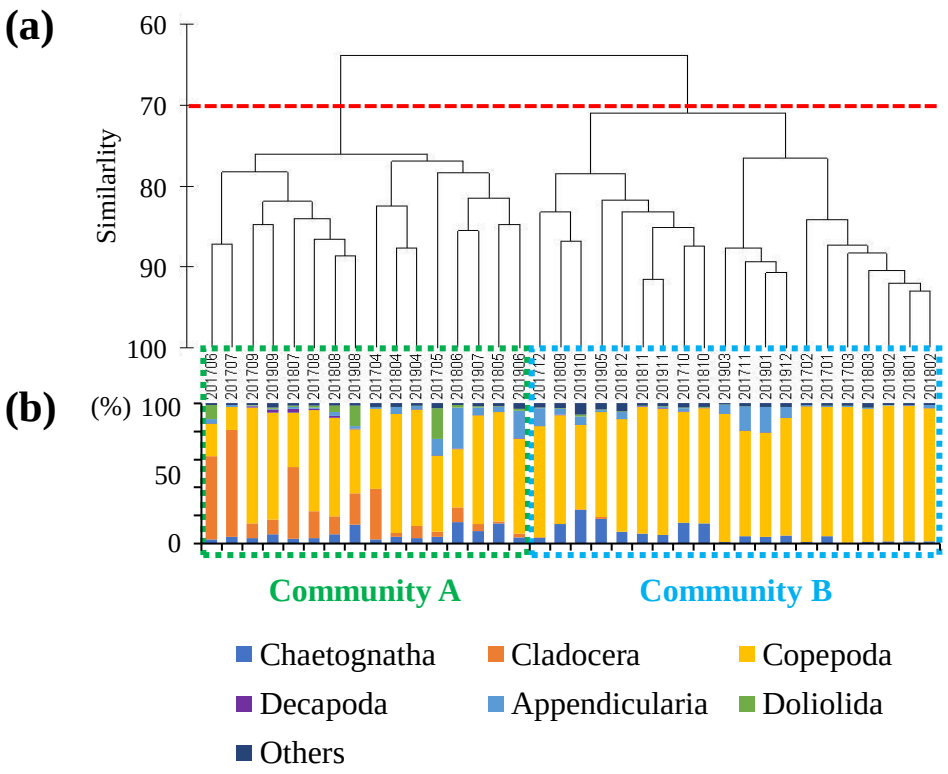

Fig. 3-2 (a) The dendrogram shows the classification of mesozooplankton communities based on the Bray-Curtis similarity index between the months of the study. (b) Mesozooplankton composition each month. The first two and last two digits indicate the sampling year and month, respectively ("1706" indicates "June 2017").

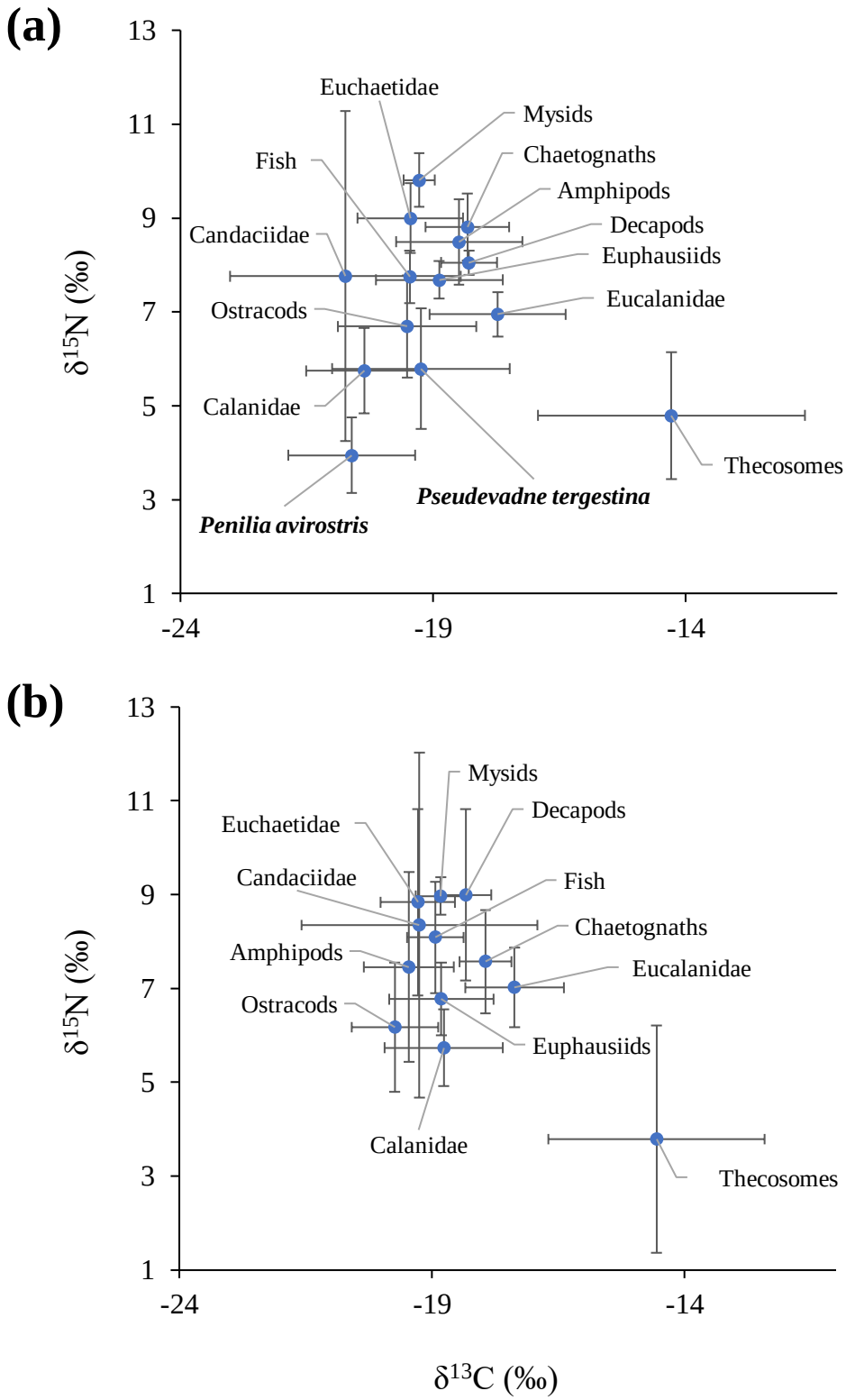

Fig. 3-3 Stable isotope compositions (Mean ± SD) of main zooplankton groups in community A (a) and community B (b). Numbers of samples used the analysis are shown in Table 3-1.

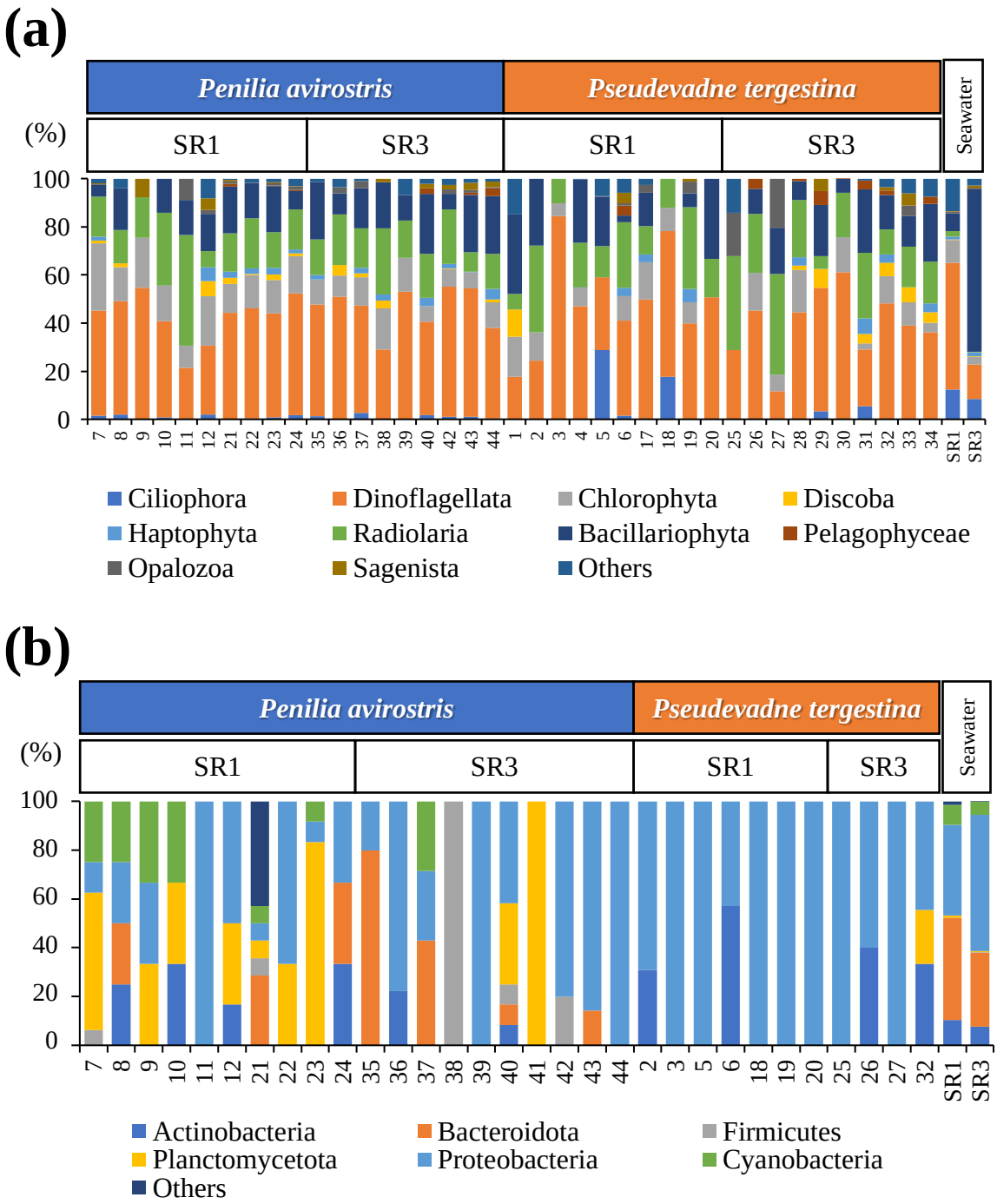

Fig. 3-4 Taxonomic composition of organisms detected in gut samples of *Penilia* and *Pseudevadne*, in the 18S (A) and 16S analysis (B). Number, indicated in lower part of the bar, shows sample number analyzed for each individual. Composition is based on relative read abundances.

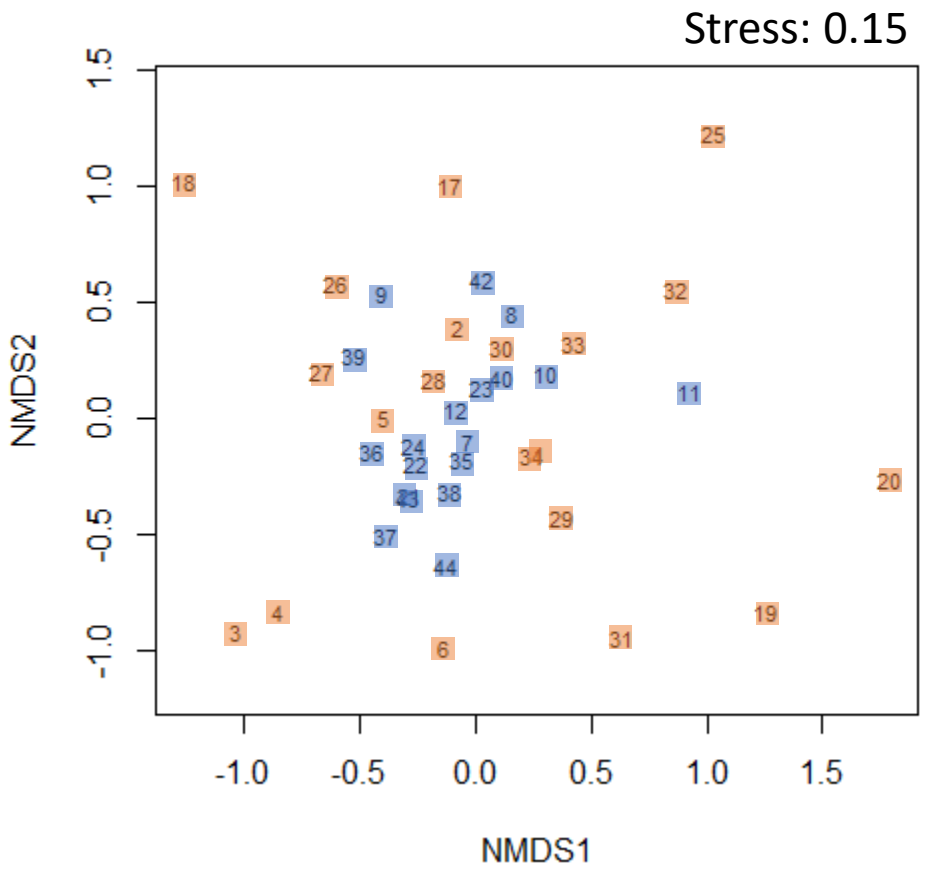

Fig. 3-5 NMDS plot based on OTUs in the 18S analysis. Blue and orange colors indicate *Penilia* and *Pseudevadne*, respectively. Number, indicated in the square, shows sample number analyzed for each individual (see Fig. 3-4 also).

Table 3-1. Stable isotope composition (mean ± SD) of each taxonomic group at station SR1 during study period.

Taxon	*n*	Stable isotope compositions	
		$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Copepods			
Calanidae	9	-19.66 ± 1.41	5.74 ± 0.87
Eucalanidae	15	-17.58 ± 1.23	6.98 ± 0.65
Candaciidae	10	-20.12 ± 2.51	8.31 ± 3.66
Euchaetidae	15	-19.38 ± 0.94	8.94 ± 1.38
Cladocerans			
Penilia avirostris	3	-20.61 ± 1.26	3.95 ± 0.80
Pseudevadne tergestina	7	-19.24 ± 1.76	5.79 ± 1.28
Ostracods	12	-19.59 ± 1.23	6.52 ± 1.22
Amphipods	13	-18.86 ± 1.22	8.10 ± 1.53
Eupahusiids	9	-18.86 ± 1.19	7.38 ± 0.70
Mysids	7	-19.08 ± 0.46	9.45 ± 0.66
Decapods	6	-18.31 ± 0.53	8.52 ± 1.39
Chaetognaths	15	-18.17 ± 0.74	8.32 ± 1.08
Thecosomes	14	-14.40 ± 2.44	4.36 ± 1.95
Pisces	5	-19.14 ± 0.51	7.95 ± 1.00

Table 3-2. Comparison of nitrogen and carbon stable isotope ratios of main zooplankton groups in offshore zooplankton communities, defined by cluster analysis. The Kruskal-Wallis test was used.

Taxon	$\delta^{15}N$ (‰) (Mean ± SD)		*p*
	Community A	Community B	
Eucalanidae copepods	6.95 ± 0.47	7.02 ± 0.85	> 0.1
Euchaetidae copepods	9.00 ± 0.74	8.84 ± 1.99	> 0.1
Ostracods	6.69 ± 1.09	6.17 ± 1.38	> 0.1
Amphipods	8.50 ± 0.91	7.46 ± 2.02	> 0.1
Chaetognaths	8.81 ± 0.71	7.57 ± 1.10	0.06
Thecosomes	4.79 ± 1.35	3.79 ± 2.42	> 0.1

Table 3-3. 18S metabarcoding analysis of gut contents of *Penilia avirostris* and *Pseudevadne tergestina* . Occurrence rates of each taxonomic group are shown.

Taxon	Occurrence rate (%)	
	Penilia	*Pseudevadne*
Ciliophora	73.7	40
Dinoflagellata	100	100
Chlorophyta	100	85
Discoba	63.2	35
Haptophyta	68.4	40
Radiolaria	100	100
Bacillariophyta	100	85
Pelagophyceae	31.6	40
Opalozoa	57.9	35
Sagenista	57.9	25

Table 3-4. Dominant OTUs in the guts of *Penilia avirostris* and *Pseudevadne tergestina* based on 18S analysis. Asterisks indicate OTU overlap between the two species.

Species	Average percentages (%)	Putative taxon	BLAST result		
			Coverage (%)	Identity (%)	Accession No.
Penilia avirostris	5.4	Unidentified Metamonada*	100	79	KT388048.1
	5.0	Unidentified Cercozoa*	100	80	AY449716.1
	3.7	*Steginoporella* (Bryozoa)*	100	100	FJ009098.1
	3.2	Unidentified Chlorophyta*	100	89	KY780193.1
	2.0	*Thalassiosira* (Bacillariophyceae)*	100	100	KY980238.1
	2.0	Unidenified Radiozoa	100	89	OK562151.1
	2.0	*Blastodinium* (Dinophyceae)	100	97	JX473667.1
	1.9	Unidentified Cercozoa*	100	85	AY449716.1
	1.8	*Chloropicon* (Chlorophyta)*	100	100	MW441228.1
	1.7	*Wangodinium* (Dinophyceae)	100	100	MH732688.1
	1.4	*Cyclotella* (Bacillariophyceae)	100	98	JF790990.1
	1.3	*Chaetoceros* (Bacillariophyceae)	100	99	KX253958.1
	0.9	*Prorocentrum* (Dinophyceae)	100	100	MK405477.1
Pseudevadne tergestina	7.2	Unidentified Metamonada*	100	79	KT388048.1
	6.5	Unidentified Cercozoa*	100	80	AY449716.1
	2.4	*Steginoporella* (Bryozoa)*	100	100	FJ009098.1
	1.8	Unidentified Chlorophyta*	100	89	KY780193.1
	1.7	*Chloropicon* (Chlorophyta)*	100	100	MW441228.1
	0.9	*Thalassiosira* (Bacillariophyceae)*	100	100	KY980238.1
	0.9	Unidentified Cercozoa*	100	85	AY449716.1
	0.9	*Cladococcus* (Radiozoa)	100	99	HQ651782.1

Table 3-5. 16S metabarcoding analysis of gut contents in *Penilia avirostris* and *Pseudevadne tergestina* . Occurrence rates of each taxonomic group are shown.

Taxon	Occurrence rate (%)	
	Penilia	*Pseudevadne*
Actinobacteria	30	36.4
Bacteroidota	35	0
Dependentiae	5	0
Firmicutes	25	0
Planctomycetota	45	9.1
Proteobacteria	85	100
Cyanobacteria	35	0

Table 3-6. Summary of the feeding habits of *Penilia avirostris* and podonid cladocerans.

Species	Detected food items	Method	Reference
Penilia avirostris	Diatoms, Ciliates	Mg	Turmer et al. (1988)
	Diatoms, Cryptophytes, chlorophytes	Pi, Ce	Wong et al. (2006)
	Diatoms, Dinoflagellates, Ciliates	Ce	Atienza et al. (2006)
Podonidae	Phytoplankton, small animals	Mo	Nival and Ravera (1979)
	Diatoms	Ce	Jagger et al. (1988)
	Nanoflagellates, Ciliates, Dinoflagellates	Ce	Sanchez et al. (2011)

Mg: Gut content analysis with microscope, Mf: Morphorogical study on gut contens or appendages of cladocerans, Pi: Gut pigment analysis, Ce: Culture experiment

Table. 4-1. List of cladoceran species in the north Pacific and Arctic regions.

Area	Latitude (N)	Species (number)	Reference
Barents Sea	69˚	EN, PL (2)	Dvoretsky and Dvoretsky (2010)
Bering Sea / Chukchi Sea	62˚–69˚	PA, EN, PL (3)	Onbé et al. (1996)
Akkeshi Bay	43˚	EN, PL (2)	Saigusa (2000)
Otsuchi Bay	39˚	PA, PT, EN, ES, PP, PS, PL (7)	Onbé (1996)
Onagawa Bay	38˚	PA, PT, EN, PP, PL (5)	Uye (1982)
Toyama Bay	37˚	PA, PT, EN, ES, PP, PS, PL (7)	Onbé and Ikeda (1995)
Chinhae Bay	35˚	PA, PT, EN, PP, PL (5)	Yoo and Kim (1987)
Suruga Bay	34˚–35˚	PA, PT, EN, ES, PP, PS, PL (7)	This study
Omura Bay	32˚–33˚	PA, PT, EN, PP, PL (5)	Itoh and Iizuka (1980)
Tolo Harbor	22˚	PA, PT, PS (3)	Tang et al. (1995)
Daya Bay	22˚	PA, PT, PP (3)	Li et al. (2021)

PA: *Penilia avirostris* , PT: *Pseudevadne tergestina* , EN: *Evadne nordmanni* , ES: *Evadne spinifera* , PP: *Pleopis polyphemoides* , PS: *Pleopis schmackeri* , PL: *Podon leuckartii*

Table 4-2. Clearance rates of P*enilia avirostris* reported in previous studies.

Clearance rate (ml inds.$^{-1}$ day^{-1})		Reference
Range	Mean	
4.8–26	-	Paffenhöfer and Orcutt (1986)
18–56	-	Turner et al. (1998)
0.1–20.3	2.2	Wong et al. (1992)
0–54.9	25.5	Katechakis and Stibor (2004)
5.9–24.9	15	Atienza et al. (2006)
3.6–7.8	5.9	Atienza et al. (2007)

Table 4-3. Secondary production rates of *Penilia avirostris* and copepods.

Secondary producer	Production rate (mg C m^{-3} d^{-1})		Area	Reference
	Range	Mean		
Cladocera				
Pe. avirostris	<0.01–0.11	0.04	Suruga Bay, Japan	This study
Copepoda				
Calanus sinicus	0.02–3.67	0.91	East China Sea	Kang and Kim (2021)
Calanus helgolandicus	0.02–1.50	-	English Channel	Ray-Rassat et al. (2004)
Acartia omori	<0.01–0.20	0.09	Ilkwang Bay, Korea	Kang et al. (2007)
Acartia steuri	<0.01–0.31	0.07	Ilkwang Bay, Korea	Kang and Kang (2005)
Temora turbinata	<0.01–1.12	-	Cananéia Lagoon, Brazil	Ara (2002)
Microsetella norvegica	<0.01–4.9	-	Seto Inland Sea	Uye et al. (2002)

Table 4-4. Predators of marine cladocerans reported in previous studies.

Predator	Prey cladoceran species	Area	Reference
Fish			
Japanese anchovy (*Engraulis japonicus*)	PA, PT	Seto Inland Sea	Yamamoto and Katayama (2012)
Japanese sardine (*Sardinops melanostictus*)	PA, PT	Seto Inland Sea	Yamamoto and Katayama (2012)
Pacific saury (*Cololabis saira*)	PT	Pacific Ocean	Morita and Arima (2022)
Fish (larvae)			
Pacific bluefin tuna (*Thunnus orientalis*)	POD	Sea of Japan	Kodama et al. (2020)
Southern bluefin tuna (*Thunnus maccoyii*)	POD	Northwest Australia	Uotani et al. (1981)
Yellowfin tuna (*Thunnus albacares*)	POD	Northwest Australia	Uotani et al. (1981)
Bigeye tuna (*Thunnus obesus*)	POD	Northwest Australia	Uotani et al. (1981)
Albacore (*Thunnus alalunga*)	POD	Northwest Australia	Uotani et al. (1981)
Bullet tuna (*Auxis rochei*)	ES, POD	Sea of Japan	Kodama et al. (2022)
Auxis sp.	POD	Northwest Australia	Uotani et al. (1981)
Blue marlin (*Makaira nigricans*)	POD	Straits of Florida	Llopiz and Cowen (2008)
Snailfish (*Istiophorus platypterus*)	POD	Straits of Florida	Llopiz and Cowen (2008)
Flatfish (*Pseudorhombus pentophthalmus*)	PA	Wakasa Bay	Kuwahara and Suzuki (1983)
Japanese sea bass (*Lateolabrax japonicus*)	PL	Seto Inland Sea	Iwamoto et al. (2010)
Red mullet (*Mullus barbatus*)	PA, POD	Mediterranean Sea	Sabatés et al. (2015)
Black seabream (*Acanthopagrus schlegeli*)	PA, POD	Tolo Harbour	Nip et al. (2003)
Japanese seaperch (*Lateolabrax japonicus*)	PA, POD	Tolo Harbour	Nip et al. (2003)
Halfbeak (*Hyporhamphus sajori*)	EN	Toyama Bay	Oya et al. (2002)
Lanternfish (*Diaphus garmani*)	POD	Pacific Ocean	Sassa and Kawaguchi (2004)
Lanternfish (*Myctophum asperum*)	POD, PA	Pacific Ocean	Sassa and Kawaguchi (2004)
Chaetognaths			
Flaccisagitta enflata	PA, POD	Mediterranean Sea	Kehayias and Kourouvakalis (2010)
Mesosagitta minima	PA, POD	Mediterranean Sea	Kehayias and Kourouvakalis (2010)
Parasagitta friderici	PA, POD	Off Ubatuba, Brazil	Liang and Vega-Pérez (1995)
Ferosagitta hispida	PA, POD	Off Ubatuba, Brazil	Liang and Vega-Pérez (1995)
Jellyfish			
Aurelia aurita	EN, POD	Baltic Sea	Barz and Hirche (2005)
Aurelia labiata	CL	Alaska	Purcell (2003)
Mnemiopsis leidyi	PA, PP	Black Sea	Finenko et al. (2013)
Cyanea capillata	CL	Alaska	Purcell (2003)
Aequorea aequorea	CL	Alaska	Purcell (2003)

PA: *Penilia avirostris*, PT: *Pseudevadne tergestina*, EN: *Evadne nordmanni*, ES; *Evadne spinifera*, PL: *Podon leuckartii*, PP: *Pleopis polyphemoides*, POD: Podonidae, CL: cladocerans (not identified)

Table S1. List of taxa used for stable isotopic analysis. Black circles indicate the taxa sorted, for which stable isotope ratios were measured.

	2017							2018				2019						
	Apr.	May	Jun.	Jul.	Aug.	Sep.	Nov.	May	Jun.	Sep.	Nov.	Jan.	Feb.	Mar.	Apr.	Jul.	Aug.	Sep.
Sampling and measurement																		
Wire out (m)	2000	2000	2000	2000	2000	2000	2000	200	200	200	200	200	200	200	200	200	200	200
Taxon																		
Cladocera																		
Penilia avirostris				●	●													
Pseudevadne tergestina		●	●	●											●	●	●	●
Copepoda																		
Eucalanidae		●		●		●	●	●	●	●	●	●	●	●	●	●	●	●
Euchaetidae		●		●		●	●	●	●	●	●	●	●	●	●	●	●	●
Calanidae								●	●	●	●		●	●	●		●	●
Candaciidae								●	●	●	●	●	●	●	●	●	●	●
Amphipoda		●		●		●	●	●			●	●	●	●	●	●	●	●
Ostracoda		●		●				●	●		●	●	●	●	●	●	●	●
Chaetognatha		●		●		●	●	●	●	●	●	●	●	●	●	●	●	●
Thecosomata		●		●		●	●	●	●	●	●	●	●	●	●		●	●
Heteropoda						●												●
Mysida				●								●	●	●	●	●	●	
Euphausiacea		●		●		●		●	●	●	●		●	●				●
Decapoda	●	●				●						●	●	●				
Pisces												●	●	●	●		●	

Table S2. Mean stable isotope ratio of zooplankton groups in the two communities (see also Fig. 3-2 and Table 3-1).

	Community A		Community B	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Copepods				
Eucalanidae	-17.72 ± 1.35	6.95 ± 0.47	-17.36 ± 0.97	7.02 ± 0.85
Euchatidae	-19.45 ± 1.04	9.00 ± 0.74	-19.28 ± 0.74	8.84 ± 1.99
Calanidae	-20.37 ± 1.15	5.75 ± 0.91	-18.77 ± 1.17	5.73 ± 0.81
Candaciidae	-20.74 ± 2.29	7.77 ± 3.52	-19.25 ± 2.33	8.35 ± 3.68
Cladocerans				
Pseudevadne tergestina	-19.24 ± 1.76	5.79 ± 1.28	N. D.	N. D.
Penilia avirostris	-20.61 ± 1.26	3.95 ± 0.80	N. D.	N. D.
Ostracods	-19.51 ± 1.37	6.69 ± 1.09	-19.74 ± 0.86	6.17 ± 1.38
Amphipods	-18.49 ± 1.25	8.50 ± 0.91	-19.46 ± 0.89	7.46 ± 2.02
Decapods	-18.29 ± 0.55	8.05 ± 0.26	-18.33 ± 0.50	8.99 ± 1.83
Mysids	-19.28 ± 0.31	9.81 ± 0.57	-18.83 ± 0.49	8.97 ± 0.40
Euphausiids	-18.88 ± 1.26	7.69 ± 0.39	-18.82 ± 1.03	6.77 ± 0.77
Chaetognaths	-18.32 ± 0.83	8.81 ± 0.71	-17.94 ± 0.52	7.57 ± 1.10
Thecosomes	-14.28 ± 2.64	4.79 ± 1.35	-14.56 ± 2.14	3.79 ± 2.42
Heteropods	-13.13 ± 0.82	6.23 ± 0.38	N. D.	N. D.
Fish	-19.46 ± 0.02	7.75 ± 0.56	-18.93 ± 0.56	8.09 ± 1.19

Ecological studies on marine cladocerans in Suruga Bay, Japan: mass occurrence in offshore waters, formation mechanisms, and roles in the offshore food-web

（駿河湾における海産枝角類の生態学的研究：
沖合域における大量出現とその形成メカニズムおよび食物網における役割）

剣持　瑛行

キーワード：海産枝角類　駿河湾　粒子追跡実験　安定同位体比　メタバーコーディング

海産枝角類は一般に「ミジンコ」と呼ばれる体長 1 mm 程度の小型の甲殻類である．600 種を超える高い種多様性をもつ淡水種に対して，海産種は 8 種のみが報告されている．海産枝角類の生態に関する研究は沿岸域を中心に行われてきており，沖合域における分布や個体群動態，食性などの知見は限られている．本研究は，5 年間にわたる調査により，駿河湾沖合域における枝角類の個体群動態を明らかにした（第 1 章）．また，物理モデルを用いた粒子追跡実験と，駿河湾沿岸・沖合における調査を組み合わせることにより，沖合に周期的に形成される枝角類群集の個体群形成メカニズムを解明した（第 2 章）．さらに，沖合食物網における枝角類の位置や役割を解明するため，安定同位体比分析による食物網解析と枝角類の消化管内容物のメタバーコーディングによる食性解析を行った（第 3 章）．本研究で得られた知見に基づき，駿河湾沖合域における枝角類の種多様性や摂餌インパクト，二次生産などについて議論した（第 4 章）．

個体群動態を調べた調査は，沖合域の定点 SR1（水深 1000 m）において，2014 年 6 月から 2019 年 12 月にかけて基本的に毎月行った．プランクトンネット（口径 160 cm，目合 335 μm）を用いて動物プランクトンの採集を行い，得られた試料は中性ホルマリン海水で固定し，実体顕微鏡を用いて各種の個体数密度（inds. m^{-3}）を算出した．調査期間中，枝角類はすべての年で春期から夏期にかけて高い個体数密度で出現し，夏期の沖合メソ動物プランクトン群集において優占した．このことから，枝角類は同湾沖合域において定常的に季節的な大量出現をすることが明らかとなった．また，これまでに日本近海で報告されている 7 種全てが出現し，*Evadne nordmanni*，*Penilia avirostris*，*Pseudevadne tergestina* が比較的高い個体数密度で出現した．その一方で，*Podon leuckartii*，*Pleopis polyphemoides* の出現は極めて稀であった．枝角類の個体群は，海底に堆積した休眠卵に由来することから，沖合の個体群は沿岸域から供給されていると推測された．

このような沖合域における枝角類の定常的な大量発生のメカニズムを明らかにするために，2018 年 4 月から 2019 年 3 月にかけて沿岸域および沖合域各 2 地点において調査を行った．ネット（口径 45 cm，目合 100 μm）を用いたプランクトン採集を行い，各種の個体数密度を推定した．さらに，粒子追跡実験により，枝角類の沿岸から沖合への輸送過程を推定した．実験は，Regional Ocean Modeling System を基盤とした物理モデルにより，2014 年–2017 年までの海況シミュレーションを実施し，得られた表層流動場の月平均値を用いた．駿河湾の沿岸域各所から枝角類に見立てた 106

個の粒子を放流し，各月 30 日間の粒子の挙動を解析した．沿岸域と沖合域における同時的な調査の結果，沿岸と沖合の個体群は類似した出現パターンを示したが，(1) 沖合域で稀であった 2 種（*Po. leuckartii*, *Pl. polyphemoides*）は沿岸域では高密度（> 100 inds. m^{-3}）で出現したこと，(2) *Pe. avirostris* の個体数密度は，沿岸域よりもむしろ沖合域で高い値を示したこと，など種による違いがみられた．粒子追跡実験の結果，3 月から 8 月にかけては沿岸域に配置された粒子は湾内で反時計回りに移動し，湾奥部沖合域に集積した．一方，それ以外の時期には，粒子は湾奥部に集積せず，湾外に流出した．このことから，春期から夏期にかけて卓越する駿河湾特有の循環流が，同湾沖合域における枝角類個体群の形成に寄与していることが示唆された．また，すべての種が沖合域に輸送・集積されるのではなく，各種の好適水温や塩分，餌生物などの生物学的要因が沖合個体群の形成に影響していると考えられた．

観察された枝角類の沖合個体群が沖合の食物網にどのような影響を与えているのか，安定同位体比解析および消化管内容物メタバーコーディング解析の 2 つの方法によって解明を試みた．2017 年から 2019 年にかけて沖合域において得られたプランクトン試料を用いて，枝角類 2 種（*Pe. avirostris*, *Ps. tergestina*）を含む主要分類群の窒素（$\delta^{15}N$）および炭素安定同位体比（$\delta^{13}C$）を測定した．クラスター解析の結果，沖合の動物プランクトン群集は枝角類が出現する群集 A とカイアシ類が優占する群集 B に大別されたが，群集間における主要分類群の $\delta^{15}N$ 値および $\delta^{13}C$ 値には明瞭な差はみられなかった．枝角類優占 2 種 *Pe. avirostris* と *Ps. tergestina* の $\delta^{15}N$ 平均値は，それぞれ 4.0±1.4‰，5.8±1.3‰であり，調べた動物プランクトンにおいて最も低い栄養段階に位置すること，また前者の方が後者に比べて低い値を取り，2 種の栄養段階，食性が異なることが示唆された．さらに，優占 2 種の消化管内に含まれる餌生物の DNA を抽出，PCR 増幅し，次世代シーケンサーによるシーケンシングを行った（18S および 16S 領域）．得られた配列データに基づき，バイオインフォマティックスにより両種の餌生物組成や種による違いを調べた．18S 解析の結果，*Penilia* と *Pseudevadne* の餌組成（真核生物）は類似しており，主として珪藻や渦鞭毛藻，放散虫を摂餌していることが示唆された．一方，原核生物を主対象とした 16S 解析では，プランクトマイセスやシアノバクテリアなどが *Penilia* の消化管からのみ検出され，種により食性が異なった．本種によるシアノバクテリアの摂餌により，微小プランクトンと高次捕食者とを直接繋ぐ役割をもつことが示唆された．

駿河湾沖合域における枝角類の個体群濾水速度や生産速度を推定したところ，カイアシ類と比べて低く，摂餌による影響やそれらによる二次生産は限定的であると推測された．一方，枝角類は様々な仔稚魚の消化管内から見つかっており，魚類にとって重要な初期餌料である可能性がある．地球規模の気候変動により予想されている海水温の上昇と黒潮の弱まりは，黒潮流域における動物プランクトン生産を弱め，駿河湾沖合域における枝角類の個体群形成に影響をもたらすかもしれない．